ADVANCES IN THE SYNTHESIS AND APPLICATION OF QUINONES

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Abstract

Chapter 1 presents a brief overview of sustainable methodologies in chemical synthesis, together with the role of quinones.

In **Chapter 2** the biological activity of twelve 5-aminoindolequinones against NQO2, an underexplored enzyme, was assessed and three new 5-amino indolequinone derivatives displayed good inhibitory profile against this enzyme. Besides, the UV-visible absorbance curves and the nucleophilic trapping of the activated electrophilic iminium intermediate following reductive activation of the indolequinone scaffold were effectively carried out for two substrates. These experiments were used in order to gather evidence that elimination of the leaving group, required for the activity of these compounds, would happen. The interaction of 5-aminoindolequinone **37** with NQO2 was analysed by mass spectrometry and the formation of the adduct **37**/FAD suggests a mechanism-based inhibition of the enzyme.

In **Chapter 3** the experiments were performed to advance towards a more sustainable synthesis of 5-aminoindolequinones. A range of reaction conditions, such as time, metal loading, solvent, and concentration of the solution were adjusted throughout the synthetic sequence but the main focus was on the optimisation of the conditions for the reaction between 2-bromo-5-methoxy-1,4-benzoquinone **156** and enamine **158** through a copper(II)-mediated process in the presence of a more environmentally benign solvent system in which water was used as the main solvent. There are no precedents in the literature for the synthesis of these derivatives in water. At the end of the synthetic sequence, sixteen 5-amino substituted indolequinones were prepared.

In **Chapter 4** the experiments were designed to develop a more sustainable synthesis of halogenated phenols. A range of reaction conditions were optimised in the reactivity of quinone diazides towards nucleophilic substitution. The concentration of the solution was increased from 0.5 to 1 M, the catalyst loading was reduced from 10 to 0.5 mol% and the reaction time decreased from 1 h to approximately 10 min. Thirteen halogenated phenols were successfully prepared, typically in low to moderate yields.

Finally, **Chapter 5** contains details of all experimental procedures, and describes the chemical elimination reductive studies, examined in Chapter 2, the synthesis of 5-aminoindolequinones and their precursors, discussed in Chapter 3, and the synthesis of halogenated phenols and corresponding precursors, quinone diazides, analysed in Chapter 4.

KEYWORDS: Indolequinones, Quinone Diazides, Synthesis, Sustainability, NQO2.

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Abbreviations

Standard abbreviations are used throughout the text presented in the following pages. Below are summarised some of the non-standard abbreviations mentioned in this thesis.

CFCs	Chlorofluorocarbons
FAD	Flavin adenine dinucleotide
IQ	Indolequinone
MTBE	Methyl *tert*-butyl ether
MCRs	Multi component reactions
NRH	Dihydronicotinamide riboside
NQO1	NAD(P)H quinone oxidoreductase 1
NQO2	NRH quinone oxidoreductase 2
VOCs	Volatile Organic Compounds

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1 Sustainable Organic Synthesis: a framework

The escalating social demands that have been driving the development of modern chemistry have allowed us to convert natural resources and to create new materials typically from non-renewable and non-biodegradable feedstocks and through unsustainable processes. Thus, contemporary living is enriched and the quality of life of our society improved. Nevertheless, these accomplishments reveal inherent downsides as the wider impacts have not been considered. Global natural resources are quickly running low and waste associated with the cycle of use and transformation of these resources as well as from chemical products that have reached their lifespan are accumulating at a fast pace. Furthermore, there are unwanted properties of chemical products, such as CFCs and leaded petrol, which could not be foreseen by their inventors and have been triggering environmental, health and social concerns.[1] As a result, sustainability has become one of the greatest challenges of our time and the use of *getting there* as a synonym of indisputable success in traditional chemistry fields has been slowly replaced by the importance of *how* they are getting there.[2] Hence, it is crucial to raise the awareness regarding the consequences that might become more frequent and aggravated as the longer we continue to utilise unsustainable chemical processes. Developing efficient chemical syntheses of complex, high added value, molecules from simpler subunits through more sustainable and greener synthetic approaches has become a more established field. Reshaping the way in which chemists perceive synthesis, recognising the importance of not focusing only on developing novel methods but also on creating more sustainable alternatives to the existing ones is a priority.

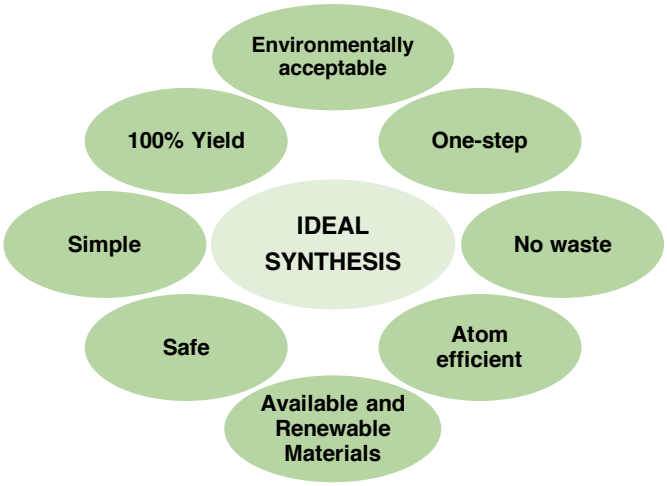


Figure 1.1 The ideal chemical synthesis.

This introduction on sustainability is not intended to be comprehensive and it presents just a very brief overview of some of the main focus of study in the field of chemistry such as synthetic methodologies and solvents.

1.1 Synthetic Methodologies

A series of factors regarding the design of synthetic, low impact and environmentally benign protocols should be considered when attempting to develop greener and more sustainable chemistry. Aspects such as energy consumption, atom efficiency and overall metrics of a chemical process should be worthy of a careful reflection and be clearly evaluated. A selection of new advances in synthetic chemistry have been discussed in the literature as greener alternatives to existing methodologies, such as aqueous chemistry, biocatalysis, telescoping and flow chemistry.[3] A recent review on multicomponent reactions (MCRs) analyses the performance of this type of reactions (in which at least three reactants are combined in the same pot to obtain a product containing most atoms of the starting materials) in view of the green chemistry recommendations.[2] A significant connection between this chemistry and sustainable organic synthesis was justified based on MCRs' atom economy, mild conditions, efficiency, high convergence and step economy, in addition to their general compatibility with "green" solvents. Additionally, despite the demonstration of the general value of MCRs for greener process developments, the authors have identified the weaknesses and challenges for future research.

Modern pharmaceutical industry should be mentioned as its success has been largely due to the remarkable achievements of organic synthesis over the last century. Until recent times, the pharmaceutical industry has relied exclusively on synthetic chemistry methodology to prepare compounds for small-molecule drug discovery programmes. The concept of lead-oriented synthesis has been introduced in order to broaden and improve the molecules used to produce new medicines.[4] Likewise, the promotion of the concept of one-pot reaction (as a sequence of chemical transformations carried out in a single flask) for the synthesis of active pharmaceutical ingredients is noteworthy, allowing savings in time and resources between individual steps within a multistep synthesis. The combination of this process with organocatalysis has been invoked as an emerging technique that chemists might apply for the accelerated production of biologically active molecules.[5] In spite of the changes, the scarcity of sustainable methodologies within the drug discovery process emphasises the importance of choosing wisely the chemistry of a first synthesised compound since it might provide a foundation for widespread implementation of these procedures by pharmaceutical industries.

The intensive use of chemicals, solvents, energy and technologies based on stoichiometric processes is a common practice in the pharmaceutical industry which has led to high waste generation per kg of product compared to other segments of the chemical industry.[6] Since the mid-1990s the pharmaceutical industry has been implementing the adoption of sustainable practices and several key green chemistry research areas have been identified by pharmaceutical manufacturers and can be grouped into three major categories:

i) reactions that are currently used but would require greener and more sustainable reagents, these include: amide formation avoiding poor atom economy reagents; OH activation for nucleophilic substitution; reduction of amides without hydride reagents and oxidation/epoxidation methods without the use of chlorinated solvents.

ii) more ambitious reactions: C-H activation of aromatics; formation of chiral amines through an aldehyde/ketone + NH_3 + "X" path; asymmetric hydrogenation of unfunctionalised olefins/enamines/imines; original greener fluorination methods and *N*-centred chemistry avoiding azides, hydrazine, etc.[7]

iii) solvents

1.2 Solvents

Ideally, the best solvent for a chemical reaction is no solvent. Nonetheless, solvents play a key role in extractions, reaction mixture solubility, stabilisation of transition states, washing and separation of the final product.[8] In the past decade, a great effort was devoted to create innovative solvent systems, such as supercritical fluids, ionic liquids, water and polyethylene glycol. In order to decrease our environmental footprint, targeting the real challenges that need to be addressed is urgently required.

In considering ways to minimise the environmental impact of medicinal chemistry, it is wise to focus on those areas that have the largest impact. As an addition to the above mentioned, the third category which has been underlined by pharmaceutical manufacturers as one of the main green chemistry research areas comprises solvents. Solvents generally are the biggest contributors to waste in pharmaceutical production, representing typically around 56% of the total mass used in a reaction.[9,10]

In medicinal chemistry, the use of solvents in the purification processes far outweighs the solvents used in the reaction and column chromatography significantly contributes to organic solvent waste produced in medicinal chemistry laboratories. Reducing the volume of solvent used in the first place and running the reactions at a more concentrated molarity should be a priority besides including the use of alternatives to chromatography such as crystallisation and

ion exchange columns when possible. Also, to select the most benign solvent, with a particular focus on using greener solvents for chromatographic purification reducing dichloromethane usage. For example, TLC evaluation of crude reaction mixtures with alternative green solvent systems is an easy first step toward reduction of dichloromethane in chromatography.[3,11]

In pharmaceutical companies the focus has been on recycling, using single solvent systems, avoidance of toxic and harmful solvents, such as chlorinated solvents and the replacement of these for more environmentally benign options such as lower alcohols, esters and some ethers like methyl *tert*-butyl ether.[12,13]

A compilation of the data from the solvent guides published by two pharmaceutical companies, AstraZeneca and GlaxoSmithKline, and ACS Green Chemistry Institute Pharmaceutical Roundtable has been made and combined to allow a ranking comparison. Of the 51 solvents considered, a ranking into four categories was established: recommended, problematic, hazardous and highly hazardous (**Table 1.1**).[14]

Table 1.1 Overall ranking of solvents.

Recommended	Water, EtOH, *i*-PrOH, *n*-BuOH, EtOAc, *i*-PrOAc, *n*-BuOAc, anisole, sulfolane
Recommended or problematic?	MeOH, *t*-BuOH, benzyl alcohol, ethylene glycol, acetone, MEK, MIBK, cyclohexanone, MeOAc, AcOH, Ac_2O
Problematic	MeCN, Me-THF, heptane, Me-cyclohexane, toluene, xylenes, chlorobenzene, DMPU, DMSO
Problematic or hazardous?	MTBE, THF, cyclohexane, CH_2Cl_2, formic acid, pyridine
Hazardous	Diisopropyl ether, 1,4-dioxane, DME, pentane, hexane, DMF, DMAc, NMP, methoxy-ethanol, TEA
Highly hazardous	Diethyl ether, benzene, $CHCl_3$, CCl_4, DCE, nitromethane

The selection of the greenest solvent for a process is a compromise. As a result, when searching for a solvent having certain desirable properties, it is probable that a chemist will often be unable to select a greener alternative.

Methods for assessing the environmental impact of solvents are of the greatest importance. Solvents can be compared through a general method considering i) the energy required to their manufacture as exemplified in **Figure 1.2a**; ii) cumulative energy demand and iii) impact on health and environment. Moreover, it is also possible to compare solvents based upon greenhouse gas emissions as displayed in **Figure 1.2b**.

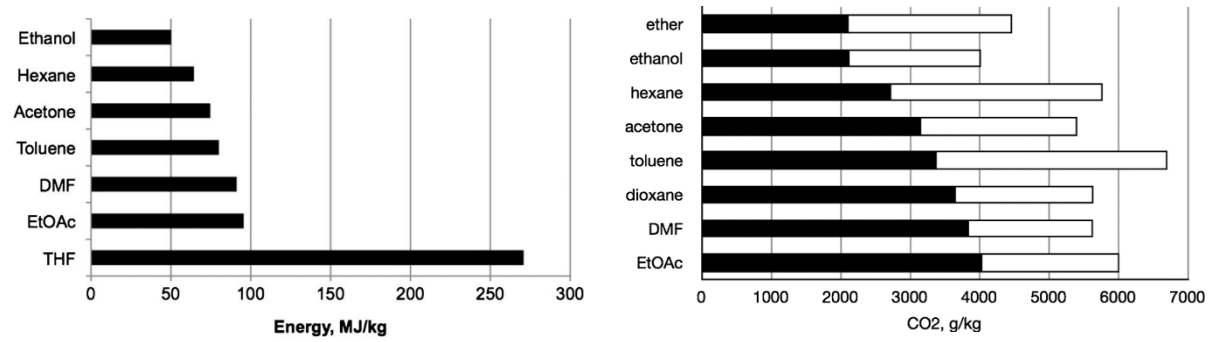


Figure 1.2 Charts presenting a) the energy required to manufacture 1 kg of solvent and b) greenhouse gas emissions resulting from the energy consumed during the manufacture (solid bars, assuming 0.042 g CO_2 emissions per KJ) and from the eventual oxidation or incineration (hollow bars) of 1 kg of solvent.[15]

An additional focus has been devoted to bio-based solvents that have been promoted as the next generation of alternative solvents to the conventional fossil fuel based ones. Bio-based solvents are normally derived from agricultural crops thus one of its most notable advantages should be the lower environmental impact.[8,16,17] Bio-based solvents also have lower toxicity, higher biodegradability, inferior volatile organic compounds (VOCs) emissions and generate less pollution during manufacture than conventional solvents. These bio-based solvents also benefit from reduced disposal costs and improved worker safety.

Ethanol and methyl tetrahydrofuran are becoming popular as bio-based alternative solvents. Glycerol, D-limonene, lactic acid and its derivative ethyl lactate are also being promoted as a reaction media, the latter being produced by combining two innocuous renewables.[8,12] Although organic bio-based solvents possess the same VOC issues as the conventional ones, the use of a renewably sourced solvent stands as a great step towards the growing social and legislative demands with regard to sustainability.[3]

Bio-based solvents revealed great potential in the promotion of more sustainable chemical processes, albeit further developments of this chemistry are required and several obstacles need to be overcome. First, accessibility, diversity and versatility of presented bio-based solvents are limited. Second, many of these solvents are expensive at this stage, limiting their use for large scale applications.[8]

In conclusion, even though there has been a great success with some green solvents, there is not yet a universal green solvent as the sustainability of a solvent is most likely to be correlated to the considered reaction.

Sustainability as a whole, including social, environmental and economic aspects, has become one of the greatest challenges of our time. Choosing wisely the chemistry when a molecule is

first synthesised can have a tremendous impact on the chemistry of compounds that progress into development in the pharmaceutical industry. Thus, the development of synthetic methodologies in a more mindful and responsible way is of the highest importance in view of contributing to a more sustainable world.

In the words of Albert Einstein, considered by many as the greatest *scientist* of the twentieth *century,* "*A new type of thinking is essential if mankind is to survive and move toward higher levels*" (Atomic Education Urged by Einstein, p.13, *New York Times*, 25 May 1946).

1.3 Aims

This thesis covers two main compound classes: the nitrogen-containing hetarene quinones, indolequinones **1**, discussed briefly in Chapter 2, and in more detail in Chapter 3. In Chapter 2, the goal is to further understand the role of these structures in the inhibition of NRH quinone 2. Diazocyclohexadienones **2-4** will be analysed in Chapter 4 (**Figure 1.3**). Taking into consideration more sustainable approaches to the synthesis of a particular scaffold is an important current theme in organic chemistry. The aims of this work are: 1) to reduce the environmental impact of the synthesis of chemicals by replacing or reducing the use of stoichiometric reagents and additives; 2) to avoid the use of rare and expensive metals; 3) to substitute harmful organic solvents with less detrimental alternatives.

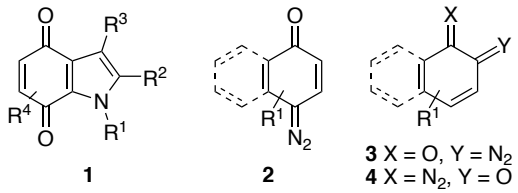


Figure 1.3 General structures for indolequinones **1** and diazocyclohexadienones **2-4**.

2 NRH quinone oxidoreductase 2

The flavoprotein quinone oxidoreductase mammalian family is divided into two major members, the first one termed NAD(P)H quinone oxidoreductase 1 (E.C.1.6.5.2 also referred to as NQO1, QR1, DT-diaphorase) and the other named NRH: quinone oxidoreductase 2 (E.C.1.10.5.1 also referred to as NQO2, QR2). NQO1 and NQO2 are mainly cytosolic enzymes whose function is to catalyse the two-electron reduction of quinones **5** to hydroquinones **7** without the formation of reactive oxygen intermediates, as semiquinones **6**, as shown in **Figure 2.1**.[18] This role has probably developed as a protective mechanism, which is present in all mammalian species, against potentially toxic quinones in the environment.[19,20] NQO2 also catalyses four-electron reduction reactions.[21] In spite of being expressed in many of the same tissues as NQO1, they do not appear to be expressed in conjunction and has not yet been clarified the precise degree of function overlapping.[22] Besides, NQO2 contains a specific metal binding site which is not present in NQO1. NQO2, like NQO1, has been implicated in the activation of both quinones and anticancer drugs leading to toxicity and cell death. Nevertheless, NQO1 has been an object of extensive studies over the past years although NQO2's cellular role has yet to be fully described and comprehended.[23]

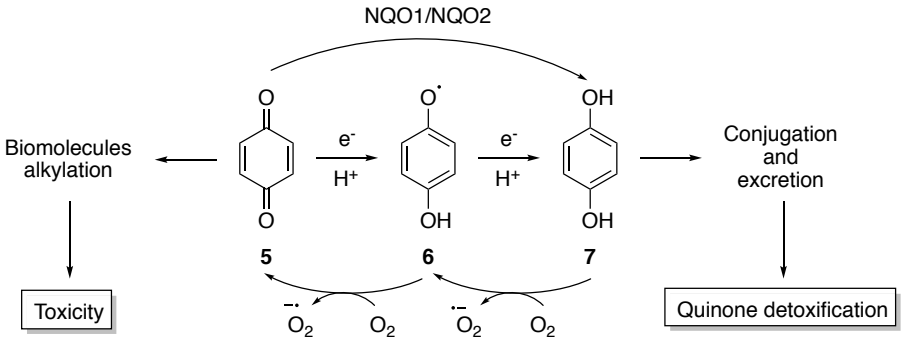


Figure 2.1 The role of NQO1 and NQO2 in the detoxification of quinones.

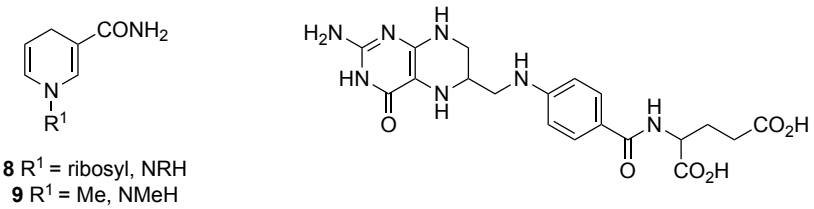
2.1 Characterisation

The screening of a human liver cDNA library, in 1990, led to the isolation of NQO2, which was initially described as an unknown mammalian cytosolic FAD-dependent flavoprotein in 1961 by Liao. After this, NQO2 has been completely forgotten over three decades. Later, it has been identified as the melatonin-binding site MT3 and as a promising target of melatonin therapeutic actions.[24,25] The "long-forgotten flavoenzyme" is widely expressed in various human tissues such as in the skeletal muscle, liver, kidney and heart. A minimal expression is detected in brain and pancreas and no expression in placenta has been yet detected.[26] This selective

expression might suggest a tissue-specific action of the enzyme.[21] It is noteworthy to point out that NQO2 is expressed at modest levels in human blood cells, in particular red blood cells while NQO1 expression is absent.[27] It has been suggest that NQO2 protects tumour suppressors p53, p33 and p63 against 20S proteasomal degradation.[28,29] Several reports stated that genetic polymorphisms of the NQO2 gene are associated with various neurological disorders, such as Parkinson disease, schizophrenia, alcohol withdrawal syndrome, clozapine-induced granulocytosis and methamphetamine psychosis.[30]

2.1.1 CO-FACTORS

NQO2 presents a distinct pyridine nucleotide-binding specificity from NQO1 and uses nonphosphorylated dihydronicotinamides, for example, natural *N*-ribosyl (NRH) **8** and *N*-methyl-dihydronicotinamide (NMeH) **9** or the synthetic *N*-benzyl-dihydronicotinamide (BNAH) **10** as the source of reducing equivalents (**Figure 2.2**). Yet, it can also utilise NAD[P]H but not so effectively as NQO1.[19,20,31] Tetrahydrofolic acid **11** was also found to act as a co-substrate for the reduction of menadione catalysed by NQO2, even with a low potency (K_m ~2 mM).[32]



8 R^1 = ribosyl, NRH
9 R^1 = Me, NMeH
10 R^1 = -CH_2Ph, BNAH

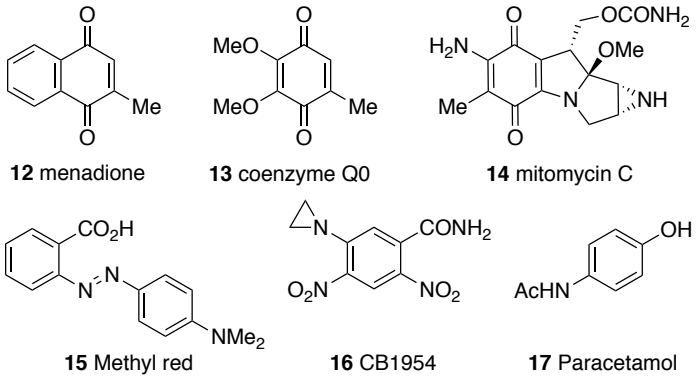
11 tetrahydrofolic acid

Figure 2.2 Examples of reported electron donating co-factors of NQO2.

2.1.2 SUBSTRATES

Endogenous substrates of NOQ2 have remained largely elusive, although dopamine has been proposed.[18,33] Exogenous substrates of NQO2 include menadione **12**, a cytotoxic synthetic analogue of vitamin K (**Figure 2.3**). Cells deficient in NQO2 and also NQO2 knock-out mice are resistant to menadione toxicity suggesting the action of this target as a bio-activating enzyme.[26,34,35] Moreover, toxic effects of menadione are potentiated through the activation of NQO2 with NRH. On the other hand, animals with NQO1 gene disruption displayed increased sensitivity to menadione toxicity even though this enzyme presents similar catalytic property towards menadione reduction when compared to the homologous enzyme NQO2.[18] Coenzyme Q0 **13** was the only commercially available quinone to be recognised as NQO2 substrate, in the presence of BNAH.[32] Metabolic activation of mitomycin C (MMC) **14** by cytosolic NQO2 has displayed enhanced cytotoxicity. The increase in NQO2 levels in Chinese

hamster ovarian (CHO) cells showed a proportional rise in MMC toxicity. Furthermore, this effect was considerably higher in the presence of NRH. Since the intracellular level of NRH is low, the exogenous addition of this electron donating co-factor might have activated NQO2 and caused greater metabolic activation of MMC and consequently, increased cytotoxicity.[36] Another study has analysed the reduction of MMC by NQO2, at pH 5.8 in the presence of NADH.[34] In both examples, along with the NQO2 inhibition, the generation of species that react with DNA to form cross-links was implicated into MMC toxicity. This enzyme also catalyses a four-electron reduction, using methyl red **15** as the electron acceptor.[37] A remarkable characteristic of NQO2 is its ability to act as a nitroreductase with high specificity for selected substrates; such is the case of 5-[aziridin-1-yl]-2,4-dinitrobenzamide (CB1954) **16**. This nitroreductase feature is common in bacteria but quite rare in mammals. Human NQO2 was found to be 3000 times more effective in reducing this prodrug in comparison with human NQO1.[21,32,35] Paracetamol **17** has recently been identified as a weak substrate for NQO2, which binds very close to FAD ring in the active site. NQO2 has been considered an off-target for this drug, having a role in superoxide production and modulation of Ca^{2+} levels in cultured HeLa cells.[33]



12 menadione **13** coenzyme Q0 **14** mitomycin C

15 Methyl red **16** CB1954 **17** Paracetamol

Figure 2.3 Examples of reported substrates for NQO2.

2.1.3 STRUCTURAL DESCRIPTION AND COMPARISON WITH NQO1

Both NQO2 and NQO1 are homodimers, which display a similar fold for the bulk of the structure, with two interlocked catalytic domains with similar topology. NQO2 dimer buries 4004 $Å^2$ of solvent accessible areas in contrast to 5428 $Å^2$ presented by NQO1 (**Figure** 2.4).[20] NQO2 is a protein containing 231 aminoacids (25,821 Da) per monomer, NQO2 contains the *N*-terminal catalytic domain of NQO1 (1-220 residues), with 5 central, parallel β-strands flanked on both sides by helices. However, it lacks NQO1 C-terminal in 43 residues. In this case, the 10 C-terminal residues have no sequence homology with corresponding residues in NQO1. Additionally, it forms a well-defined loop, within which is contained the cysteine residue that takes part in the coordination with the metal atom, zinc.[27,38]

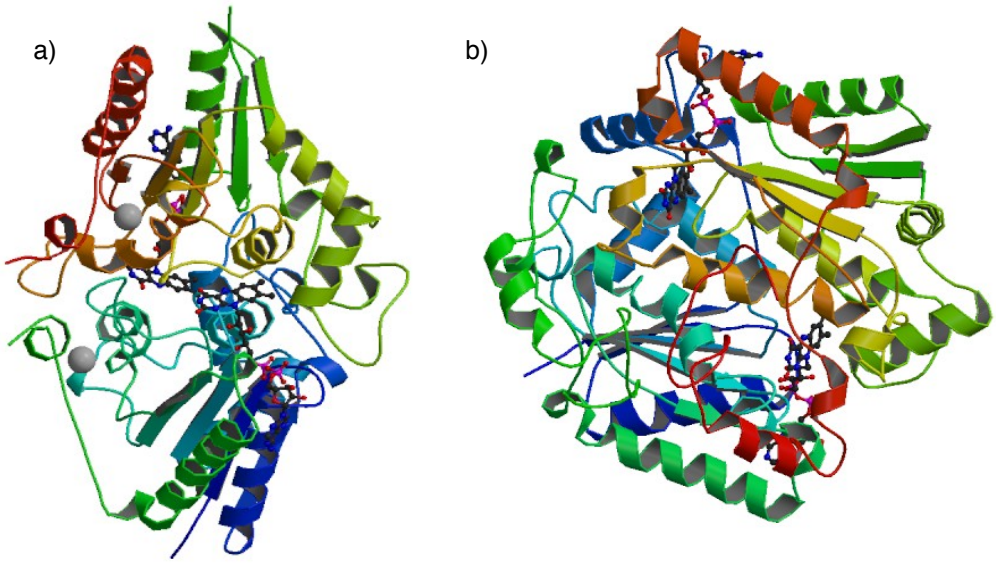


Figure 2.4 a) PDB ID: 1QR2 (C. E. Foster, M. A. Bianchet, P. Talalay, Q. Zhao, L. M. Amzel, (1999) Crystal structure of human quinone reductase type 2, a metalloflavoprotein. Biochemistry 38: 9881-9886).[38] b) PDB ID: 1D4A (M. Faig, M. A. Bianchet, P. Talalay, S. Chen, S. Winski, D. Ross, L. M. Amzel (2000) Structures of recombinant human and mouse NAD(P)H:quinone oxidoreductases: species comparison and structural changes with substrate binding and release. Proc. Natl. Acad. Sci. USA 97: 3177).[39]

2.1.4 Coordination and role of zinc

The metal binding site is tetracoordinated, with two histidine and one cysteine ligands. The fourth ligand is the main-chain carboxyl oxygen atom of the same cysteine residue. The complex displays a distorted tetrahedral geometry. This 3-residue motif was consistently present in all NQO2 sequences studied but not in NQO1 sequences. The two histidine residues, His^{173} and His^{177}, are in adjacent turns of the same α-helix, with their side chains exposed on the same face of the helix. The cysteine ligand is within the unique C-terminal loop.[38] This coordination system can be compared with the metal sites of other enzymes. Regardless of the greatest analogy with type I copper site, the metal site of NQO2 also exhibits similarities with Zn sites. In this case, the coordination profile is mostly analogous to those displayed by a Type I Cu centre, whose main role is to promote electron-transfer in the enzymes. Likewise NQO2, typical Type I Cu site involves two histidine, one cysteine and a fourth variable ligand. Nonetheless, in NQO2, the cysteine residue contributes with two ligands besides the histidine residues are unusually close, which is uncharacteristic for type I copper sites. In conclusion, it is likely that NQO2 is a type I copper enzyme and the metal site may be interconnected with the active site by an electron-transfer route. Additionally, the location of the metal on the protein surface is more consistent with a role in electron transfer rather than in structural stabilisation.[38] Nevertheless, this premise was more recently challenged. Finally,

it was demonstrated that EDTA does not interfere significantly with NQO2 activity, suggesting that the metal might not participate in the enzymatic mechanism.[40]

2.1.5 PROTEIN SEQUENCE

Human NQO1 and NQO2 cDNAs and proteins share 54% and 49% sequence identity, respectively. Both have an approximate length of 20 kb coding for a protein of 274 aminoacids for NQO1 and a shorter overall protein of 231 aminoacids for NQO2.[41] The human NQO2 gene locus is highly polymorphic and is precisely located to chromosome 6p25.[27,41,42] There are two common forms of the NQO2 gene in the human population, the difference being present at codon 47, which can either encode leucine or phenylalanine. As a result, there are two corresponding forms of the enzyme, one bearing phenylalanine at position 47 and the other with leucine. Residue 47 is not present at the catalytic site of NQO2 but is close to the dimer interface.[38,43] NQO2 gene induction is increased by antioxidants.[19] In addition, NQO2 can be induced in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and β-naphthoflavone (β-NF).[37,41,44]

2.1.6 CATALYTIC SITE

NQO2 is a dimeric enzyme. As a consequence, it possesses two equivalent catalytic sites, located far from each other at opposite ends of the dimer interface, that work independently. Each site is a large cavity lined by residues from both monomers. The FAD isoalloxazine rings are deeply bound inside the crevices forming the floor. Phe^{126} and Ile^{128}, as opposed to Tyr^{126} and Tyr^{128} in NQO1, are positioned within the active sites, with their side chain being perpendicular to the plane of the FAD isoalloxazine ring. Moreover, Met^{131} residue present in NQO1 is replaced by Phe^{131} in NQO2 catalytic site. As a result, these changes make the catalytic site of NQO2 slightly larger and more hydrophobic than NQO1, which is a 360 $Å^3$ pocket and they should be responsible for the divergences in substrate and inhibitor specificity between the two enzymes.[20,38]

2.1.7 COFACTOR BINDING SITE

NQO2 contains two molecules of FAD, which are bound at opposite ends of the dimer interface. Both conformations and locations of NQO2- and NQO1-bound FAD are very similar. Tight non-covalent binding of FAD is achieved in both cases through multiple protein contacts being almost all of these specific interactions identical in these homologous enzymes. Furthermore, FAD molecules do not come off the enzyme under native conditions.

The isoalloxazine ring of FAD is anchored by hydrogen bonds to the main chain NH groups of Trp^{105}, Phe^{106}, Gly^{149} and Gly^{150} and to the side chain of Tyr^{155}. The N5 of this moiety also participates in a hydrogen bond with the carbonyl oxygen of Leu^{103}. Stacking with the dimethyl ring of the flavin is the side chain of Tyr^{104}. Interactions between oxygen atoms O2* and O4* in the ribitol ring of FAD with the main chain carbonyl oxygen of Leu^{103} and the side chain of Thr^{147}, respectively, is also present. In NQO2 is verified an additional contact between O3* and the side chain of Glu^{193}, which is not present in NQO1. NQO2 also preserves protein interactions with the first phosphate group, O1P contacts with the side chain of His^{11} and a water molecule as well as two hydrogen bonds link O2P to the backbone and side chain nitrogens of Asn^{188}. The replacement of Gln^{66} present in NQO1 by Asn^{66} in NQO2 leads to a loss of interaction mediated through a hydrogen bond between Gln^{66} and the second phosphate group of FAD in the NQO1 enzyme. Finally, the side chains of Arg^{200} and Glu^{197} in addition to the main chain NH group of Phe^{17} interact with the adenine ring.[38] To summarise, only one of the 13 residues used by NQO1 for FAD binding is mutated in NQO2.

2.1.8 Electron donor binding

In contrast to what occurs with FAD-protein interactions in NQO2, which are very similar to those stablished in NQO1, there are several different residues comprising the NAD(P)H binding site, especially among those involved in binding the adenoside diphosphate moiety. Along, the truncation of C-terminal in NQO2 is associated with loss of protein-cofactor contacts.[38]

2.1.9 Substrate binding

In the case of menadione, it does not appear to induce significant conformation changes in the protein; the structures of native and menadione-bound NQO2 are very similar. No specific interactions are stablished between protein and substrate although they are linked through water molecules.[38]

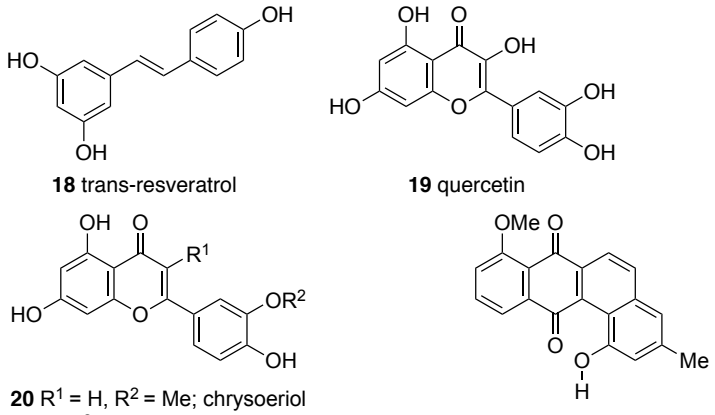
2.1.10 Ping pong catalytic mechanism

The conservation of catalytic site residues, suggest that both NQO2 and NQO1 operate under analogous catalytic mechanisms. Similarly to NQO1, the ping pong (as is used the same catalytic site for both substrate and electron donor) catalytic mechanism of NQO2 means that the active cycle progressions through an oxidised state and a reduced state for each catalytic turnover. The isoalloxazine ring of FAD is the responsible for this variation in the oxidation state. The first step takes place through the transfer of two electrons from an electron donor,

such as NRH, onto the isoalloxazine ring of FAD. $FADH_2$ presents the N5 atom protonated and a change in its orbital is observed, from planar sp^2 to trigonal pyramidal sp^3 hybridisation. As a result, the planarity of the isoalloxazine ring of FAD is disrupted, which put a negative charge mainly centred on N1 and O2 atoms. Besides, the N5 atom can act as a hydrogen bond donor.[38,45] Subsequently, occurs the dissociation of oxidised nicotinamide and binding of a quinone substrate.[20]

2.1.11 Reversible inhibitors of NQO2

NQO2 possesses a single active site, two per dimer, and it has been suggested that the inhibitor competes with the cosubstrate during the early stage of the ping-pong mechanism.[31] The binding pocket of NQO2 is hydrophobic and known inhibitors of NQO2 have planar aromatic ring systems. NQO2 binds several ligands (**Figure 2.5**), with inhibitory properties, for example natural flavonoids such as resveratrol **18**, quercetin **19** and chrysoeriol **20**. Quercetin **19** is a potent competitive inhibitor with respect to NRH, with a $K_i = 21$ nM and $IC_{50} = 80$ nM.[37]



18 trans-resveratrol

19 quercetin

20 R^1 = H, R^2 = Me; chrysoeriol

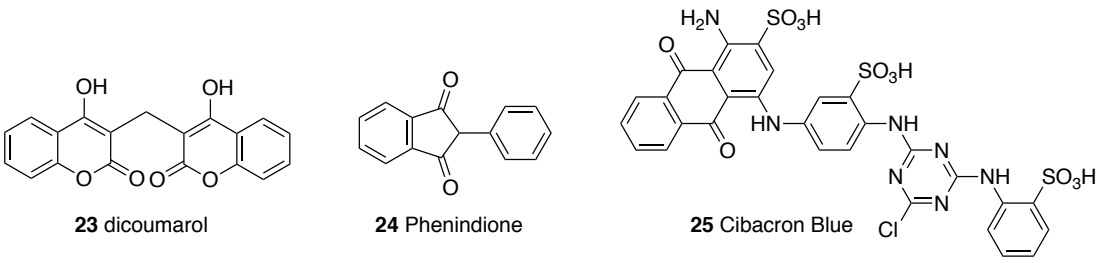
21 R^1 = glucose, R^2 = H; quercetin-3-*O*-β-D-glucoside

22 tetrangulol methyl ether

Figure 2.5 Examples of reported natural inhibitors of NQO2.

Chyrsoerisol **20** has been reported to inhibit NQO2 with an IC_{50} value of ca. 300 nM in the presence of BNAH and menadione.[32] Recently, a quercetin glycoside **21**, isolated from the fern *Lepisorus contortus*, has also demonstrated inhibition against NQO2, with an IC_{50} value of 3.84 μM.[46] A screening of natural products, in this particular case from extracts of marine sediment bacteria, using ultrafiltration LC-MS has found tetrangulol methyl ether **22** to display an inhibitory activity against NQO2 with an IC_{50} value of 0.16 μM.[47]

On another note, this enzyme displays resistance to typical NQO1 inhibitors such as dicoumarol **23**, phenindione **24** and Cibacron blue **25** (**Figure 2.6**).[41]



23 dicoumarol **24** Phenindione **25** Cibacron Blue

Figure 2.6 Examples of reported inhibitors of NQO1.

Additionally, several synthetic molecules have been described in the literature to act as NQO2 inhibitors and a few examples are represented in **Figure 2.7**. Benzo[*a*]pyrene **26** is a well-known inhibitor of this enzyme presenting an IC_{50} value in the 100 nM range. Similarly, the anticancer drug imatinib **27**, an AbI kinase inhibitor, inhibits NQO2 with an IC_{50} value of 82 nM. [48] The thrombin inhibitor dabigatran **28**, competitively and concentration-dependently, inhibits NQO2 with a $K_i = 70\ \mu M$ and $IC_{50} = 60\ \mu M$. Moreover, its corresponding methyl ester **29** displays an enhanced inhibitory profile with a $K_i = 0.9\ \mu M$ and $IC_{50} = 0.8\ \mu M$.[49] *N*-(2-(2-methoxy-6*H*-dipyrido[2,3-*a*:3',2'-*e*]pyrrolizin-11-yl)ethyl)furan-2-carboxamide (NMDPEF) **30**, a melatonin-related inhibitor of NQO2, with an IC_{50} value of 14 nM has been recently reported to protect against paraquat (PQ)-induced toxicity both *in vitro* and *in vivo*.[50] This protective effect was due mainly to the prevention of reactive oxygen species (ROS) formation induced by PQ, implying a role for NQO2 in the regulation of oxidative stress.[35] Furthermore, NQO2 is the only known human target of the antimalarial drugs chloroquine (CQ) **31** and primaquine **33** with IC_{50} values of $0.6 \pm 0.1\ \mu M$ and $1.0 \pm 0.4\ \mu M$.[40] The two molecules inhibit NQO2 through different mechanisms. The first bind preferentially to the oxidised form of NQO2 thus, exhibiting a competitive inhibitory action whereas the former molecule exerts its action binding preferentially to the reduced form of NQO2.[51] Quinacrine **32** revealed an $IC_{50} = 0.51 \pm 0.17\ \mu M$. All of these antimalarial compounds are competitive inhibitors of NQO2. Also 4,5,6,7-tetrabromo-1*H*-benzo[*d*]imidazole (TBBz) **34**, 4,5,6,7-tetrabromo-*N*,*N*-dimethyl-1*H*-benzo[*d*]-imidazol-2-amine (DMAT) **35** and 4,5,6,7-tetrabromo-1*H*-benzo[*d*][1,2,3]triazole (TBB) **36** are some of the most widely used inhibitors of protein CK2 and have also shown to be effective NQO2 inhibitors. DMAT **35** exhibited a $K_D = 36.4 \pm 1.7$ nM and $IC_{50} = 484 \pm 80$ nM. Values of K_D and IC_{50} for TBBz **34** and TBB **36** were 18.1 ± 0.4 nM and 79.4 ± 12 nM and $7.11 \pm 0.04\ \mu M$ and $12.8 \pm 2\ \mu M$, respectively. In this manner, DMAT **35** is the most effective ligand for NQO2 and TBBz **34** the best inhibitor of NQO2. Despite the IC_{50} were all higher than the dissociation constants, the difference was dependent on the compound suggesting that these CK2 inhibitors are inhibiting NQO2 through different mechanisms.[45]

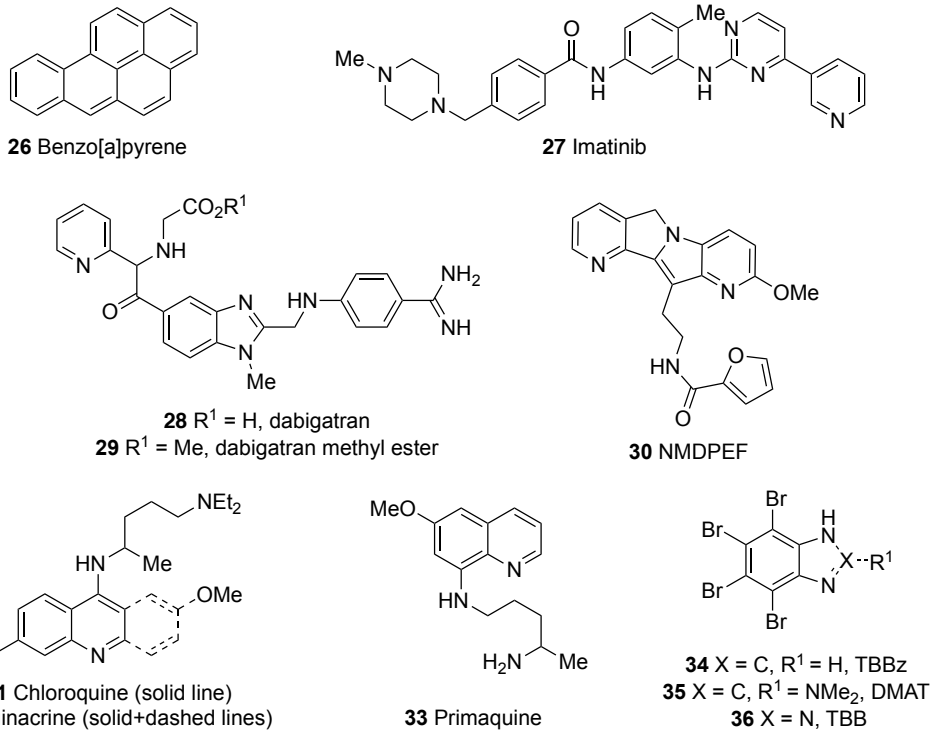


Figure 2.7 Examples of reported synthetic NQO2 inhibitors.

2.1.12 MECHANISM BASED INHIBITORS

Apart from our own work,[52,53] there are no known mechanism-based inhibitors of NQO2 as opposed to its counterpart NQO1. The development of molecules with the ability to inhibit this enzyme would be of the greatest importance in order to characterise the role of NQO2 in the diseases that were associated to it namely, leukaemia, malaria and neurodegenerative diseases.[54] Yan and co-workers have reported a series of 5-aminoindolequinones as mechanism-based inhibitors of NQO2 (**Figure 2.8**).[53] Compounds **37** and **38** were the most efficient NQO2 inhibitors with K_i values of 2.39 μM and 1.64 μM, respectively.

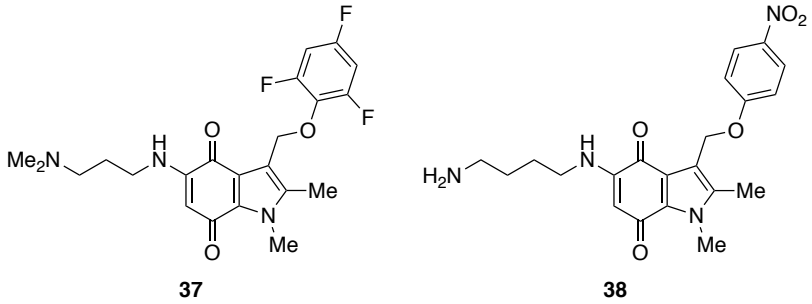
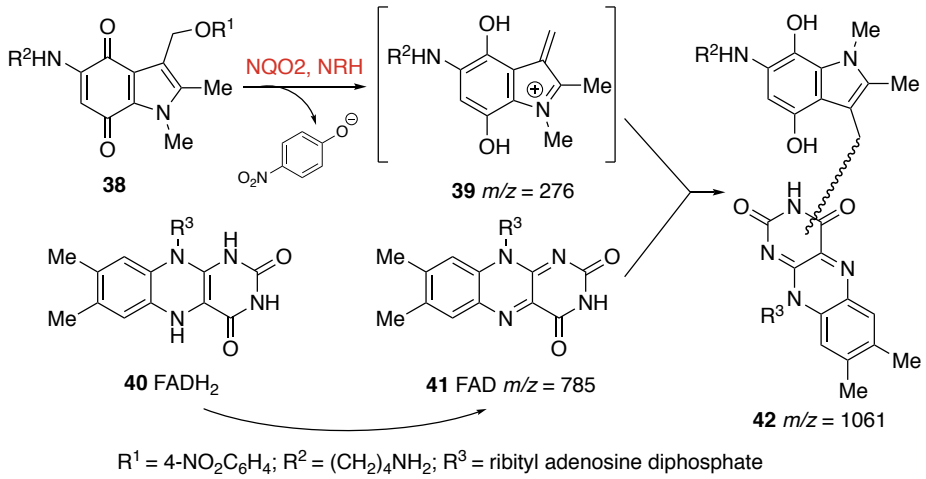


Figure 2.8 5-Aminoindolequinones **37** and **38** as mechanism-based inhibitors of NQO2.

The mechanism of inhibition of NQO2 by aryl ether indolequinone **38** was based on FAD adduction, in both cell-free and cellular systems. This irreversible process should start with reductive activation of the indolequinone **39** and loss of the phenoxide moiety (O-4-$NO_2C_6H_4$)

then the nucleophilic attack by FAD **41** on the electrophilic iminium species **39** (**Scheme 2.1**). The mass spectrum confirmed the presence of a FAD adduct of indolequinone and also suggested the formation of the hydroquinone form of the iminium-FAD adduct **42** based on the m/z ratio. Three additional compounds were analysed using ESI-MS with R^1 = 2,4,6-$F_3C_6H_2$ and R^2 = -$(CH_2)_3NMe_2$ (**37**), -$(CH_2)_3NH_2$ and -$(CH_2)_4NH_2$.



Scheme 2.1 Proposed mechanism of inhibition of NQO2 by **38**.

2.2 Aims & Objectives

NQO2 has been associated with various diseases including carcinogenesis, neurological disorders and infectious diseases. The shortage of known mechanism-based inhibitors of NQO2 in contrast to the homologous enzyme NQO1 has created an opportunity for the development of molecules with potential to inhibit this enzyme. Such biological tools would represent a major advance in the elucidation of the role of intracellular NQO2.

This Chapter is divided in four major sections. In **Section 2.3** preliminary UV-visible kinetics studies are described, in order to assess the relative rates of reductive elimination of 4-nitrophenoxide in indolequinones. The reductive elimination of the leaving group of indolequinones are also reported, with the trapping of the activated electrophilic iminium intermediate with potassium *O*-ethyl xanthate. (**Section 2.4**). The nucleophilic reactivity of riboflavin tetraacetate is briefly described in **Section 2.5**. Finally, mass spectrometry analysis, the assessment of NQO2 catalytic activity and an inhibitor screening of the indolequinones against NQO2 are presented in **Section 2.6**.

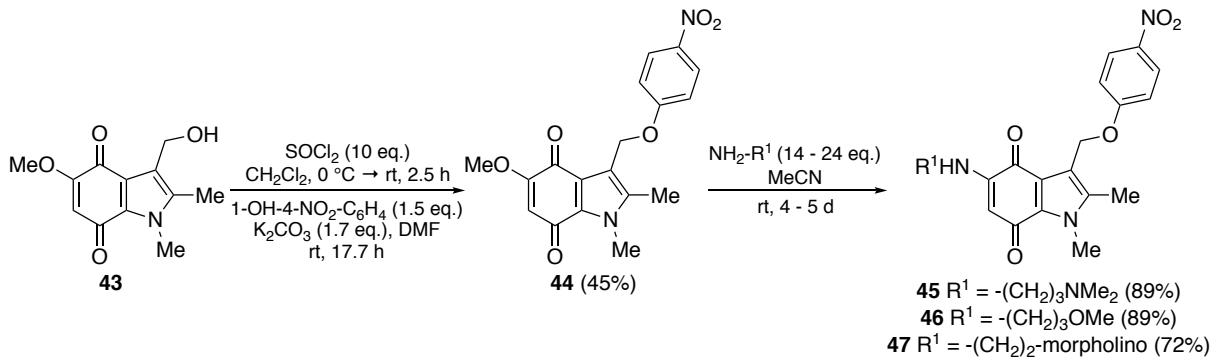
2.3 UV-Vis kinetic studies

A reduction method with sodium borohydride was used in order to gather evidence that elimination of the leaving group would happen and the rates of reductive elimination of leaving groups of different compounds could be compared through UV-visible monitoring. Therefore, structures bearing 4-nitrophenoxy aryl ethers were required to allow monitoring the release of the 4-nitrophenoxide group, following reductive activation of the indolequinone, at a wavelength of 410 nm. Despite nitro (NO_2) groups not being suitable in potential drug molecules due to their metabolism, 4-nitrophenol has a similar pKa to 2,4,6-trifluorophenol (7.2 and 7.5, respectively) and therefore, the rates of reductive elimination of 4-nitrophenol might be extrapolated and the kinetic properties compared with that of 2,4,6-trifluorophenol.

2.3.1 Synthesis of 4-nitroaryl ethers

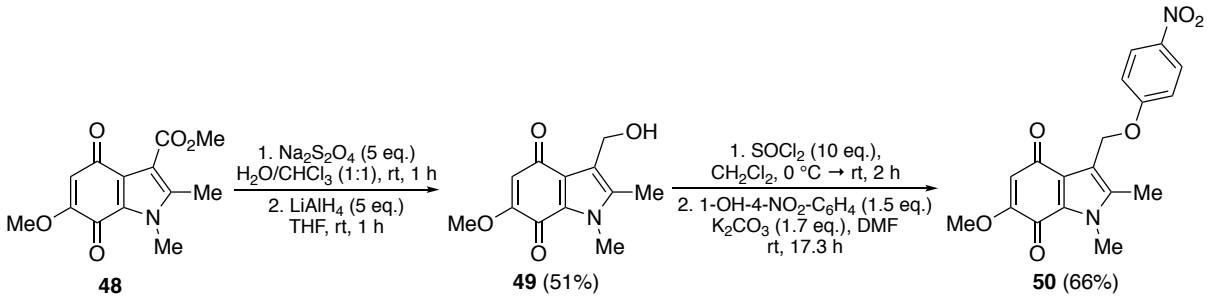
With this purpose, five analogues **44-47** and **50** were synthesised, with a 4-nitrophenoxy leaving group.

Alcohol **43**, whose synthesis is described in Chapter 3, was converted into the corresponding 4-nitro substituted aryl ether **44** in 45% yield (**Scheme 2.2**). This intermediate was then reacted with three primary amines to give 5-amino indolequinones **45-47**, in good to excellent yields.



Scheme 2.2 Synthesis of 4-nitro aryl ether substituted 5-amino IQs **45-47**.

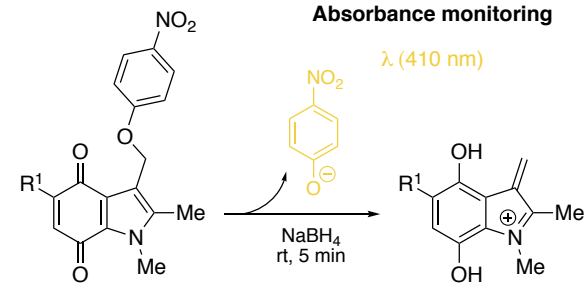
Indolequinone **48** was reduced to the corresponding alcohol **49** in 51% yield, using the traditional protocol that relied on the super-stoichiometric use of sodium dithionite followed by the reduction of the ester functionality with an excess of lithium aluminium hydride (**Scheme 2.3**). The last step was the conversion of this derivative into the corresponding alkyl chloride and then introduction of the 4-nitrophenol, in the presence of potassium carbonate, in DMF gave aryl ether **50** in 66% yield.



48 **49** (51%) **50** (66%)

Scheme 2.3 Synthesis of 4-nitro aryl ether substituted 6-methoxy IQ **50**.

With the UV-visible kinetic studies, the aim was to compare the influence of the substitution at the C-5 position by either a methoxy group **44,** or a derivative bearing an amino-alkyl chain **46** as well as to compare 5- vs. 6-methoxy substituted indolequinones, **44** and **50**. The wavelength was set at 410 nm for monitoring the release of the 4-nitrophenoxide following reduction by sodium borohydride and the reaction was carried out at room temperature for 5 minutes (**Scheme 2.4**). Since the starting material and the other reaction product (the hydroquinone) do not absorb at this wavelength, the ultraviolet absorption is a direct measure of the concentration of the 4-nitrophenoxide in this reductive system. From the data presented (**Chart 2.1**), it is possible to infer that **46** presents a faster rate of releasing the 4-nitrophenoxide than **44**. Derivatives **45, 47** and **50** were also analysed however, it was not possible to plot the data for those compounds due to the formation of hydrogen during the reaction, which created bubbles in the middle of the solution, interfering with the path of the light beam through the samples.



44 R^1 = OMe
46 R^1 = $NH(CH_2)_3OMe$

Scheme 2.4 Reductive elimination of the leaving group from species **44** and **46**.

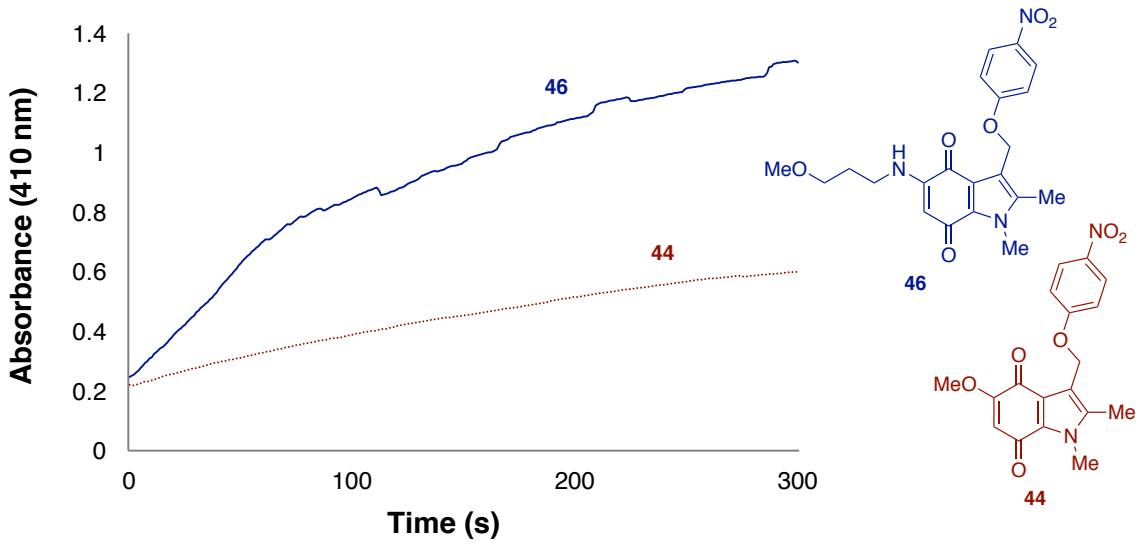


Chart 2.1 Absorbance monitoring at 410 nm for IQs **44** and **46**.

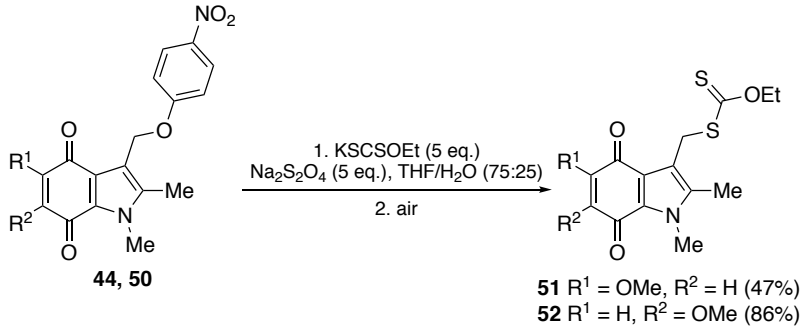
In summary, the data obtained from the UV-visible kinetic studies show that chemical elimination of the leaving group (4-nitrophenoxide) of the analysed compounds (**44** and **46**) occurred upon reductive activation with sodium borohydride.

2.4 Chemical reductive elimination studies

The ability of the 3-indolylcarbinyl substituents to undergo elimination is particularly important and such reactivity is only observed upon reductive activation of the indolequinone. Sodium dithionite has been reported as a convenient method to activate mitomycin C.[55] The use of chromium(II) dichlorate has also been subject of studies for the reductive activation of mitomycin C.[56,57] As mentioned beforehand, the irreversible NQO2 inhibition by indolequinones starts with the reductive activation of the quinone and loss of the phenoxide moiety to generate an electrophilic iminium species. Then the nucleophilic attack by FAD present in the enzyme generates an indolequinone-FAD adduct that inhibits NQO2.

A set of reactions was carried out to prove that chemical elimination of the leaving group would happen upon reductive activation. The quenching of the reactive electrophilic iminium intermediate by a thiol, such as *O*-ethyl xanthate, gave the substituted thioester derivatives **51** and **52**, in moderate to good yields (**Scheme 2.5**). Water-soluble potassium *O*-ethyl xanthate was chosen because not only are thiols good nucleophiles, the quenching of the reactive electrophile by a thiol would mimic the scavenging role of cellular thiols such as glutathione in biological systems. Following previously described protocols,[58,59] the reduction of

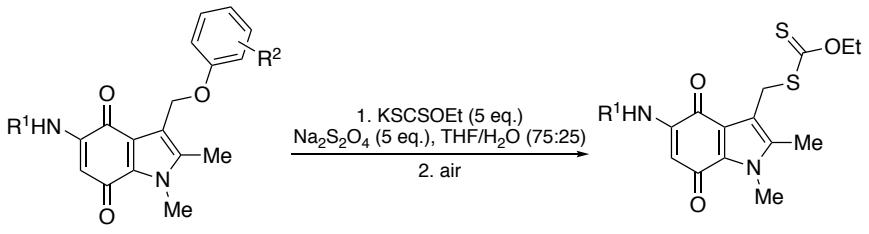
indolequinones **44** and **50** was carried out using a large excess of sodium dithionite in the presence of the thiol (5 eq.) in degassed aqueous THF. The elimination of ArO^- occurred and it was observed by the appearance of ArOH in the TLC analysis of the reaction mixture. Concurrent with the elimination of ArO^-, the formation of the thiol-trapped product was also observed by TLC in all cases. Oxidative workup of the reaction mixture allowed the isolation of **51** and **52**.



Scheme 2.5 Nucleophilic trapping of the activated iminium intermediate from **44** and **50**.

This reaction was also attempted for 5-aminoindolequinones **45** and **53** (**Scheme 2.6**) however it was not possible to isolate the product despite its formation as suggested by TLC and ESI-MS analysis of the crude material. For instance, the ESI-MS analysis of the crude product for the reaction of **53** with potassium *O*-ethyl xanthate found a peak with $M+H^+$, 397.1254 providing evidence of product formation.

To conclude, the information above described confirm that chemical elimination of the leaving group (4-nitrophenoxide or 2,4,6-trifluorophenoxide) of the tested compounds (**44**, **45**, **50** and **53**) happened upon reductive activation with sodium dithionite.



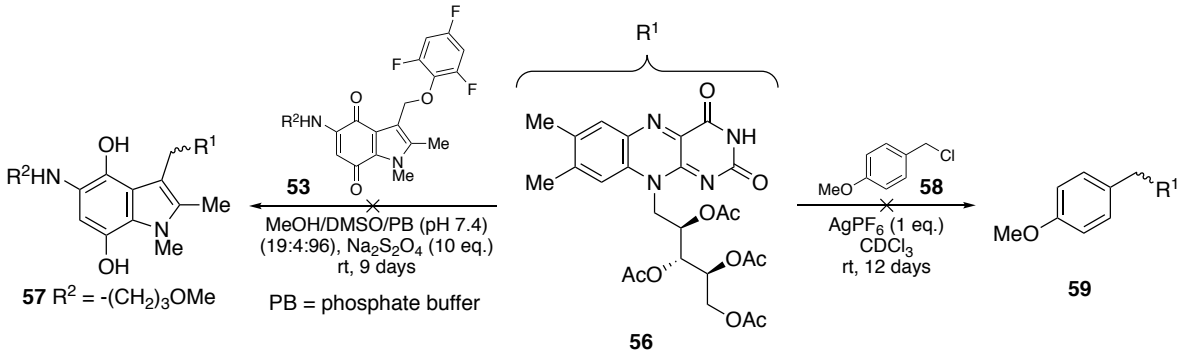
45 $R^1 = -(CH_2)_3NMe_2$, $R^2 = 4\text{-}NO_2$
53 $R^1 = -(CH_2)_3OMe$, $R^2 = 2,4,6\text{-}F_3$

54 $R^1 = -(CH_2)_3NMe_2$
55 $R^1 = -(CH_2)_3OMe$

Scheme 2.6 Attempts to generate indolequinones thioesters derivatives **54** and **55**.

2.5 Reactivity of riboflavin tetraacetate

From the several possible nucleophilic sites in FAD, the adenine moiety has been previously ruled out by mass spectrometric data and the isoalloxazine ring nitrogens have been most likely considered to be the reactive point. Besides, it has been shown that the modification happens on the riboflavin core.[52,53] In order to gather some chemical-based evidence to support this theory, the aim was to synthetically mimic the enzymatic mechanism-based inhibition of NQO2 by the 5-amino indolequinones. Therefore, if an adduct was formed, the product might be isolated and characterised, which might clarify what happens enzymatically in NQO2, when the indolequinone inhibits the enzyme through a FAD irreversible modification. Therefore, two reactions have been tried with riboflavin tetraacetate **56** (**Scheme 2.7**). Firstly, the reaction was tried with the indolequinone **53** in reducing conditions, using sodium dithionite. After 9 days, at room temperature, there was no evidence of reaction and the starting materials **53** and **56** have been recovered.



Scheme 2.7 Attempted reactions with flavin tetraacetate **56**.

In the second experiment, 4-methoxybenzylchloride **58** has been used. The reaction was carried out in deuterated chloroform and monitored by ^{1}H NMR. Firstly, the benzyl chloride **58** was combined with 1 eq. of silver hexafluorophosphate, targeting the formation of the methide intermediate, which should be susceptible of nucleophilic attack from the FAD-derivative to give **59**. Upon combination of the silver salt and the benzyl chloride, a precipitate was formed, which from the analysis of the ^{1}H NMR data, was identified as the product of the anionic exchange of the reagents, benzyl hexafluorophosphate. No further attempts were made to optimise these conditions.

2.6 Biochemical assays

2.6.1 NQO2 CATALYTIC ACTIVITY

We then moved on to the MS studies, in which several experiments were conducted. After initial experiments, simply on the detection/quantification of the FAD present in the enzyme released by denaturation it was possible to verify that the detected quantity of denatured FAD was much lower than the expected values. Three different columns were tested - Atlantis T3 5 μm, 4.6 x 150 mm (Waters); SunFire C18 3.5 μm, 2.1 x 30 mm (Waters) and X-Select HSS T3 3.5 μm, 2.1 x 50 mm (Waters). Variations on NQO2 denaturation processes were also tested, ranging from the use of 100% methanol and mixed systems of methanol and acid, namely methanol and 0.1% formic acid and methanol and 0.1% TFA. Followed in all the cases by centrifugation at 13000 rpm for 5 min, at 4 °C. Nonetheless, no significant differences were observed using the different techniques. Whenever commercially available FAD was used, the problem was not present and a calibration curve was plotted (**Chart 2.2**).

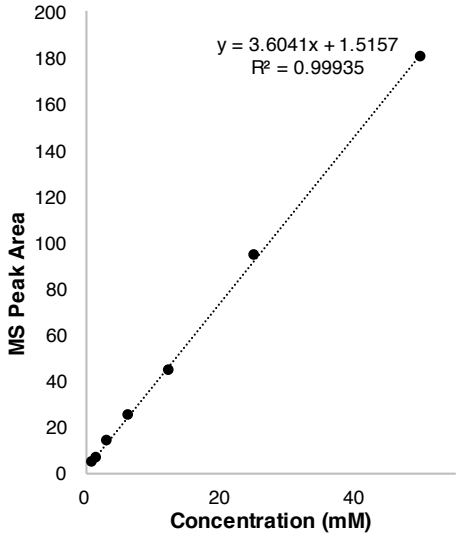
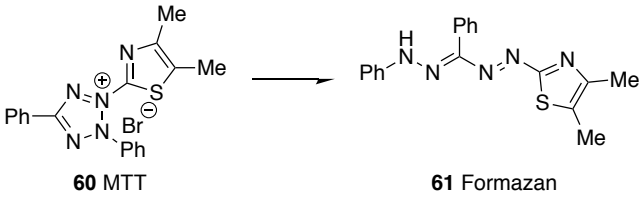


Chart 2.2 Calibration curve for commercially available FAD, concentration vs. MS peak area.

Considering the unsuccessful attempts to detect the FAD released from NQO2, thus not being able to detect the adduct FAD-**37**, an investigation of the catalytic activity of the enzyme was envisaged. If the enzyme had lost its catalytic ability, that would have explained the results obtained. The catalytic activity of the NQO2 was assessed through a MTT-formazan assay, which relies on the ability that an active enzyme has to turn MTT **60** into its corresponding formazan derivative **61** (**Scheme 2.8** and **Chart 2.3**). NQO2 activity was measured as NRH-dependent menadione reductase activity using MTT as the final electron acceptor.[37] Initially

menadione (quinone) is reduced by NQO2/NRH to menadiol (hydroquinone), then menadiol reduces MTT **60** (yellow) to the MTT formazan **61** (purple).



60 MTT **61** Formazan

Scheme 2.8 MTT-formazan catalytic assay.

In this NQO2 inhibition experiment change in absorbance vs time (da/dt, slope) was measured from 0 to 120 sec (linear region). The slope (da/dt) is about 0.78 abs/min/μg rhNQO2. In the previous inhibition experiments, the slope is about 1.25 abs/min/μg rhNQO2, thus suggesting that the rate for the enzyme used is about 1.6-fold slower.

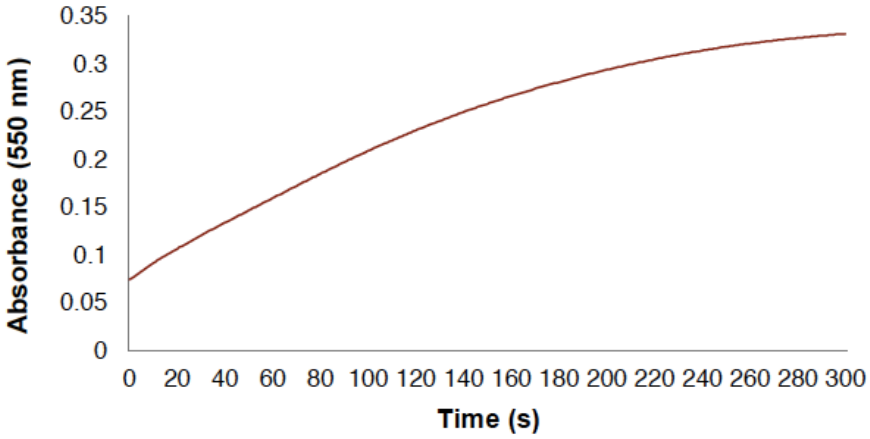
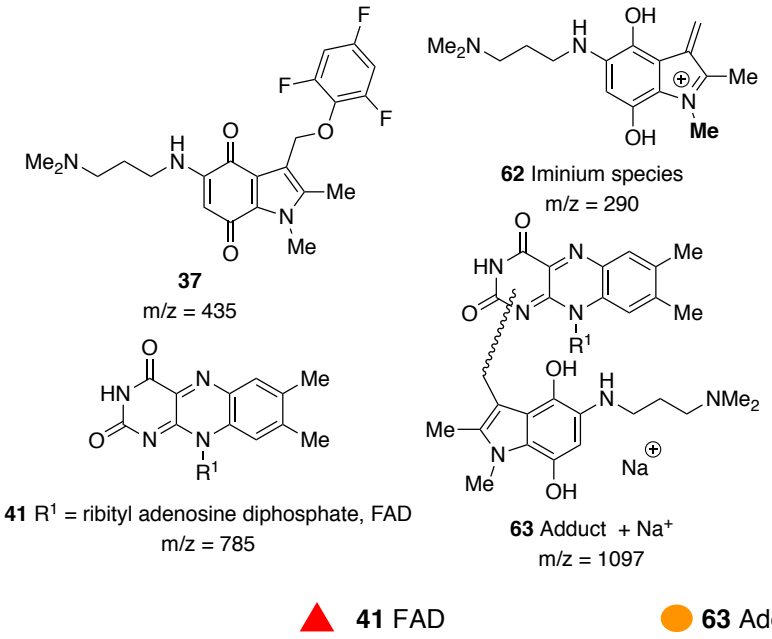


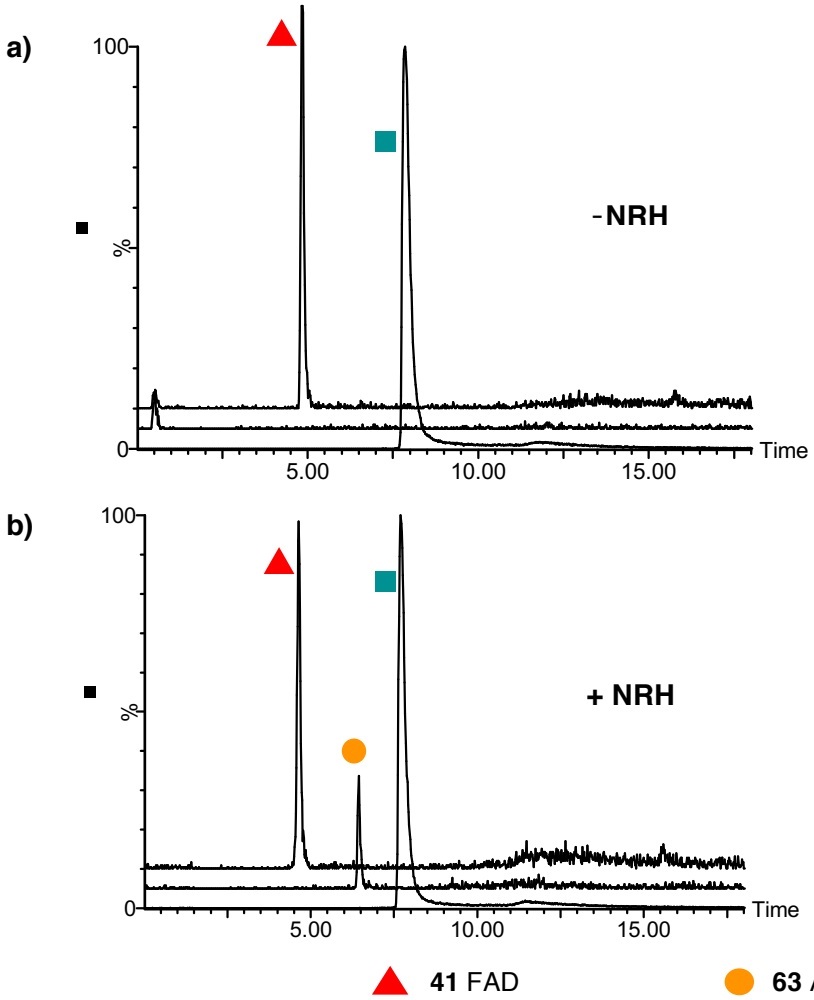
Chart 2.3 Absorption monitoring at 550 nm for MTT formazan.

Previous data using compound **37** suggested a novel mechanism of inhibition of NQO2 by the indolequinone involving FAD adduction where the indolequinones were adducted to the non-covalently bound FAD cofactor.[53] To confirm and extend these observations, the same compound was examined as a template and the aim was to study other compounds using ESI-LC-MS. The rhNQO2 protein was incubated with indolequinone **37** in the presence and absence of NRH, and the liberated FAD was then analysed using HPLC and ESI-LC-MS for potential indolequinone derived adduct. In **Figure 2.9** the structure and m/z of some of the species detected/generated in MS spectrometry are presented.



37 **41** FAD **63** Adduct + Na^+

Figure 2.9 Structure and m/z of some of the species detected/generated in MS spectrometry.



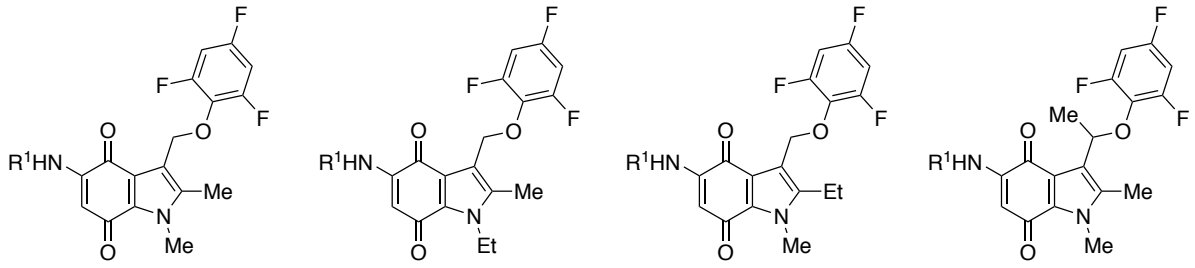
37 **41** FAD **63** Adduct + Na^+

Figure 2.10 Extracted ion chromatograms for a) control (-NRH) and b) reaction (+NRH).

HPLC analysis of FAD extracted from NQO2 following incubation with indolequinone **37** in the absence of NRH is presented as the control overlay; which represents the three extracted ion chromatograms overlaid: top is FAD, middle is the adduct, and bottom is indolequinone **37** [**Figure 2.10** (top)]. On the other hand, HPLC analysis of FAD extracted from NQO2 following incubation with indolequinone **37** and NRH showed the appearance of a new, slightly more hydrophobic peak, in relation to FAD, when compared to incubation performed in the absence of NRH. HPLC-MS analysis (positive mode) of the new peak gave the reaction overlay, which is the same as previously but for the latest reaction [**Figure 2.10** (bottom)]. The mass spectrum confirms the presence of an iminium-FAD adduct (FAD, m/z 785, and the reactive iminium intermediate of **62**, m/z 289) and suggests the formation of the hydroquinone form of the iminium-FAD adduct **220** (**Figure 2.9**). In **Figure 2.10** both the FAD and 1097 (**63** adduct+Na^+) extracted ion chromatograms are magnified 50x but indolequinone **37** extracted ion chromatogram remains the same.

2.6.2 INHIBITOR SCREENING FOR NQO2

The compounds synthesised were sent to our collaborators, Dr David Siegel and Professor David Ross, at Denver University, in the USA for biological screening (**Figure 2.11**). With a potential mechanism-based inhibitor of NQO2 available, **37** we explored the structural requirements for this enzyme inhibition. The advantages of a side chain with a terminal amino group was already clear from previous data. Compounds containing shorter three- or two-carbon substituents terminating in dimethylamino groups have previously proved to be potent inhibitors of NQO2 although a longer side chain, as in the 8-aminooctylamino substituted indolequinone, was less satisfactory.



37 R^1 = $(-CH_2)_3NMe_2$
64 R^1 = $-(CH_2)_4NMe_2$
65 R^1 = $-(CH_2)_2$-piperidinyl
66 R^1 = $-(CH_2)_3$-1-imidazolyl

67 R^1 = $-(CH_2)_3NMe_2$
68 R^1 = $-(CH_2)_4OH$
69 R^1 = $-(CH_2)_2$-piperidinyl
70 R^1 = $-(CH_2)_2$-morpholino

71 R^1 = $-(CH_2)_3NMe_2$
72 R^1 = $-(CH_2)_3OH$
73 R^1 = $-(CH_2)_2$-morpholino

74 R^1 = $-(CH_2)_3NMe_2$

Figure 2.11 Indolequinones screened as potential NQO2 inhibitors.

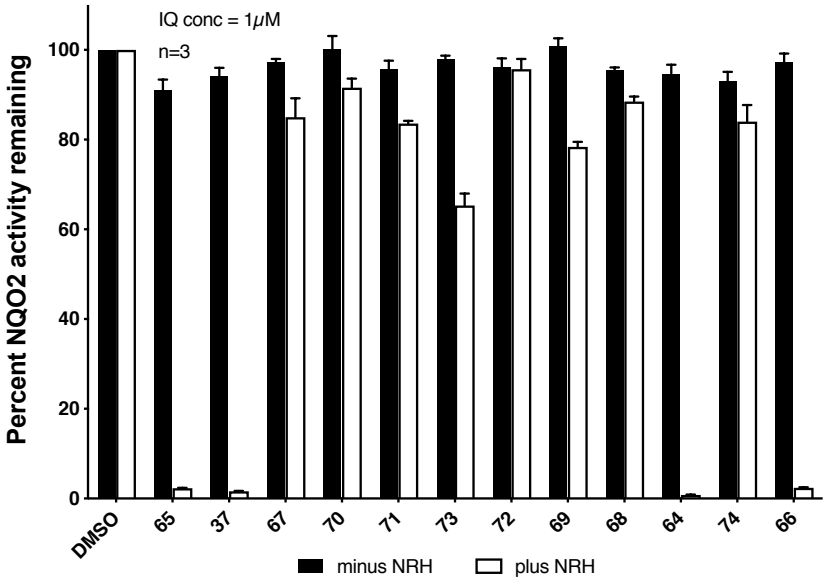


Figure 2.12 NRH-dependent inhibition of NQO2 by indolequinones. Purified rhNQO2 was incubated with 1 μM indolequinones in the absence (filled bars) or presence (hatched bars) of NRH, and after 5 min, an aliquot was removed and assayed for NQO2 catalytic activity. Results are expressed as percentages of NQO2 activity remaining compared to DMSO-treated controls. Reaction conditions are described in Materials and Methods. Results represent the mean standard deviation of three independent determinations.

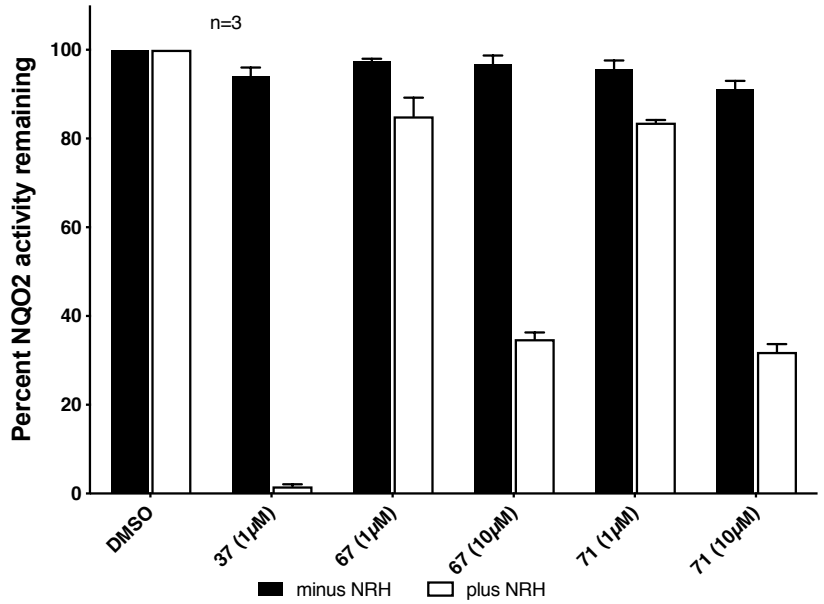


Figure 2.13 NRH-dependent inhibition of NQO2 by indolequinones. Purified rhNQO2 was incubated with 1 and 10 μM indolequinones in the absence (filled bars) or presence (hatched bars) of NRH, and after 5 min, an aliquot was removed and assayed for NQO2 catalytic activity. Results are expressed as percentages of NQO2 activity remaining compared to DMSO-treated controls. Reaction conditions are described in Materials and Methods. Results represent the mean standard deviation of three independent determinations.

Briefly, a four-carbon substituent terminating in dimethylamino group **64** was also tested and proved to effectively inhibit NQO2. The role of the 1- and 2-substituents were also briefly

investigated through the N-1 and C-2 methyl and ethyl substituted compounds **37**, **67** and **71**. The addition of an ethyl group at either N-1 or C-2 position on the indole ring decreases the efficiency of inhibition of NQO2 (**Figure 2.12** and **Figure 2.13**). Also, the incorporation of an extra methyl at the carbinyl position (**74**) did not improve the inhibitory activity against NQO2. The addition of a heterocyclic ring at the end of the amino-alkyl chain, **66**, did not prevent inhibition of NQO2.

2.7 Conclusions

In **Section 2.3** it was only possible to plot the UV-visible absorbance curves for two indolequinones. The trapping of the activated electrophilic iminium intermediate generated in situ following reductive activation of the quinone with a nucleophile was effectively carried out for two substrates (**Section 2.4**). Two other substrates were also tested however; the isolation of the final products was unsuccessful. The reaction of riboflavin tetraacetate with potential in situ generated electrophiles did not give a positive result too (**Section 2.5**). In the final part (**Section 2.6**) several issues arose, including the inability to detect the FAD released from the enzyme as well as the FAD-**37** adduct while conducting the mass spectrometry studies. This prevented further studies of the NQO2-indolequinone interaction and the establishment of a mechanism for this reaction. Nonetheless, it was possible to identify the formation of the adduct/FAD by mass spectrometry, following incubation of 5-amino indolequinone **37** and NQO2 and denaturation. Following a MTT-formazan assay to assess NQO2 catalytic activity, it was possible to conclude that the enzyme was active. Finally, the inhibitor screening of indolequinones against NQO2 showed that besides the reported NQO2 mechanism-based inhibitor **37**, three new 5-amino indolequinone derivatives (**64**-**66**) displayed good inhibitory profile against NQO2.

3 Indolequinones

3.1 Quinones

Quinones constitute a vast and important class of organic molecules that display diverse and interesting chemistry. These structures are common components of biologically significant molecules frequently acting as electron acceptors in electron transport chains, thus assuming a crucial role in electron transport in the respiratory and photosynthetic elements as well as various redox processes in Nature. Quite often they serve as useful intermediates in organic synthesis, biological chemistry, inorganic chemistry, and materials science.[60] While biological processes and applications in materials science commonly rely on the redox properties of the *p*-quinone/hydroquinone or *o*-quinone/catechol systems, in coordination chemistry these compounds act as redox active, strongly electron-accepting ligands. In organic synthesis, *p*- and *o*-quinones present a class of extremely versatile building blocks as well as very good one-electron acceptors and mild oxidants. Moreover, in spite of the wide variety of colours they display, their contribution as natural colouring agent is remarkably frail. Commonly, their concentration is low and therefore they are masked by other pigments.[61]

A number of compounds containing benzo-1,4-quinone as well as benzo-1,2-quinone moieties are broadly distributed in nature: they have been isolated from marine organisms but also from fungi lichens, roots and bark and are found to be biologically and pharmaceutically active.[62,63] Natural quinones span in structure from the simple benzoquinone to greatly complex heterocyclic quinones. *N*-heterocyclic quinones are frequently found in alkaloids and represent a family of closely related compounds.

Ubiquinones (coenzymes Q) **75**, for example, occur in all aerobic organisms from bacteria to higher plants and animals, including humans. They play an active part in electron transport in the respiratory chain with mitochondria.[64] Plastoquinones **76** and phylloquinone **78** are typically present in green plants due to their function in the electron transport pathways in photosynthesis.[65] Doxorubicin **77**, a natural quinone, exhibits antibiotic properties and it is related to the anthracycline family and it is utilised as a drug in cancer chemotherapy; acting as a DNA intercalating agent (**Figure 3.1**).[66]

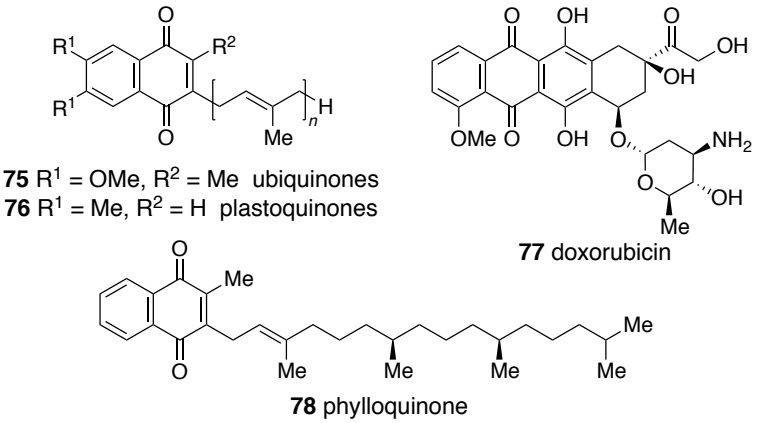


Figure 3.1 Examples of naturally occurring quinones.

3.2 Indolequinone as biologically relevant scaffolds

Indolequinones constitute the core structure in a number of classes of *N*-heterocyclic natural products possessing interesting biological properties. For example, murrayaquinone A **79**,[67,68] mitomycin C **14**, exiguamine A **80**,[69] BE 10988 **81**,[70] have been reported to present powerful anticancer activity (**Figure 3.2**).

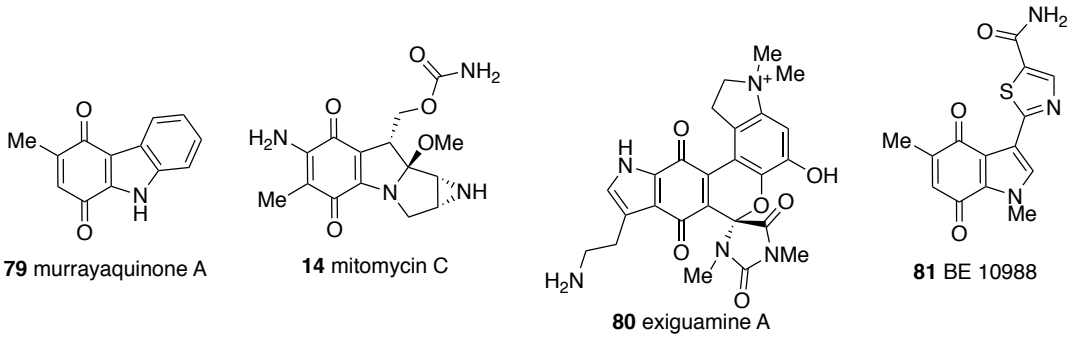


Figure 3.2 Examples of natural indolequinones with anticancer properties.

In synthetic medicinal chemistry, they are usually referred to as effective bioreductive prodrugs and the indolequinone scaffold has been used to incorporate various active agents such as 5-fluorodeoxyuridine, camptothecin, and naloxone (**Figure 3.3**).[71–76]

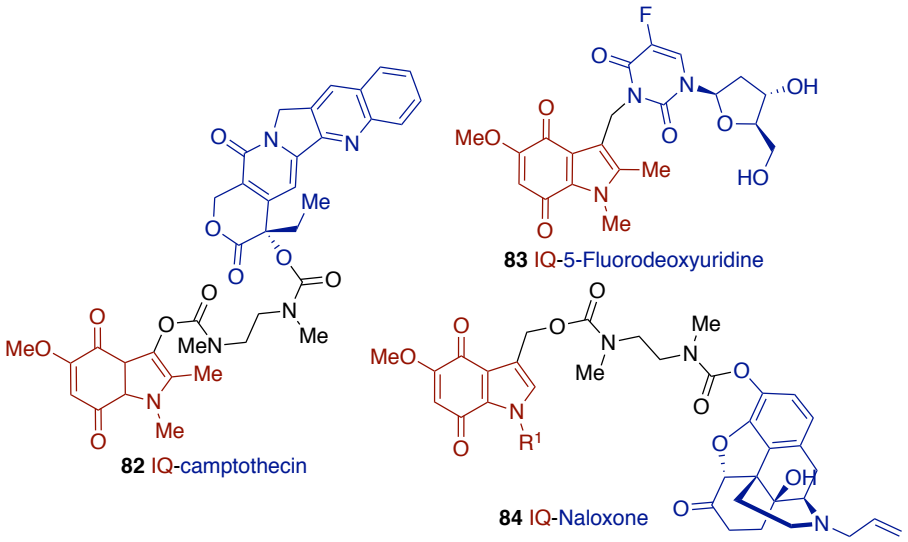


Figure 3.3 Examples of indolequinone-active agents' complexes.

Indolequinone structures ES936 **44** and **85** have been revealed to be as potent and selective inhibitors of the human quinone reductases NQO1 and NQO2, respectively (**Figure 3.4**). [52,77] Related indolequinones, such as **86,** show promising activity against pancreatic cancer.[78] Also, mitomycin C's synthetic analogue EO9 **87** is currently in clinical trials for the treatment of bladder cancer.[79]

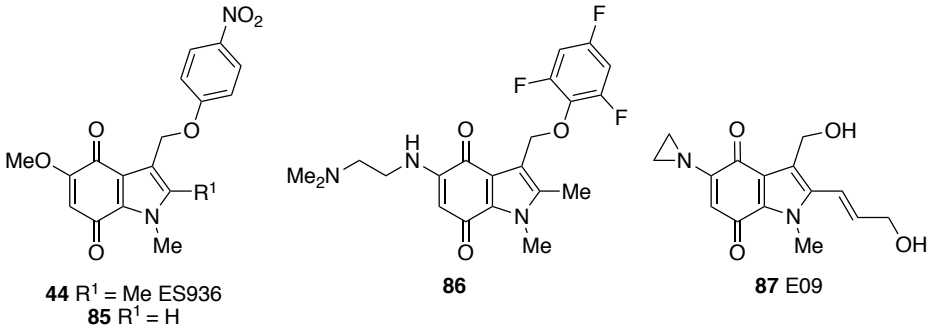


Figure 3.4 Biologically relevant synthetic indolequinone scaffolds.

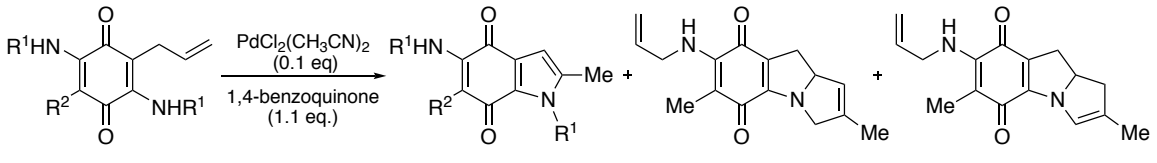
3.3 Synthesis of indolequinones

The study of the chemistry and biology of indolequinones requires a synthetic route that would provide rapid access to a range of structures with wide substrate scope and this task has attracted synthetic chemists over the last few decades. A vast number of reports have described the synthesis of indolequinones predominantly from the oxidation of indoles [80–96] and from the cyclisation of quinones.[97–113] However, the methods that have been widely used to obtain this core are often carried out using undesirable solvents such as halogenated solvents and produce great quantities of waste.

Indolequinones can be classified into two types of compounds based on their quinone structures: *p*-indolequinones (indole-4,7-diones) and *o*-indolequinones (indole-4,5- or 6,7-diones). Traditionally, indolequinones have been obtained by the oxidation of indole derivatives, which usually contain at least one hydroxy, methoxy, or amino group at the 4- and 7-position.[87] Typically, the oxidation of indoles has been accomplished by utilising metal salts and complexes as single electron transfer agents. Likewise, other reagents including inorganic acids, hypervalent iodines, bromine, DDQ, and air have also been used. Despite the usual effectiveness of these methods, they require access to highly substituted indole precursors that commonly involve an overall laborious synthetic route.[114] The biggest advantage of an intramolecular cyclisation approach on a pre-formed quinone to the typical oxidative process is avoiding the oxidation step of indoles, which sometimes is problematic with regards to regioselectivity and functional group tolerance. The synthesis of indolequinones from quinones has been under development since the late 1970s and this section will comprise a brief analysis of the existing literature, focused on the synthesis of *p*-indolequinones, starting from 1,4-benzoquinones. The majority of the methods for the cyclisation of quinones use transition metals such as palladium, copper, gold and silver to promote the annulation of quinones with enamines, alkynes or aniline derivatives. Other metals such as manganese and cerium, have also been employed in the cyclisation of quinones via oxidative free radical reactions. Nevertheless, the use of these metals is not desirable and moving towards the use of substoichiometric quantities and preferably avoiding the use of rare and expensive metals such as palladium, rhodium, gold and silver should be advantageous. Some methods using bases, acids, heat, and UV light for cyclisation have also been reported and proved to be as efficient as metal reagents.[102,115]

3.3.1 CYCLISATION WITH PALLADIUM

Hegedus *et al.* reported the palladium(II)-mediated cyclisation of various allyl-containing diaminobenzoquinones **156** (**Scheme 3.1**).[116] The indolequinone **89** was obtained in excellent yield. When R^1 = allyl, the cyclisation gave a mixture of **90**, **91** and **92** in low yields.



88

89 R^1 = Bn, R^2 = H (A: 93%)
90 R^1 = $-CH_2CH{=}CH_2$, R^2 = Me (B: 20%)

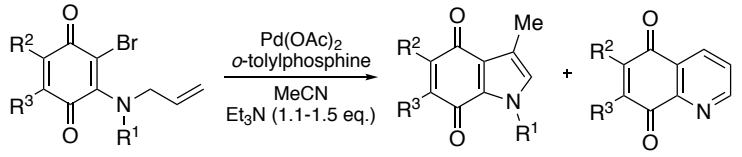
91 (B: 25%)

92 (B: 15%)

Method A: Na_2CO_3 (1 eq.), LiCl (10 eq.), THF, 110 °C, sealed tube, 20 h; Method B: THF/DMF (67:23), 65 °C, 2 h

Scheme 3.1 Synthesis of indolequinones **89-92** through a palladium(II)-mediated reaction.

The research was further extended by using catalytic Pd(0) for the cyclisation of *N*-allyl-2-bromo-1,4-benzoquinones **93** (**Scheme 3.2**).[113] With this method, indolequinones **94-97** were obtained along with quinolinequinones **98-99**, depending on the substrate, in low to moderate yields.



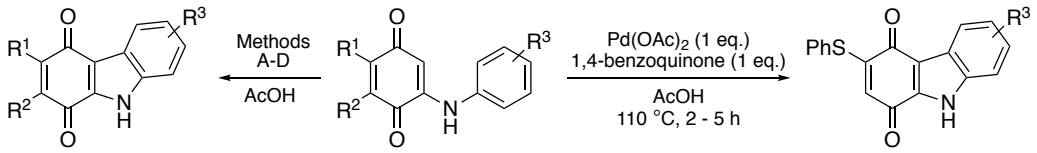
93 **94-97** **98-99**

Method A: 20 mol% Pd(0), 75 °C, 15 h
Method B: 5 mol% Pd(0), 95 °C, 18 h
Method C: 8 mol% Pd(0), 65 °C, 15 h
Method D: 6 mol% Pd(0), 105 °C, 75 min

$R^1 = R^2 = H$, $R^3 = Me$ (A: **94**, 31%)
$R^1 = R^3 = H$, $R^2 = NHCH{=}CH_2$ (B: **95**, 43%; **98**, 26%)
$R^1 = H$, $R^2 = NHCH{=}CH_2$, $R^3 = Me$ (C: **96**, 49%; **99**, 23%)
$R^1 = -CH_2CH{=}CH_2$, $R^2 = H$, $R^3 = Me$ (D: **97**,14%)

Scheme 3.2 Synthesis of indolequinones **94-97** through a palladium(0)-mediated reaction.

Bittner and co-workers carried out the cyclisation of anilinoquinones **100**, in boiling acetic acid, to give carbazoloquinones **101** (**Scheme 3.3**). The reaction required stoichiometric quantities of $Pd(OAc)_2$ even with the use of 1,4-benzoquinone as re-oxidant.[98] Cyclisation of **100** gave **102** and **103** in good yields, with both stoichiometric (method A) and catalytic (methods B and C) quantities of $Pd(OAc)_2$ using $Cu(OAc)_2$ as a re-oxidant.[117,118] Derivative **102** was then converted into carbazomycin G and carbazoquinocin C and derivative **103** into carbazomycin H. This method was later improved by Hagelin *et al*. by the use of molecular oxygen at atmospheric pressure (method D) to yield **36** in good yields.[119] All of the methods were energy intensive reactions. Besides that, Method A used stoichiometric quantities of the palladium complex, which implies a greater consumption of this expensive and rare metal therefore, generating more palladium waste by the end. Methods C and D, in spite of using sub-stoichiometric quantities of the $Pd(OAc)_2$ had longer reaction times (3 and 2 days, respectively).



102 R^1 = OMe, R^2 = Me, R^3 = H (A: 83%, B: 71%, C: 91%)
103 R^1 = OMe, R^2 = Me, R^3 = 6-OMe (A: 71%, B: 73%)
104 R^1 = Me, $R^2 = R^3$ = H (D: 86%)
105 $R^1 = R^2 = -C_4H_4-$, R^3 = 6-Br (A: 40%)
106 $R^1 = R^2 = -C_4H_4-$, R^3 = 8-OMe (D: 87%)
107 $R^1 = R^2 = -C_4H_4-$, R^3 = 6-OMe (A: 54%, D: 89%)

100

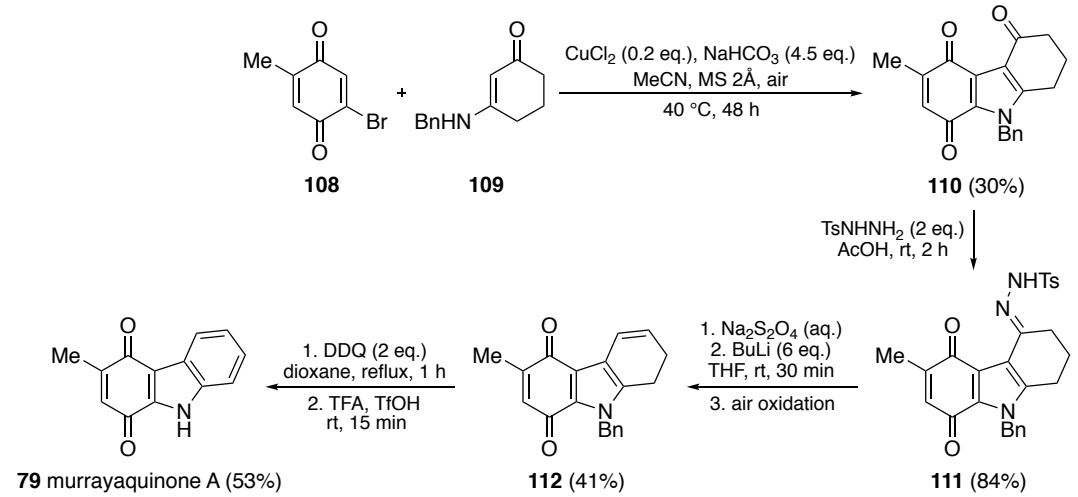
101
5 examples, 20 - 94%
R^3 = H, Me, OMe, Cl

Method A: $Pd(OAc)_2$ (1 eq.), 117 °C, 5 or 17 h. Method B: $Pd(OAc)_2$ (0.1 eq.), $Cu(OAc)_2$ (2.5 eq.), 117 °C, 16 or 19 h
Method C: $Pd(OAc)_2$ (0.3 eq.), $Cu(OAc)_2$ (2.5 eq.), 117 °C, 72 h. Method D: $Pd(OAc)_2$ (5 mol%), O_2, 95 °C, 48 h

Scheme 3.3 Synthesis of indolequinones **101-107** through a $Pd(OAc)_2$ mediated reaction.

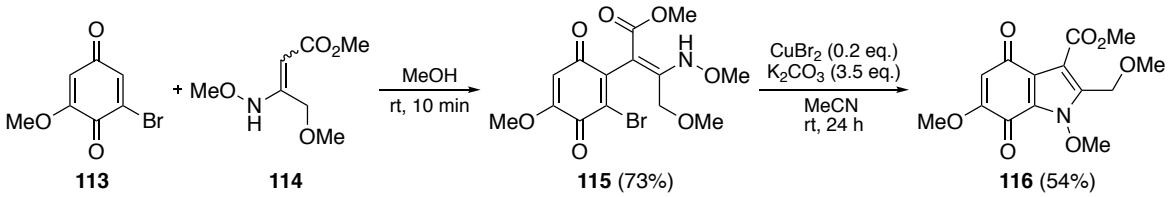
3.3.2 CYCLISATION WITH COPPER

Murphy *et al.* reported the annulation of 2-bromo-1,4-benzoquinone **108** and amino crotonate **109** mediated by $CuCl_2$ in the presence of sodium hydrogen carbonate to give the intermediate **110** (**Scheme 3.4**).[104] This method was applied to the synthesis of murrayaquinone A **79**, an important carbazole alkaloid.



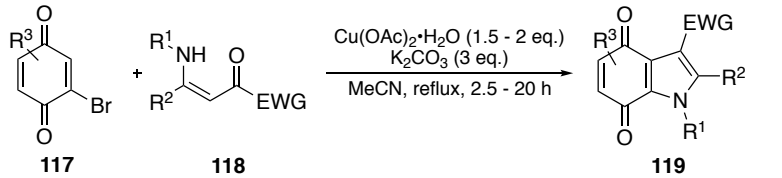
Scheme 3.4 Synthesis of murrayaquinone A **79** from the copper-mediated intermediate **110**.

In the same research group, the one-pot reaction was separated into two steps aiming to improve the yield.[103] Michael addition between 2-bromo-6-methoxy-1,4-benzoquinone **113** and enamine **114** was carried out, at room temperature, in methanol to give the adduct **115** in 73% yield (**Scheme 3.5**). The intermediate was then treated with copper(II) bromide in the presence of potassium carbonate for 24 h and gave the indolequinone **116**, in an overall 39% yield.



Scheme 3.5 Synthesis of indolequinones **116** via a copper-mediated reaction.

More recently, Inman and Moody have further investigated this method.[102,120] A series of indolequinones **119** was prepared from the reaction of 2-bromo-1,4-benzoquinones **117** with amino-crotonates **118** in the presence of super-stoichiometric quantities of $Cu(OAc)_2 \cdot H_2O$ and potassium carbonate, under reflux in acetonitrile (**Scheme 3.6**).

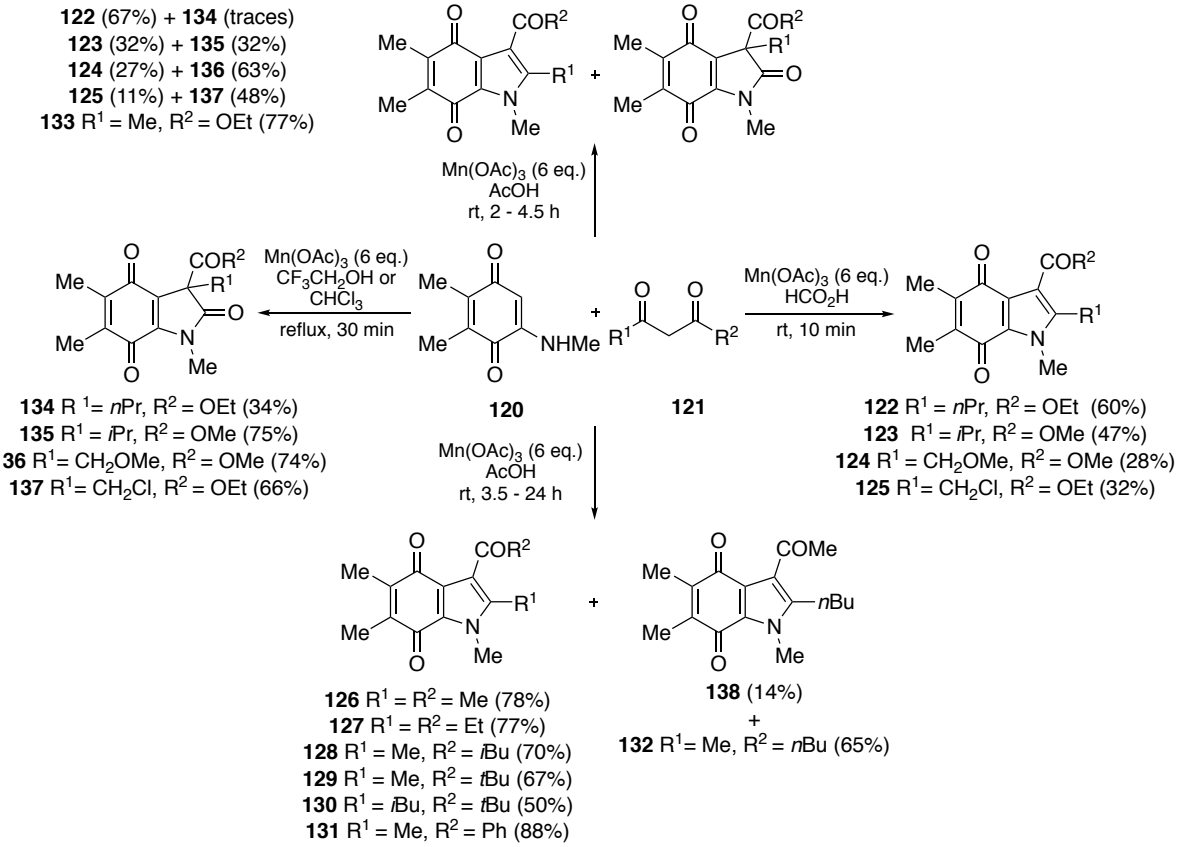


R^1 = alkyl, aryl; R^2 = alkyl, aryl, CO_2Me, $CH_2OCH_2CH{=}CH_2$;
R^3 = H, Et, OMe, O*i*Pr, NHBoc, Br; EWG = CO_2Me, CO_2Et, CO_2tBu, etc

Scheme 3.6 Synthesis of indolequinones **119** via a copper-mediated reaction.

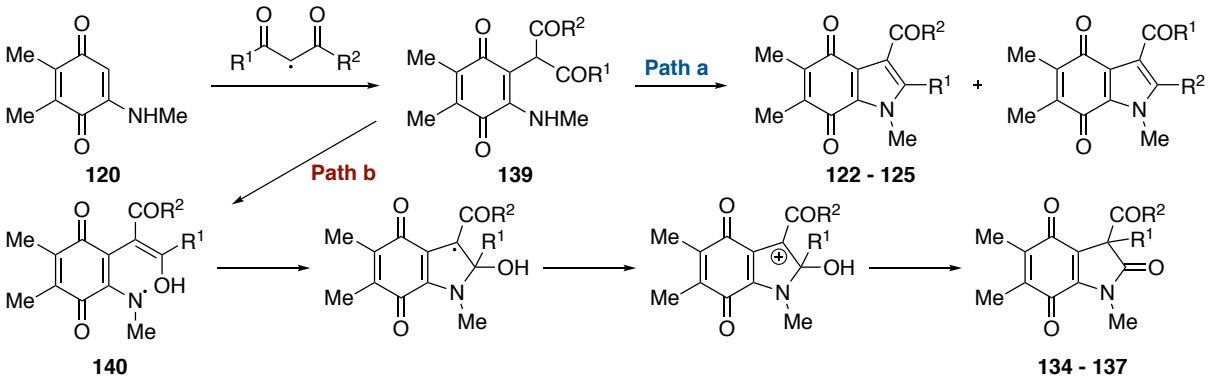
3.3.3 CYCLISATION WITH MANGANESE

A manganese(III) acetate mediated oxidative free radical reaction between 2-amino-1,4-benzoquinones **120** and 1,3-dicarbonyl compounds **121** was reported (**Scheme 3.7**).[121] With β-keto esters, by changing the solvent, the condensation product indole-4,7-dione **122-125** and **133** and rearrangement product indole-2,4,7-trione **134-137** can be generated in high chemo-selectivity. With 1,3-diones, the condensation product was the only product. The authors also tried the reaction with simple ketones yielding the condensation product and it gave better results in neutral solvents. Initially, the authors observed that when **121** were β-keto esters, the **134-137/122-125** ratio increased as the size of R^1 increased.



Scheme 3.7 Manganese-mediated synthesis of indolequinone-based motifs **122-138**.

Besides steric hindrance, solvent also plays an important role in the final result of this reaction. Performing the reaction in neutral solvents such as CF_3CH_2OH or chloroform resulted in the selective formation of the rearrangement product **134-137**. Finally, the reaction yield decreases as the size of R^1 and R^2 increases. The authors hypothesised that this is due to the steric effect exerted by the R^1 group (**Scheme 3.8**) - the condensation of **139** (path a, in blue) is retarded by the larger R^1 group and the oxidation of **139** to produce radical **140** occurred (path b, in red).

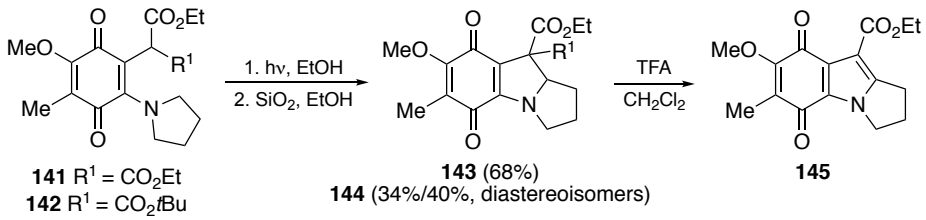


Scheme 3.8 Proposed reaction mechanism for the synthesis of **122-125** and **134-137**.

This procedure was carried out in the presence of 4 eq. of 1,3-dicarbonyl compounds **121** and 6 eq. of the manganese salt. Taking into consideration the sustainability of this process, both reagent and metal salt should be used in lower quantities and an alternative to halogenated solvents would be preferred. Besides, manganese salts are hazardous to the environment, especially to aquatic organisms.

3.3.4 Photochemical cyclisation

Akiba *et al.* successfully prepared dehydro indolequinones **143** and **144** from the photochemical reaction of aminobenzoquinones **141** and **142** (**Scheme 3.9**).[109] Product **144** was obtained as a mixture of diastereoisomers in a 56:44 ratio (at the time structural assignments were not made).

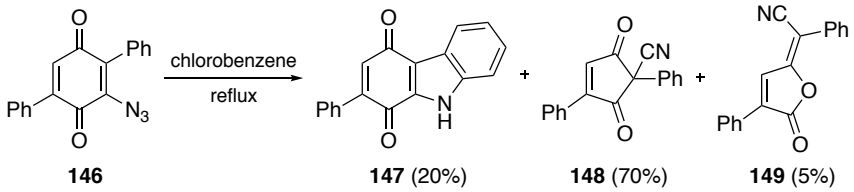


Scheme 3.9 Synthesis of indolequinone 145 via a photochemical reaction.

Treatment with trifluoroacetic acid (TFA) resulted in oxidative de-esterification to give indolequinone **145**.

3.3.5 THERMAL CYCLISATION

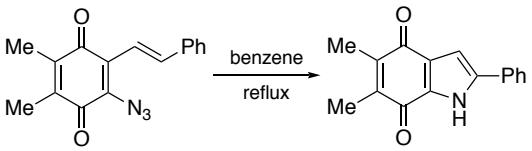
In 1969, Moore *et al.* reported the formation of indolequinones by thermal decomposition of azidoquinones **146** (**Scheme 3.10**).[122] Indolequinone **147** was obtained as a by-product, in 20% yield, along with the major product **148** and **149**.



146 **147** (20%) **148** (70%) **149** (5%)

Scheme 3.10 Synthesis of indolequinone **147** via thermal decomposition.

The group further developed this synthetic strategy.[108,123] Thermolysis of azidoquinone **150** in benzene under reflux gave indolequinone **151** in good yield (**Scheme 3.11**).



150 **151** (66%)

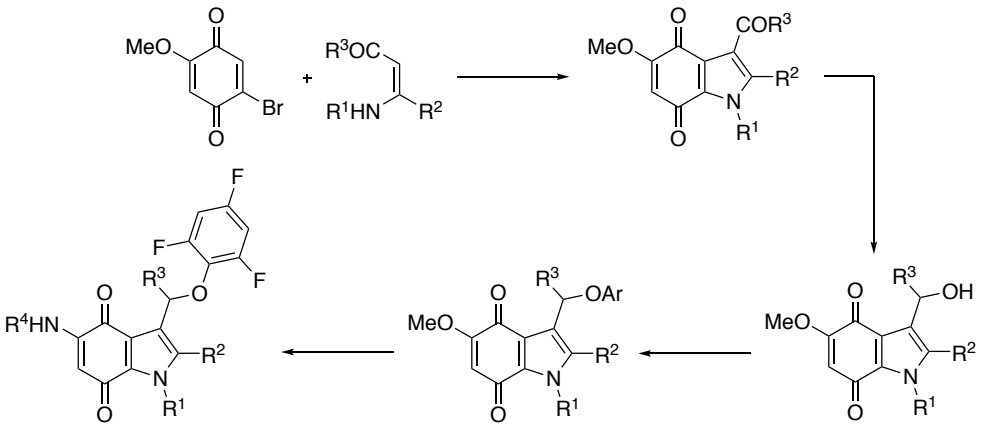
Scheme 3.11 Synthesis of indolequinone **151** via thermal decomposition.

In both cases, the reaction was carried out in a benzene-derived solvent. Chlorobenzene specifically being a halogenated organic solvent offers extra environmental and health hazards and its use should be avoided.

3.4 Aims & Objectives

This project aims at the development of a more sustainable methodology for the synthesis of potential 5-aminoindolequinone-based NQO2 inhibitors, whose structures were based on structures previously identified. In addition, the focus will be on carrying out the reactions under milder conditions and minimising the volume of solvent used and waste formation. The synthetic sequence consists of four main stages, represented below in **Scheme 3.12**. The

synthetically accessible derivatives with functionalisable handles, obtained in the first step, will provide a rapid access to a range of substrates.



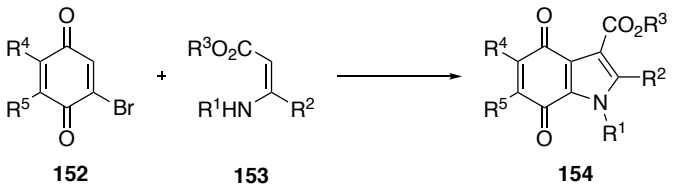
Scheme 3.12 General synthetic sequence to prepare 5-aminoindolequinones.

The first part of the project, presented in **Section 3.5**, will describe the synthesis of the indolequinone precursors, namely 2-bromo-1,4-benzoquinones and enamines. **Section 3.6** is focused on the synthesis of indolequinones from the combination of the previously prepared indolequinone precursors, with the optimisation of the reaction conditions being carried out. Next, the substrate scope for this reaction is discussed in **Section 3.7** and the optimisation studies carried out for the synthesis of a hydroquinone by-product will be discussed in **Section 3.8**. The subsequent section will be focused on the optimisation studies for the reduction of the carbonyl functional group to the corresponding alcohols derivatives. Again, optimisation of the reaction conditions will be performed (**Section 3.9**). The next stage will be the transformation of these derivatives into the corresponding aryl ethers, focusing primarily on the reduction of the quantity of thionyl chloride used and replacement of dimethylformamide (**Section 3.10**). The last step of this sequence will be the functionalisation of the C-5 position with the introduction of a primary amine (**Section 3.11**). Finally, other reactions attempted that do not fall into any of the previously mentioned sections are presented in **Section 3.12**.

3.5 Synthesis of indolequinones

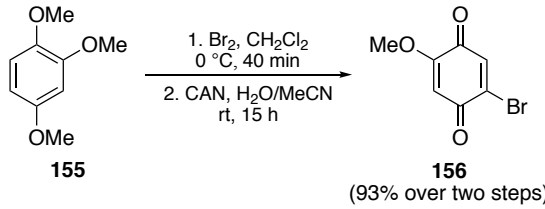
3.5.1 Synthesis of indolequinone precursors

Chasing a more sustainable method to synthesise indolequinones, we were attracted by the convergent nature of the reaction between 2-bromo-1,4-benzoquinones **152** and amino-crotonates **153** in addition to the ready availability of the required starting materials.



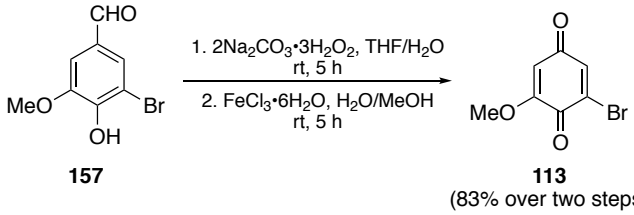
Scheme 3.13 General synthesis of indolequinones.

In this project, syntheses of the indolequinone precursors, namely 2-bromo-1,4-benzoquinones **113** and **156** and enamines **159-168** were achieved based on the previously described procedures, in excellent to good yields.[102,120] Although commercially available, the 2-bromo-1,4-benzoquinones substrates are expensive and they were synthesised in good yields from the phenolic precursor. The bromination of 1,2,4-trimethoxybenzene **155** and subsequent oxidation of the intermediate 1-bromo-2,4,5-trimethoxybenzene, in the presence of cerium(IV) ammonium nitrate (CAN) gave 2-bromo-5-methoxy-1,4-benzoquinone **156** (**Scheme 3.14**), which was isolated by filtration, in excellent yield over two steps.



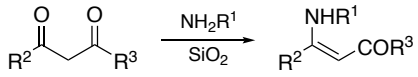
Scheme 3.14 Synthesis of 2-bromo-5-methoxy-1,4-benzoquinone **156**.

2-Bromo-6-methoxy-1,4-benzoquinone **113** was prepared through a Dakin oxidation of 5-bromovanillin **157** with sodium percarbonate, followed by an oxidation with iron(III) chloride (**Scheme 3.15**).



Scheme 3.15 Synthesis of 2-bromo-6-methoxy-1,4-benzoquinone **113**.

Enamines **158-167** were prepared in good to excellent yields from the addition of primary alkyl amines to 1,3-dicarbonyl compounds **121**, in the presence of silica (**Scheme 3.16**). Solid products, such as **158** and **167**, were isolated through filtration and enamines that are liquids/oils, namely compounds **159-166**, were isolated following extraction with dichloromethane or ethyl acetate and concentration under reduced pressure.



121

158 $R^1 = R^2 = Me$, $R^3 = OMe$ (92%); **159** $R^1 = Et$, $R^2 = Me$, $R^3 = OMe$ (99%)
160 $R^1 = n$Pr, $R^2 = Me$, $R^3 = OMe$ (65%); **161** $R^1 = (CH_2)_2$-morpholino, $R^2 = Me$, $R^3 = OMe$ (98%)
162 R^1 = 4-methylpiperazino, $R^2 = Me$, $R^3 = OMe$ (69%); **163** $R^1 = -CH_2CO_2Me$, $R^2 = Me$, $R^3 = OMe$ (87%)
164 $R^1 = Me$, $R^2 = Et$, $R^3 = OMe$ (88%); **165** $R^1 = Me$, $R^2 = -CH_2OMe$, $R^3 = OMe$ (94%)
166 $R^1 = R^2 = Me$, $R^3 = OEt$ (83%); **167** $R^1 = R^2 = R^3 = Me$ (100%)

Scheme 3.16 Synthesis of enamines **158-167**.

The *Z* configuration of the prepared enamines was confirmed by the high chemical shift of the –NH, typically δ_H around 8-9 ppm, indicative of the intramolecular H bonding with the oxygen from the carbonyl functionality, as displayed with the orange line in **Figure 3.5**.

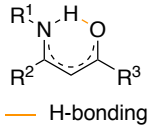


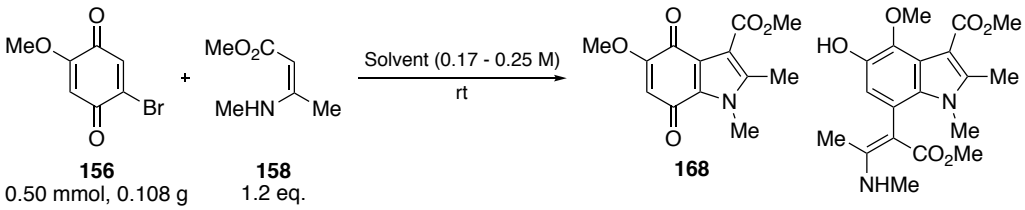
Figure 3.5 Hydrogen bond between the –NH and carbonyl functionality of the enamines.

3.5.2 Preliminary studies with 2-bromo-5-methoxy-1,4-benzoquinone

A set of metal-free reaction conditions that provide access to a range of range of substrates was sought as an alternative approach to the previous work developed in the research group,[102,120] that relied on the use of copper(II) as reaction mediator.

Solvent screening

With this objective, initial attempts focused on the reaction of 2-bromo-5-methoxy-1,4-benzoquinone **156** with methyl (methylamino)crotonate **158** were carried out in a range of solvents, in the absence of additives (metal salts or bases) (**Scheme 3.17**).



156
0.50 mmol, 0.108 g

158
1.2 eq.

168

169

Scheme 3.17 Reaction of **156** and **158** in a range of solvents.

Table 3.1 Solvent screening for the reaction of **156** and **158**.

Entry	Solvent	Time (h)	Recovery yield (%)[a]	Ratio[b]	
				168	**169**
1	MeOH	6.8	4	0	100
2	EtOH	3.1	31	0	94[c]
3	acetone	3.5	12	0	100
4[d]	DMC	72	16	100	0
5[e]	H_2O	0.5	18	n/a[f]	

a) Based on 100% being 100% recovery of the desired product **168**;
b) Calculated based on ^{1}H NMR integrations; c) Isolated as a mixture of **169/156** (94:6);
d) Dark; e) Sonication; f) Isolated as a mixture of **168/156** (93:7).

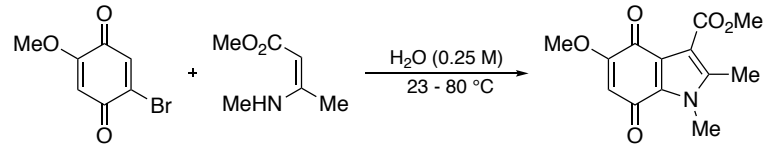
Firstly, on the basis of earlier work,[103] methanol was chosen as the solvent. Also, simple alcohols (methanol and ethanol) are environmentally preferable solvents with relatively low health hazards.[124] However, this resulted in none of the desired indolequinone **168.** Instead, a colourless solid was isolated from the dark reaction mixture, and on the basis of extensive NMR spectroscopic studies, was tentatively assigned as the 1:2 adduct **169**. A more detailed discussion about the formation of this product (**Table 3.3**) as well as a mechanistic proposal is presented below (**Scheme 3.21**).

Subsequently a range of other solvents (ethanol, acetone, dimethyl carbonate, ethyl lactate, water), selected for their "green" credentials,[10] was investigated. Dimethyl carbonate (DMC) holds properties of nontoxicity and biodegradability which makes it a good solvent to use in synthesis minimising its environmental impact at the source. In fact, since the mid-1980s it has been synthesised by a clean process using CO_2 as a building block and due to its classification as volatile organic compounds (VOC) exempt, dimethyl carbonate has grown in popularity.[16,125] Ethyl lactate is a biodegradable compound, with good properties to be used as a green solvent in organic synthesis and pharmaceutical preparations. It can easily be obtained from carbohydrate feedstocks, since it has been industrially produced as a racemic mixture through the reaction between ethanol and lactic acid, which are obtained by fermentation of biomass.[126] Water is an environmentally benign, non-flammable and readily available solvent over a wide temperature range and possesses a high heat capacity making it inherently safe. Despite being a uniquely advantageous solvent, many other parameters such as atom efficiency, yield, workup and purification processes of the reaction must be considered.[16,127]

The reactions carried out in (-)-ethyl L-lactate and an aqueous solution of potassium carbonate (0.2 M) led to degraded and/or complex mixtures. Of the solvents tried, only water and DMC

showed any promise with the formation of the desired indolequinone **168** in 18% and 16% yield, correspondingly.

Considering water's intrinsically environmental advantages, it seemed reasonable to further study the trends of the effect of using this solvent on synthesis of the indolequinone **168**.



156 0.50 mmol, 0.108 g **158** 1.2 eq. **168**

Scheme 3.18 Reaction of **156** and **158** in a water.

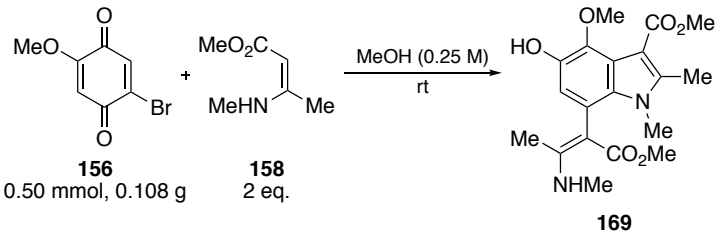
Table 3.2 Water as solvent in the reaction of **156** and **158**.

Entry	Temperature (°C)	Time (min)	Recovery yield (%)[a]	Ratio[b]	
				156	168
1	25	30	37	93	7
2	50	30	21	26	74
3	80	30	32	33	67

a) Based on 100% being 100% recovery of the desired product **168**;
b) Calculated based on ^{1}H NMR integrations.

Carrying out the reaction of 2-bromo-6-methoxyindolequinone **156** with 1.2 eq. of enamine **158** at three temperatures revealed that the best set of conditions are presented in entry 3, at 80 °C, with a comparable recovery yield as to the reaction carried at 25 °C, and similar ratio of formation of **168**, when compared to the reaction carried at 50 °C.

At this stage, and considering the results from the initial solvent screening (**Table 3.1**) the formation of product **169** in methanol was also briefly scoped.



156 0.50 mmol, 0.108 g **158** 2 eq. **169**

Scheme 3.19 Reaction of **156** and **158** in methanol.

Table 3.3 Use of methanol in the reaction of **156** and **158**.

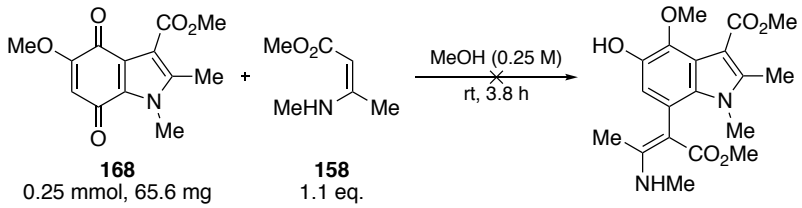
Entry	Time (h)	Isolated yield 169 (%)
1	0.5	16[a]
2	2.8	14
3	16.2	14
4	69	7
5[b]	88	19

a) Isolated as a mixture of **169**/**156** (50:50); b) Dark.

The general trends from the reactions tried show that prolonging the reaction time from 0.5 h to 2.8 h increased the formation of **169** (entries 1 and 2). Letting the reaction on for 16.2 h did not increase the isolated yield of **169** (entry 3). However, prolonging the reaction time to 69 h, did not improve **169** isolated yield (entry 4). The reaction was also tried in the dark for an extended period of time (entry 5) and the isolated yield of **169** was comparable to that obtained after 2.8 h or 16.2 h, in the usual reaction conditions.

Mechanistic proposal for the formation of 169

After realising that most of the above reactions appeared to form product **169** preferentially, an attempt to gain further understanding on how this product would be formed in the reaction was undertaken, to ascertain if the indolequinone **168** was an intermediate. Indolequinone **168** was combined with the amino crotonate **158**, in methanol and the mixture was stirred at rt for 2.3 h (**Scheme 3.20**).



168 0.25 mmol, 65.6 mg

158 1.1 eq.

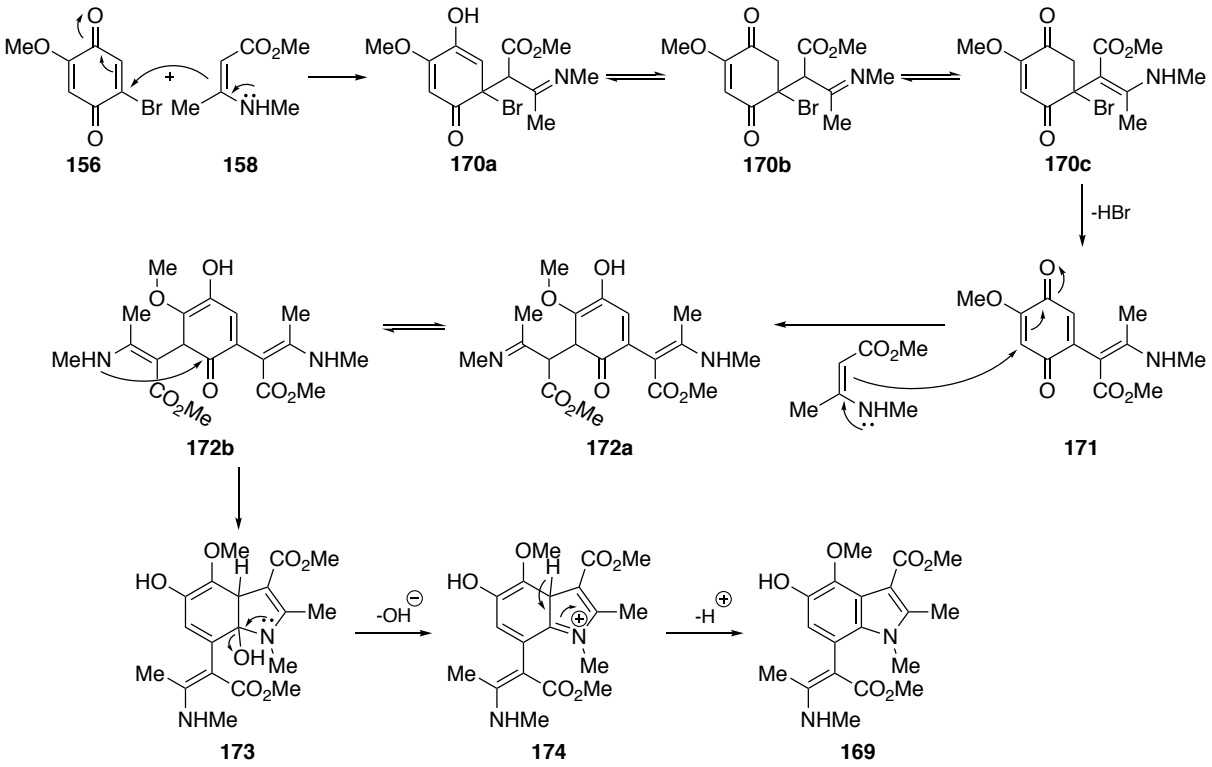
169

Scheme 3.20 Attempt to obtain **169** from the reaction of **168** with **158**.

The outcome of this reaction was the recovery of the starting material **168** in 75% yield, with no evidence for formation of **169**.

From the previous reaction and considering the lack of reactivity of **168** under these conditions, it is not likely that the formation of **169** would occur through a similar pathway to that proposed for the synthesis of **168** (**Scheme 3.20**). Presumably the 1:2 adduct **169** originates from initial displacement of the bromide in 2-bromo-5-methoxy-1,4-benzoquinone **156** by the enamine

158, then Michael addition of a second unit of the enamine **158** to quinone **172**, followed by a Nenitzescu cyclisation.



Scheme 3.21 Mechanistic proposal for the synthesis of **169**.

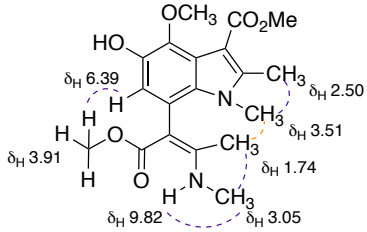
The NMR data collected for this compound, especially those obtained from two-dimensional techniques, such as HSQC, HMBC and NOESY, also suggest the formation of the product **169** considering the 2D interactions identified. From the NOESY spectrum it was possible to identify six interactions between signals with two of them being weak (**Table 3.4** and **Figure 3.6**).

Table 3.4 NOESY interactions observed in compound **169**.

Entry	δ_H (ppm) original	δ_H (ppm) interaction
1	9.82 (-N<u>H</u>)	3.05 (-NHC<u>H</u>$_3$)
2	6.39 (H-5)	3.91 (-OC<u>H</u>$_3$)
3	5.25 (-O<u>H</u>)	1.74 (-C<u>H</u>$_3$) - weak
4	3.51 (-NC<u>H</u>$_3$)	2.50 (-C<u>H</u>$_3$)
5	3.51 (-NC<u>H</u>$_3$)	1.74 (-C<u>H</u>$_3$) - weak
6	3.05 (-NHC<u>H</u>$_3$)	1.74 (-C<u>H</u>$_3$)

The –N<u>H</u> signal at δ 9.82 ppm correlates with the expected signal at δ 3.05 ppm for the adjacent -C<u>H</u>$_3$ substituent (entry 1). The proton at the C5 position interacts with the signal at δ 3.91 ppm,

which corresponds to the aromatic -OCH_3 (entry 2). The signal for the hydroxyl proton at δ 5.25 ppm correlates weakly with the signal at δ 1.74 ppm (entry 3), which is the chemical shift for the protons of the -$C\underline{H}_3$ substituent in the enamine functionality. The signal at δ 3.51 ppm, which relates to the -$NC\underline{H}_3$ interacts with the expected signal at δ 2.50 ppm for the -$C\underline{H}_3$ substituent at C-2 position (entry 4) and also, weakly, with the signal at δ 1.74 ppm (entry 5), from the -$C\underline{H}_3$ substituent in the enamine moiety. Finally, the signal at δ 3.05 ppm, which relates to the -$NHC\underline{H}_3$ substituent in the enamine correlates with the expected signal at δ 1.74 ppm from the other -$C\underline{H}_3$ substituent.



170

strong NOESY interactions weak NOESY interactions

Figure 3.6 NOESY interactions observed in compound **169**.

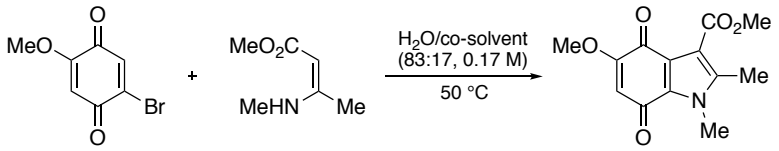
All the strong NOESY interactions (**Table 3.4**, entries 1, 2, 4 and 6), in purple (**Figure 3.6**) are common and one of the weak NOESY interactions (**Table 3.4**, entry 5) depicted in orange (**Figure 3.6**) provide evidence to support the assignment of the suggested structure for **169**, suggesting a Nenitzescu-based mechanism for this reaction (**Scheme 2.18**). X-ray crystallography would help to undoubtedly elucidate the structure, but a crystalline form of the product was not obtained.

Co-solvent screening

The next optimisation study is focused on the effect of using a mixture of water and a co-solvent in the product of the reaction. The purpose of this stage was to find a co-solvent that helped to dissolve the starting materials (especially the 2-bromo-5-methoxy-1,4-benzoquinone **156**) rather than having a heterogeneous mixture when water was used as the only solvent.

The reactions were conducted at 0.25 or 0.50 mmol scale with an excess of the enamine (1.5 eq. in general). An 83:17 ratio mixture of water and another solvent; such as DMC, 2,2,2-trifluoroethanol (TFE), acetone, dimethyl sulfoxide (DMSO), acetonitrile and methanol, was added to a mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** and enamine **158** (**Scheme 3.22** and **Table 3.5**). The screened co-solvents were selected considering their physicochemical properties and greenness associated (**Table 1.1**).

Fluoroalcohols display unique properties such as strong H-bond donation, low nucleophilicity and high ionising power make them ideal solvents for generating reactive electrophilic species and allowing them to react with the desired nucleophile.[128,129] In addition, they have been shown to be beneficial as solvents for asymmetric homogeneous catalysis of organic reactions often improving yields and reaction times while preserving enantioselectivity.[128,130] Acetone and DMSO along with acetonitrile are polar aprotic solvents having dielectric constants of 21 and 47, respectively.[131] They were selected to replace acetonitrile whose dielectric constant is 37 and therefore the effect of this property, if any, could be investigated in the reaction behaviour. In addition, acetone and DMSO occupy a similar or higher position compared to acetonitrile in the ranking of solvents as before mentioned.[14]



156 0.50 mmol, 0.108 g + **158** 1.5 eq. → **168**

Scheme 3.22 Reaction of **156** and **158** in water and a range of co-solvents.

Table 3.5 Optimisation studies of the reaction of **156** and **158** in water and a range of co-solvents.

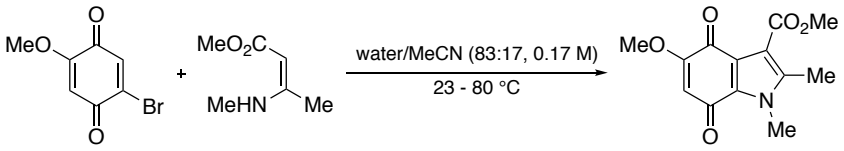
Entry	Co-solvent	Time (min)	Isolated yield 168 (%)
1	DMC	28	15
2	MeOH	28	24
3	MeCN	10	16
4	MeCN	28	26
5	acetone	16	10
6	DMSO	10	18

Overall, there are no significant differences in the outcome of the reaction. The indolequinone **168** was isolated with comparable yields and degrees of purity. Except when water/TFE mixture was used as a solvent and the product isolated was indole **169** in 18% yield.

After deciding that the combination of water/acetonitrile offered marginally the best result, taking into consideration the reaction yield, the higher boiling point of acetonitrile in comparison with methanol and not being necessary to separate the product by column chromatography, variations on the reaction conditions were attempted.

Temperature screening

The first parameter to be assessed in the reaction of **156** and **158** was the temperature (**Scheme 3.23** and **Table 3.6**) and four set temperatures were tried: 23 °C, 50 °C, 80 °C and 100 °C.



156 0.50 mmol, 0.108 g — **158** 1.5 eq. — **168**

Scheme 3.23 Reaction of **156** and **158** in water/acetonitrile at variable temperatures.

Table 3.6 Reaction of **156** and **158** in water/acetonitrile at variable temperatures.

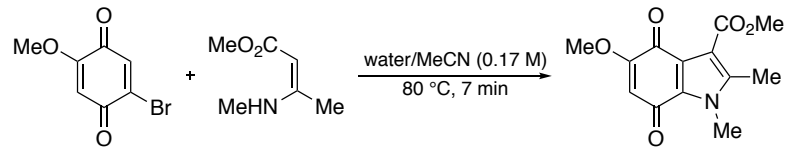
Entry	Temperature (°C)	Time (min)	Recovery yield (%)[a]	Ratio[b]	
				156	168
1	23	190	28	70	30
2	50	10	16	0	100
3	80	8	26	0	100
4[c]	100	8	18	0	100

a) Based on 100% being 100% recovery of the desired product **168**;
b) Calculated based on ^{1}H NMR integrations; c) Degraded reaction mixture.

From the data displayed (**Table 3.6**), the best set of conditions to obtain **168** are those presented in entry 3, where the reaction was carried at 80 °C for 8 min, allowing the isolation of the desired product in 26% yield.

Water/acetonitrile screening

The following step was to evaluate the effect of the ratio of the water/acetonitrile in the reaction performance and three water/acetonitrile ratios were analysed: 67:23, 83:17 and 93:7.



156 0.50 mmol, 0.108 g — **158** 1.5 eq. — **168**

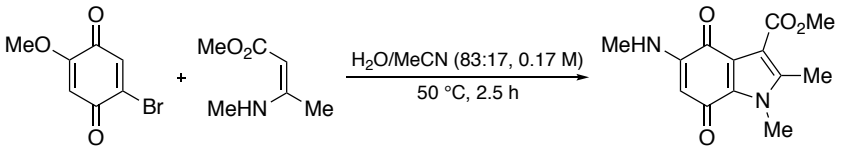
Scheme 3.24 Reaction of **156** and **158** in variable water/acetonitrile ratios.

Table 3.7 Reaction of **156** and **158** in variable water/acetonitrile ratios.

Entry	water/MeCN	Isolated yield 168 (%)
1	67:23	18
2	83:17	26
3	93:7	18

From **Table 3.7** it is possible to conclude that using 83:17 water/acetonitrile ratio (entry 2), as opposed to 67:23 and 93:7, gave indolequinone **168** in the highest yield (26%).

Finally, one attempt was made in which the reaction of 2-bromo-1,4-benzoquinone **156** was performed in the presence of a larger excess (4 eq.) of the enamine **158**, at 50 °C and for an extended period of time (**Scheme 3.25**). This led to the isolation of the 5-amino substituted indolequinone **175**, in 17% yield. This might be explained based on the hydrolysis of enamine **158**, into the corresponding methylamine and ethyl acetoacetate, followed by the displacement of the methoxy group by methylamine.

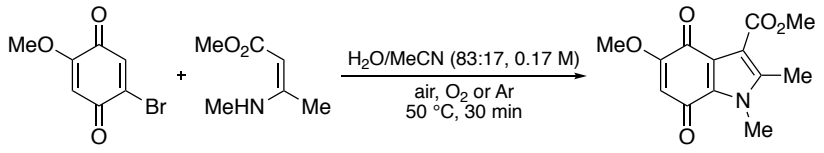


156 0.50 mmol, 0.108 g — **158** 4 eq. — **175** (17%)

Scheme 3.25 Reaction of **156** and 4 eq. of **158** in water/acetonitrile.

Air vs oxygen vs. inert atmosphere screening

A previous report stated that either air or Cu(II) could act as the terminal oxidant, but the optimum conditions utilised both.[132] To confirm this evidence, the reactions of 2-bromo-5-methoxy-1,4-benzoquinone **156** with enamine **158** (1.5 eq.) were carried out on a 0.25 mmol scale, at 50 °C in water/acetonitrile (83:17, 0.17 M) open to air, or under oxygen or argon atmosphere (**Scheme 3.26** and **Table 3.8**).



156 0.25 mmol, 54.3 mg — **158** 1.5 eq. — **168**

Scheme 3.26 Reaction of **156** and **158** under air, argon or oxygen.

Table 3.8 Optimisation studies of the reaction of **156** and **158** under air, argon or oxygen.

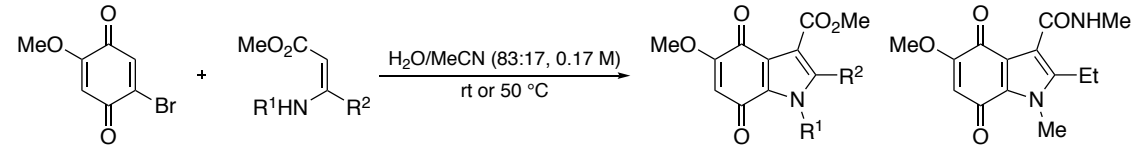
Entry	Atmosphere	Isolated yield 168 (%)[a]
1	air	18
2	O_2	17
3	O_2	23
4	Ar	15
5	Ar	20

a) Crude product isolated with impurities.

The purity degree of the isolated product in the reactions carried out under an oxygen (entries 2 and 3) or argon (entries 4 and 5) atmosphere was always lower than that observed when the reaction was carried under air (entry 1). To summarise, there was not an improvement in the yield of the product **168** when using atmospheric oxygen nor has the yield diminished in the presence of an argon atmosphere. From this point onwards, the reactions were conducted in the presence of an argon atmosphere.

Substrate scope

Despite these reaction conditions not being optimised at this stage, the reaction of 2-bromo-5-methoxy-1,4-benzoquinone **88** with enamines **92** and **96**, in water/acetonitrile at rt or 50 °C, was also attempted and the results are presented below (**Scheme 3.27** and **Table 3.9**).



156 0.50 mmol, 0.108 g

160 R^1 = *n*Pr, R^2 = Me
164 R^1 = Me, R^2 = Et
1.5 or 2 eq.

176 R^1 = *n*Pr, R^2 = Me
177 R^1 = Me, R^2 = Et

178

Scheme 3.27 Reaction of **156** with enamines **160** and **164** in water/MeCN.

Table 3.9 Preliminary studies for the reaction of **156** with enamines.

Entry	Enamine (eq.)	Enamine R^1	Enamine R^2	Temperature (ºC)	Time (min)	Product	Isolated yield (%)
1	**160** (1.5)	*n*Pr	Me	50	27	**176**	32
2	**164** (2)	Me	Et	25	16	**177**	9

Indolequinone **176** was obtained in 32% yield (entry 1). In entry 2, besides the formation of the indolequinone **177** in 9% yield, there was evidence of formation of the carboxamide by-product **178** (similarly to the copper-mediated cyclisation described below (**Table 3.10**, entries 2 and

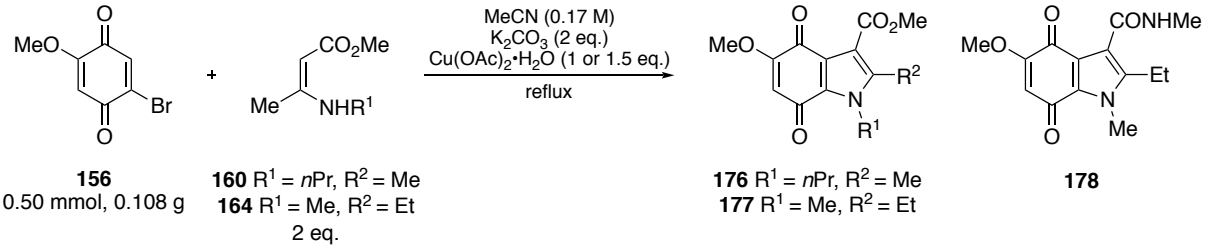
3). Nonetheless the isolation of the carboxamide **178**, in this case, was not successful. This might be explained based on the hydrolysis of enamine **164**, followed by the nucleophilic attack of methylamine to the carbonyl carbon of the ester moiety, displacing the methoxy group.

3.5.3 COPPER-MEDIATED CYCLISATION

At this point, the copper-mediated conditions developed previously in the group were applied to the synthesis of indolequinones **176** and **177** to provide a comparison with the metal-free method developed and discussed earlier.[102,120]

In the reactions presented in **Table 3.10** an attempt towards the use of smaller quantities of: 1) enamine, 2) oxidant agent, 3) cupric salt, 4) base and 5) solvent as compared with the reported protocol by Inman *et al.* was taken.

The copper(II) mediated reactions of 2-bromo-5-methoxy-1,4-benzoquinone **156** and enamines **158, 160** and **164** were carried out on a 0.50 mmol scale in acetonitrile, and 2 eq. of potassium carbonate. (**Scheme 3.28** and **Table 3.10**).



156 0.50 mmol, 0.108 g

160 R^1 = *n*Pr, R^2 = Me
164 R^1 = Me, R^2 = Et
2 eq.

176 R^1 = *n*Pr, R^2 = Me
177 R^1 = Me, R^2 = Et

178

Scheme 3.28 $Cu(OAc)_2 \cdot H_2O$ mediated reaction of **156** with enamines.

Table 3.10 Optimisation studies of $Cu(OAc)_2 \cdot H_2O$ mediated reaction of **156** with enamines.

Entry	Enamine	Enamine R^1	Enamine R^2	$Cu(OAc)_2 \cdot H_2O$ (eq.)	Time (h)	Isolated yield (%) 176/177	Isolated yield (%) 178
1	**160**	*n*Pr	Me	1	4.3	33	n/a
2	**164**	Me	Et	1.5	4.3	4	6
3	**164**	Me	Et	1	5.5	2	3

Carrying out the reaction of 2-bromo-5-methoxy-1,4-benzoquinone **156** with enamine **160** (R^1 = *n*Pr, R^2 = Me) gave **176** in low yield (33%) (entry 1). This reaction was also attempted in ethanol considering being a greener solvent as well as its comparable polarity index with acetonitrile. However, the reaction mixture was a complex mixture of products by ^1H NMR analysis. In both reactions tried with the enamine **164** (R^1 = Me, R^2 = Et), the residue was separated by column chromatography to yield sequentially the indolequinone **177** and its

corresponding carboxamide derivative **178** (entries 2 and 3). Analogously to what happened when the same enamine was used in the metal free cyclisation process (**Table 3.9**, entry 2).

Comparing the two synthetic approaches above described; the similar yields obtained in both the metal-free and in copper(II) acetate mediated reactions combined to the milder conditions of the former render the metal-free method as a more attractive alternative to pursue.

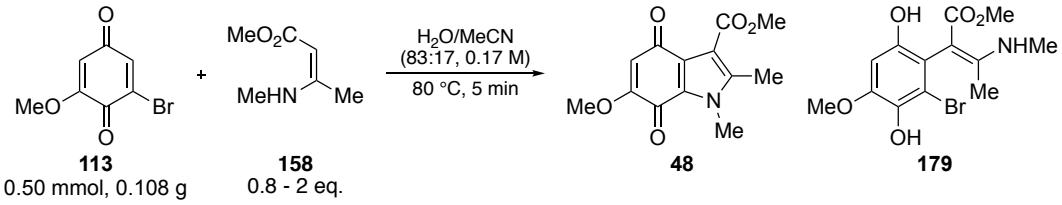
3.6 Optimisation studies

Considering the better overall synthetic performance and product yields for the reactions using 2-bromo-6-methoxy-1,4-benzoquinone **113** over its regioisomeric analogue 2-bromo-5-methoxy-1,4-benzoquinone **156**,[102,120] a set of optimisation studies was carried out using the first.

3.6.1 METAL-FREE REACTION

Enamine loading

Comparably to the optimisation studies with 2-bromo-5-methoxy-1,4-benzoquinone **156**, the effect of the enamine loading was undertaken for the 2-bromo-6-methoxy-1,4-benzoquinone derivative **113** (**Scheme 3.29** and Table 3.11).



113
0.50 mmol, 0.108 g

158
0.8 - 2 eq.

48

179

Scheme 3.29 Reaction of **113** with **158** in water/MeCN.

Table 3.11 Optimisation studies for the reaction of **113** with **158** in water/MeCN.

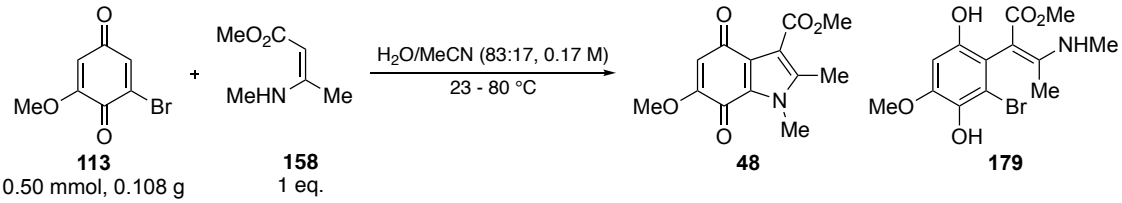
Entry	158 (eq.)	Ratio[a]	
		48	179
1	0.8	27	73
2	1.2	24	76
3	1.5	21	79
4	2	28	72

a) Calculated based on HPLC-MS integrations.

Carrying out the reaction in water/acetonitrile, with 0.8-2 eq. of amino-crotonate **158** led always to the isolation of the product as a mixture of the indolequinone **48** and the uncyclised hydroquinone **179**, in variable ratios. The best set of conditions is shown in entry 3, in which the reaction was carried out with 1.5 eq. of **168** to give a mixture of **48/179** in 21:71 ratio.

Temperature

The effect of the temperature was then evaluated in the reaction. Three reactions were performed at different temperatures (80 °C, rt and 0 °C) in water/acetonitrile with 1 eq. of **158** (**Scheme 3.30** and **Table 3.12**).



113 0.50 mmol, 0.108 g

158 1 eq.

48

179

Scheme 3.30 Reaction of **113** with **158** in water/MeCN at different temperatures.

Table 3.12 Optimisation studies for the reaction of **113** with **158** in water/MeCN at different temperatures.

Entry	Temperature (°C)	Time (min)	Recovery yield (%)[a]	Ratio[b]	
				48	179
1	80	31	53	69	31
2	23	6	110	14	86
3	0	23	87	9	81

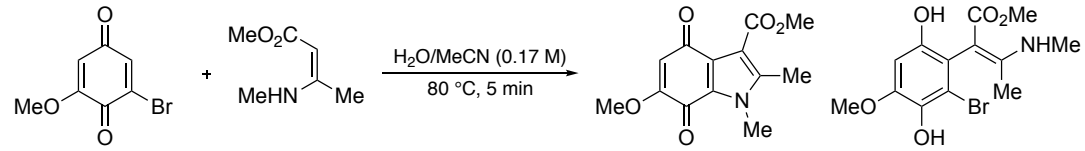
a) Based on 100% being 100% recovery of indolequinone **48**;
b) Calculated based on ^{1}H NMR integrations.

At the three set temperatures, the product was isolated as a mixture of **48**/**179**. **Table 3.12** shows that when temperature decreases the ratio of **48**/**179** drops as well. This evidence suggests that higher temperatures promote the formation of product **48** and the upcoming optimisation studies were carried at 80 °C.

The next parameter to be considered was the influence of the ratio water/acetonitrile in the reaction outcome.

Water/acetonitrile ratios

The 2-bromo-6-methoxy-1,4-benzoquinone **113** was combined with 1.5 eq. of enamine **158**, at 80 °C to evaluate the effect of increasing the water/acetonitrile ratio in the reaction performance (**Scheme 3.31**).



113 0.50 mmol, 0.108 g | **158** 1.5 eq. | **48** | **179**

Scheme 3.31 Reaction of **113** with **158** in variable ratios of water/MeCN.

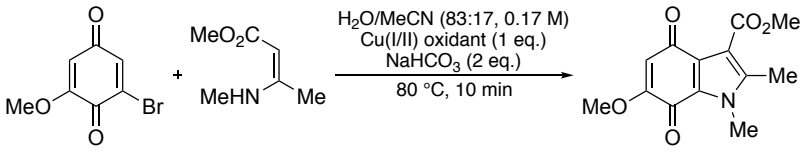
Two reactions were attempted with different ratios water/acetonitrile. In the first case, the reaction was carried out in 75:25 water/acetonitrile ratio and the product was recovered in 93% yield (based on 100% being 100% recovery of indolequinone **48**) as a mixture of indolequinone **48** and the open-structure hydroquinone **179**, in 17:83 ratio. In the second trial, the reaction was performed in 83:17 water/acetonitrile ratio and the product was recovered in 77% yield and as a mixture of 27:73 ratio of **48**/**179**. This suggests that a higher ratio of water in the solvent mixture, helps the formation of **48**.

Owing to the generally unsatisfactory yields, in the metal-free processes described above, we decided to return to investigate and optimise the metal-mediated process and the optimisation studies will be discussed below.

3.6.2 METAL MEDIATED REACTIONS

Oxidant screening

The optimisation studies of the metal mediated reaction started with a screening on the best source of copper as oxidant agent. 2-bromo-6-methoxy-1,4-benzoquinone **113** was reacted with 1.5 eq. of the enamine **158**. The reactions were carried out at 0.25 mmol scale in the presence of 1 eq. of copper(I) and copper(II) bromide and copper(II) chloride and 2 eq. of sodium hydrogen carbonate, at 80 °C, in variable ratios of water/acetonitrile (**Scheme 3.32**).



113 0.25 mmol, 54.2 mg | **158** 1.5 eq. | **48**

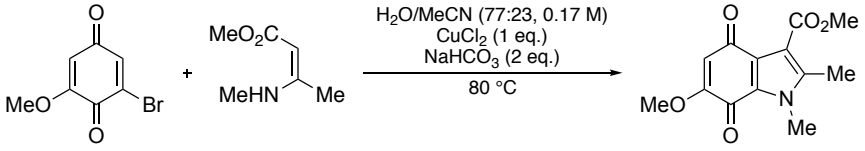
Scheme 3.32 Reaction of **113** with **158 i**n the presence of a range of Cu(I/II) oxidants.

Table 3.13 Optimisation studies for the reaction of **113** with **158** in the presence of Cu(I/II) oxidants.

Entry	Cu(I/II) oxidant	Isolated yield 48 (%)
1	CuBr	67
2	$CuBr_2$	48
3	$CuCl_2$	81

Comparing the results using copper(I) and copper(II) bromide and copper(II) chloride (**Table 3.13**), it can be established that running the reaction in the presence of copper(II) chloride (entry 4) gave the 6-methoxy indolequinone **48** with the best yield and highest purity degree by analysis of the ^{1}H NMR spectrum of the crude material. Another reaction in the presence of copper (II) bromide was also tried in which the number of eq. of sodium hydrogen carbonate were reduced to 1 and it led to the isolation of **48** in a diminished yield (17%). Considering these findings, the next optimisation studies were carried out in the presence of copper(II) chloride.

In the subsequent set of reactions, 2-bromo-6-methoxy-1,4-benzoquinone **113** was reacted with 1-1.5 eq. of enamine **158** in water/acetonitrile, at 80 °C.



113
0.25 mmol, 54.2 mg

158
1 - 1.5 eq.

48

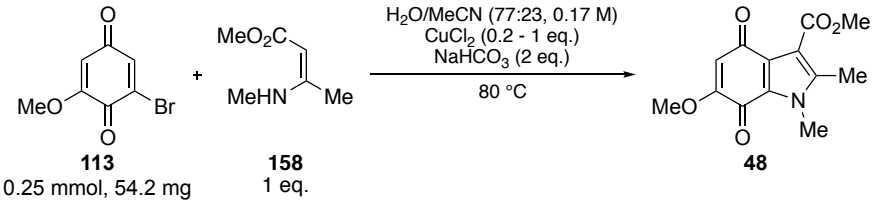
Scheme 3.33 $CuCl_2$ mediated reaction of **113** with variable quantities of **158**.

Table 3.14 Optimisation studies for the $CuCl_2$ mediated reaction of **113** with variable quantities **of 158.**

Entry	158 (eq.)	Time (min)	Isolated yield 48 (%)
1	1.5	10	65
2	1.2	8	71
3	1	8	86

From **Table 3.14** above it is possible to verify that the isolated yield of product **48** increases from 65% to 71% to 86% as the loading of enamine **158** decreases from 1.5 to 1.2 to 1 eq.

Next, the effect of the loading of copper(II) chloride in the reaction of 2-bromo-6-methoxy-1,4-benzoquinone **113** and 1eq. of enamine **158** in water/acetonitrile, at 80 °C was evaluated.



113
0.25 mmol, 54.2 mg

158
1 eq.

48

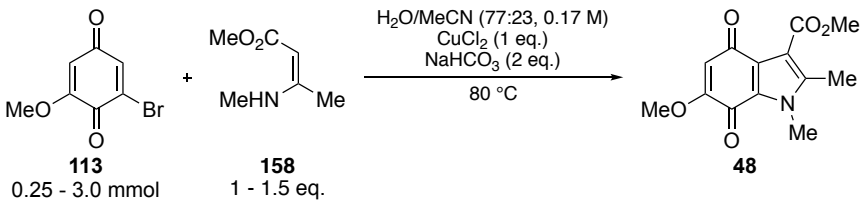
Scheme 3.34 Reaction of **113** and **158** in the presence of variable quantities of $CuCl_2$.

Table 3.15 Optimisation studies for the reaction of **113** and **158** in the presence of variable quantities of $CuCl_2$.

Entry	$CuCl_2$ (eq.)	Time (min)	Isolated yield 48 (%)
1	1	10	72
2	0.5	10	54
3	0.5	20	65
4	0.4	20	54
5	0.2	20	26

From the data displayed in **Table 3.15**, it is possible to verify that the isolated yield of **48** drops significantly when halving the number of eq. of copper(II) chloride when the reaction is carried out for 10 min (entries 1 and 2). Nonetheless, extending the reaction time improves the formation of **48** (entry 3). Reducing the quantity of copper(II) chloride and letting the reaction on, for 20 min, led to a reduction in the isolated yield of **48**.

The following parameter to be studied was the effect of scaling-up the reaction of 2-bromo-6-methoxy-1,4-benzoquinone **113** and 1 eq. of enamine **158**, from 0.25 mmol to 3.0 mmol scale.



113
0.25 - 3.0 mmol
54.2 - 652 mg

158
1 - 1.5 eq.

48

Scheme 3.35 $CuCl_2$ mediated reaction of **113** (0.25-3.0 mmol) with **158**.

Table 3.16 Optimisation studies for the $CuCl_2$ mediated reaction of **113** (0.25-3.0 mmol) with **158**.

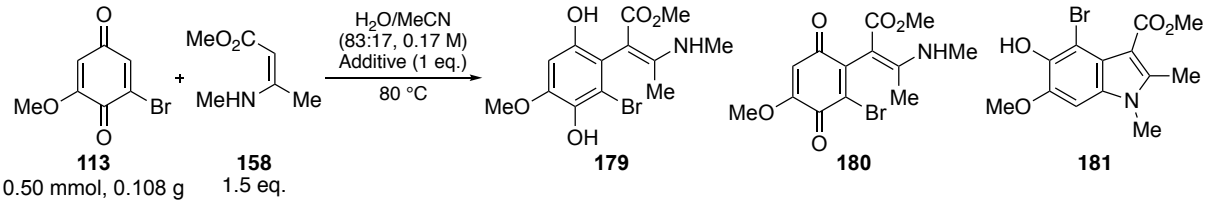
Entry	113 (mmol)	Time (min)	Isolated yield 48 (%)
1	0.25	10	72
2	0.75	20	60
3	0.75	30	62
4	3.0	20	53
5	3.0	30	58

In summary, from **Table 3.16**, it is clear that indolequinone **48** was isolated with the highest yield (72%) in entry 1, where the reaction at 0.25 mmol scale was carried out for 10 min. The general trend seems to suggest that the isolated yield of indolequinone **48** drops as the scale of the reaction increases.

Before moving on to start applying similar conditions to the regioisomeric starting material **156**, a set of control reactions was carried out and they are presented next.

Control reactions

The 2-bromo-6-methoxy-1,4-benzoquinone **113** was reacted with 1.5 eq. enamine **158**, in a mixture of water/acetonitrile (83:17) and one additive. The aim was to evaluate the individual contribution of the additive in the reaction (**Scheme 3.36** and **Table 3.17**). The reaction was tried in sodium hydrogen carbonate and potassium carbonate, Besides the effect of 1 eq. of copper(II) acetate copper(II) bromide and iron(III) chloride, a more earth-abundant metal, was also evaluated.



113 0.50 mmol, 0.108 g — **158** 1.5 eq. — **179** — **180** — **181**

Scheme 3.36 Control reactions of **113** with **158**.

The reaction using $Cu(OAc)_2 \cdot H_2O$ (entry 2) led to the exclusive formation of the 5-hydroxyindole **181**, the product from the Nenitzescu reaction. This is a widely applied strategy for the synthesis of indole derivatives with an electron withdrawing group at position 3, such as an ester or a carbonyl group.[114,133] Despite not being the target product, its spectroscopic data was collected to characterise the structure.

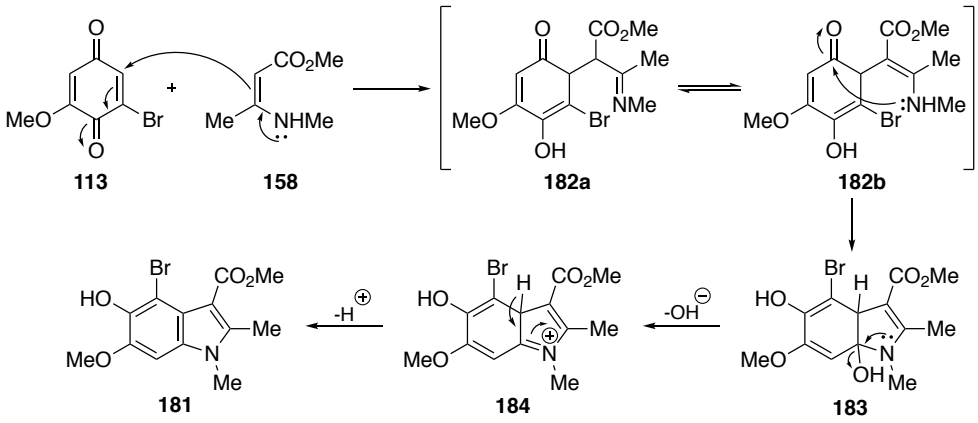
Table 3.17 Control reactions of **113** with **158**.

Entry	Additive	Time (min)	Recovery yield (%)[a]	Ratio[b]			
				113	179	180	181
1[c]	$NaHCO_3$	10	46	8	92	0	0
2	$Cu(OAc)_2 \cdot H_2O$	5	42	0	0	0	100
3[d]	$Cu(OAc)_2 \cdot H_2O$	30	60	54	0	0	46
4[c]	$CuBr_2$	10	48	3	0	58	39

a) Based on 100% being 100% recovery of product **48**;
b) Calculated based on ^{1}H NMR integrations; c) 50 °C; d) Water.

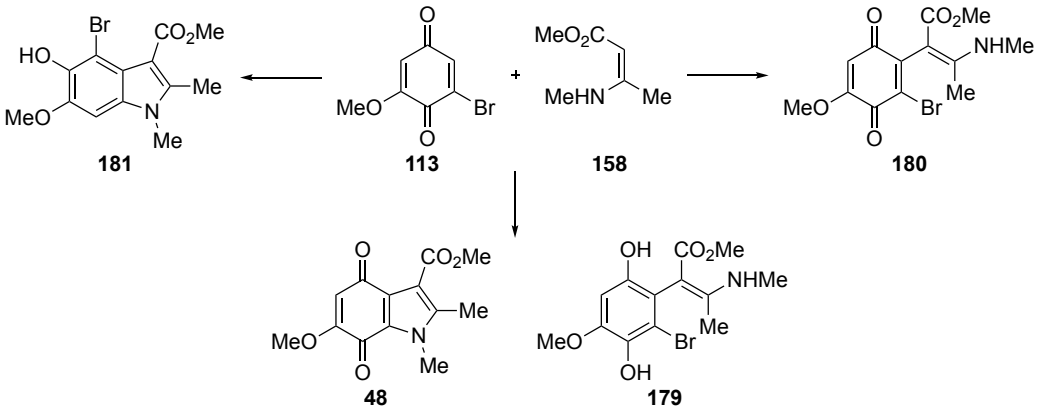
The other four reactions presented in **Table 3.17** gave mixtures of variable rations and compositions of **113** and **179-181**. In addition, two more reactions were conducted. Carrying out the reaction for 5 min, in the presence of 1 eq. of potassium carbonate, the base used previously by Inman *et al.*[102] or in the presence of 1 eq. of iron(III) chloride, for 30 min, led to degradation of the reaction mixture.

The experimental conditions used to obtain substrate **181** represent an alternative to the traditional conditions for Nenitzescu synthesis,[134,135] which may deserve further consideration. Two kinds of mechanisms are possible for this synthesis, depending on the order of carbon-carbon bond and carbon-nitrogen bond formation. Nevertheless, the one involving the initial carbon-carbon condensation is accepted as the more plausible (**Scheme 3.37**).[133] The mechanism is proposed to start with the Michael addition, followed by a nucleophilic attack by the enamine **158** and elimination to give 5-hydroxyindole **181**.



Scheme 3.37 Mechanistic proposal for the synthesis of 5-hydroxyindole **181**.

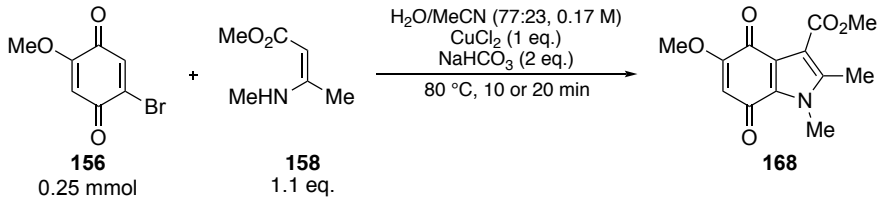
To summarise, the optimisation studies of the reaction between the 2-bromo-6-methoxy-1,4-benzoquinone **113** and the enamine **158** led to the isolation and identification of four different products, **48-181**, presented in **Scheme 3.38** below.



Scheme 3.38 Summary of the reaction of **113** with **158** in water.

5-Methoxy Indolequinone

The reactions of the isomeric 2-bromo-5-methoxy-1,4-benzoquinone **156** with 1.1 eq. of enamine **158** in the presence of stoichiometric and sub-stoichiometric quantities of copper(II) chloride (1-0.4 eq.) are presented in **Scheme 3.39** and **Table 3.18**. The reaction was set at 80 °C in a 77:23 ratio water/acetonitrile.



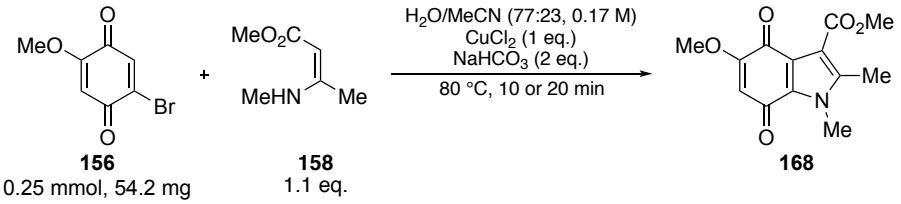
Scheme 3.39 $CuCl_2$ mediated reaction of **156** with **158**.

Table 3.18 Optimisation studies for the $CuCl_2$ mediated reaction of **156** with **158** at variable times.

Entry	Time (min)	Isolated yield 168 (%)
1	7	31
2	10	54
3[a]	19	18

a) Crude product isolated with impurities.

The reactions of the isomeric 2-bromo-5-methoxy-1,4-benzoquinone **156** with 1.1 eq. of enamine **158** in the presence of stoichiometric and sub-stoichiometric quantities of copper(II) chloride (1-0.4 eq.) are presented in **Scheme 3.40** and **Table 3.19**.



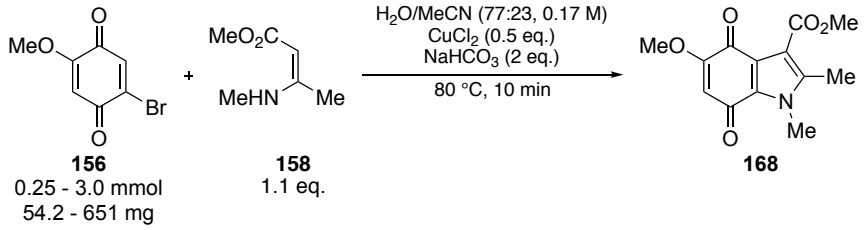
Scheme 3.40 Optimisation studies for the reaction of **156** and **158** in the presence of variable quantities of $CuCl_2$.

Table 3.19 Reaction of **156** and **158** in the presence of variable quantities of $CuCl_2$.

Entry	$CuCl_2$ (eq.)	Isolated yield 168 (%)
1	1.0	54
2	0.5	61
3	0.4	43

From the data reported in **Table 3.19**, the best set of conditions to prepare indolequinone **168**, in 61% yield, uses 0.5 eq. of copper(II) chloride (entry 2).

The following factor to be studied was the effect of scaling-up the reaction of 2-bromo-5-methoxy-1,4-benzoquinone **156** and 1eq. of enamine **158**, from 0.25 mmol to 3.0 mmol scale.



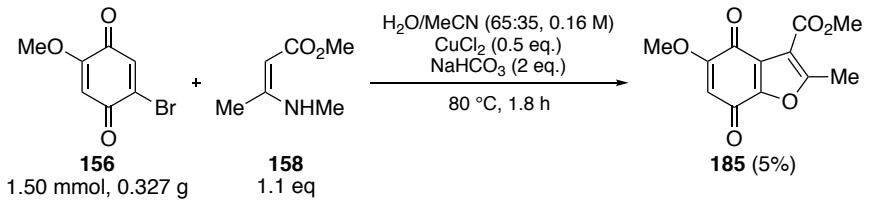
Scheme 3.41 $CuCl_2$ mediated reaction of **156** (0.25-3.0 mmol) with **158**.

Table 3.20 Optimisation studies for the $CuCl_2$ mediated reaction of **156** (0.25-3.0 mmol) with **158**.

Entry	156 (mmol)	Isolated yield 168 (%)
1	0.25	61
2	0.75	49
3	3.0	45

Product **168** was isolated with decreasing yields as the reaction scale increases, with the major drop being from 0.25 mmol to 0.75 mmol scale. The reaction was also tried at 7.5 mmol scale, however after 15 minutes, the yield dropped to 9%. Despite evident product formation, the isolated crude material had a high degree of impurities as verified by ^{1}H NMR spectra analysis.

One copper(II) chloride mediated reaction of **156** (1.50 mmol) and enamine **158** was carried out in the presence of a reduced water/acetonitrile ratio (65:35), for a prolonged period of time and it yielded benzofuranquinone **185** in 5% yield.



Scheme 3.42 Synthesis of benzofuranquinone **185**.

This may be explained based on the hydrolysis of enamine **158**, into the corresponding methylamine and ethyl acetoacetate. Then, condensation of the 2-bromo-5-methoxy-1,4-benzoquinone **156** with ethyl acetoacetate gave the by-product, based on previous evidence from the literature.[136]

To conclude, the reaction does not seem to behave equally for 2-bromo-5-methoxy-1,4-benzoquinone **156** and 2-bromo-6-methoxy-1,4-benzoquinone **48**. The synthesis of 5-methoxyindolequinone **168** happened in the presence of stoichiometric and sub-stoichiometric quantities copper(II) chloride, with no major changes in the yield. Nonetheless, 6-methoxyindolequinone **48** was exclusively formed when stoichiometric amounts of the oxidant agent were used. The reaction in the presence of sub-stoichiometric quantities of copper(II) led to a mixture of the targeted 6-methoxyindolequinone **48** and its 1,4-hydroquinone adduct **179**. Furthermore, the synthesis of 5-methoxyindolequinone **168** was usually faster than the synthesis of 6-methoxyindolequinone **48** thus suggesting that the reaction may follow different mechanisms.

The optimised conditions for the synthesis of 5-methoxyindolequinone **168**, in 61% yield, (**Table 3.20**, entry 1) are listed below.

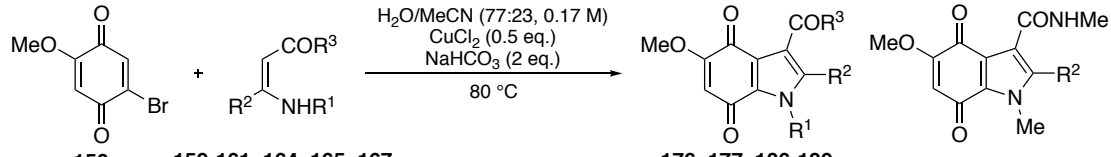
- **Eq. Enamine**: 1.1
- **Metal salt (eq.):** $CuCl_2$ (0.5)
- **Base (eq.):** $NaHCO_3$ (2)
- **Solvent/co-solvent system (ratio, concentration):** water/acetonitrile (77:23, 0.17 M)

3.7 Substrate scope

With an optimised set of conditions in hand, different enamines were combined with the two 2-bromo-methoxy-1,4-benzoquinones **156** and **113** to evaluate the substrate scope of this reaction. The results are displayed next, in separate tables, for both the derivatives 2-bromo-5-methoxy-1,4-benzoquinone **156** and 2-bromo-6-methoxy-1,4-benzoquinone **113**.

3.7.1 FROM 2-BROMO-5-METHOXY-1,4-BENZOQUINONE

The reaction of 2-bromo-5-methoxy-1,4-benzoquinone **156** (1.5-3.0 mmol) with a range of amino-crotonates was carried out in the presence of 0.5 or 1 eq. of copper(II) chloride and in a 77:23 ratio water/acetonitrile (**Scheme 3.43**).



156 3.0 mmol, 0.651 g

159-161, 164, 165, 167 1.1 eq

176, 177, 186-189

178 R^2 = Et

190 R^2 = -CH_2OMe

159, **186** R^1 = Et, R^2 = Me, R^3 = OMe; **160**, **176** R^1 = *n*Pr, R^2 = Me R^3 = OMe
161, **187** R^1 = $(CH_2)_2$-morpholino, R^2 = Me R^3 = OMe; **164**, **177** R^1 = Me, R^2 = Et, R^3 = OMe
165, **188** R^1 = Me, R^2 = -CH_2OMe, R^3 = OMe; **167**, **189** R^1 = R^2 = R^3 = Me

Scheme 3.43 Substrate scope of the $CuCl_2$ mediated reaction of **156** with enamines in water/MeCN.

Table 3.21 Effect of substrate scope of the $CuCl_2$ mediated reaction of **156** with enamines in water/MeCN.

Entry	Enamine	Enamine		Time (min)	Ester	Isolated yield (%)
		R^1	R^2			
1[a]	**159**	Et	Me	5	**186**	34
2[a]	**159**	Et	Me	10	**186**	27
3[b]	**160**	*n*Pr	Me	15	**176**	19
4	**161**	$(CH_2)_2$-morph	Me	5	**187**	1
5	**161**	$(CH_2)_2$-morph	Me	15	**187**	7
6	**161**	$(CH_2)_2$-morph	Me	108	**187**	3
7	**164**	Me	Et	5	**177**	25[c]
8	**164**	Me	Et	10	**177**	12
9	**165**	Me	CH_2OMe	10	**188**	6
10	**167**[d]	Me	Me	10	**189**	23

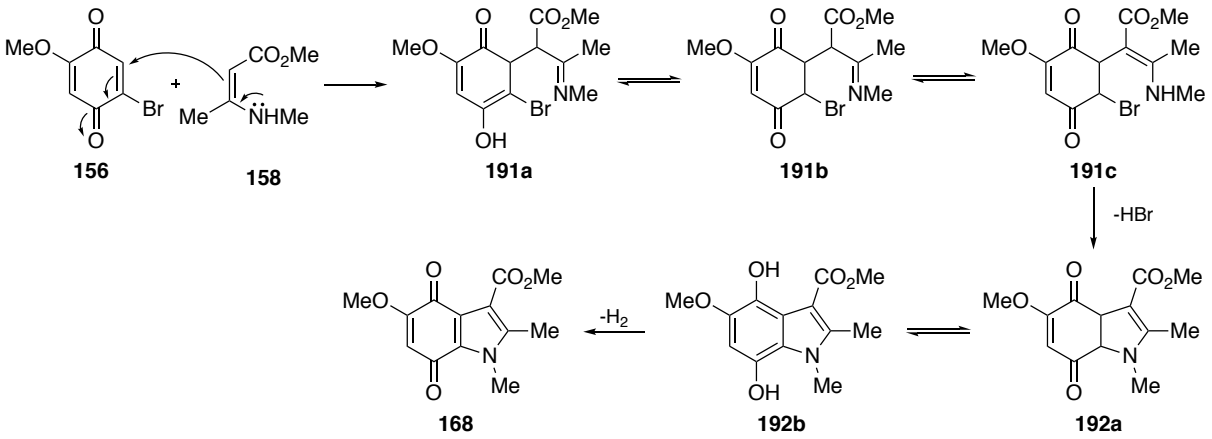
a) H_2O/MeCN (67:23); b) $CuCl_2$ (1 eq.); c) Amide **178** also isolated in 14% yield; d) Enamine **167** (R^3 = Me).

Several reactions were tried for each enamine and **Table 3.21** only summarises the best results obtained for each one of them. For example, the reaction of **165** (R^1 = Me, R^2 = CH_2OMe and R^3 = OMe) with 2-bromo-5-methoxy-1,4-benzoquinone **156** for 5 min gave amide **190** in 9% yield, however the purification of this product was unsuccessful. In entry 7, two products were sequentially isolated, namely the ester **177**, and the corresponding carboxamide **178**, in 25% and 14% yield, correspondingly. The formation of the by-product **178** was already discussed in **Table 3.10**.

Other enamines such as **162** (R^1 = 1-methylpiperazino, R^2 = Me and R^3 = OMe) and **163** (R^1 = CH_2CO_2Me, R^2 = Me and R^3 = OMe) were tried and the reactions led to the isolation of either starting material **156** in 57% yield or complex mixture of products. No further attempts to optimise the synthesis of these products were taken in the interest of time.

Mechanistic proposal

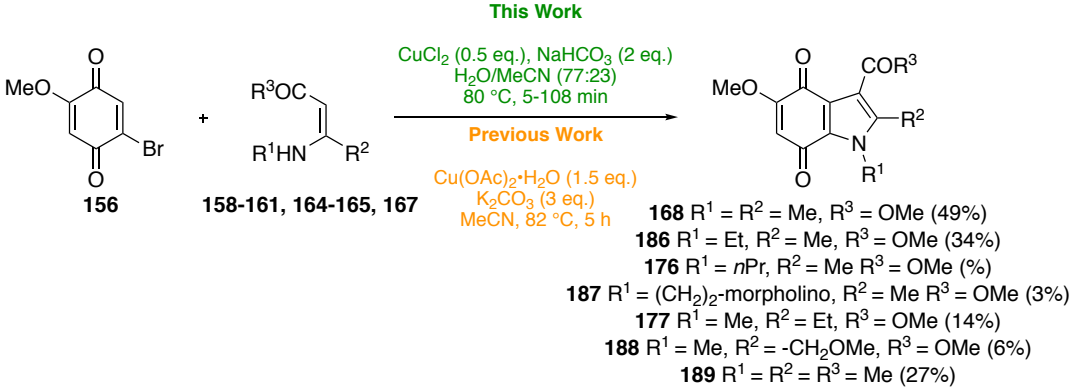
The reaction of 2-bromo-5-methoxy-1,4-benzoquinone **156** and enamine **158** proceeds under air in the absence of added copper salts, although with a diminished yield. The reaction did also proceed with sub-stoichiometric amounts of copper(II) chloride under air or with 1.5 eq. of copper(II) acetate under argon, albeit in lower yields in both cases. This indicates that either air or Cu(II) could act as the terminal oxidant, but the optimum conditions utilised both. When 2-bromo-6-methoxy-1,4-benzoquinone **113** was used, the isolation of intermediate **181**, may be indicative of a reaction mechanism that begins with a nucleophilic attack by the enamine on the more electrophilic C-3 of the quinone, which has no bromine atom.[132] The 5-methoxy substituent on the quinone generally resulted in lower yields, which was most likely the result of their deactivating influence on the 3-position. The keto-enolic tautomerism is followed by cyclisation and C–N bond formation with the loss of hydrogen bromide to give the product **192a,** which is in equilibrium with its enolic structure **192b** (**Scheme 3.44**).



Scheme 3.44 Mechanistic proposal for the synthesis of 5-methoxyindolequinone **168**.

The final step is the oxidation of this intermediate to give **168** The precise mechanism by which this occurs is unclear, although it has been attributed to the activation of the C–Br bond through the Lewis acidity of the copper salt.[103,104] Although an Ullmann–Goldberg-type reaction cannot be entirely discounted, it seems unlikely given the success of the reaction without the addition of $CuCl_2$.

In **Scheme 3.45** and **Table 3.22** below, a comparison between the route developed in this project and the method previously used is presented. Overall, in this project the loading of enamine, copper(II), based and solvent were reduced. Also, a more environmentally friendly solvent system was used and the reaction time of this reaction was much faster in the presence of water. For derivatives **168** and **188** purification of the crude material was not required, and the yield of the former was comparable to that obtained through the previously developed method.[102,120]



Scheme 3.45 Synthesis of 5-methoxy indolequinones precursors.

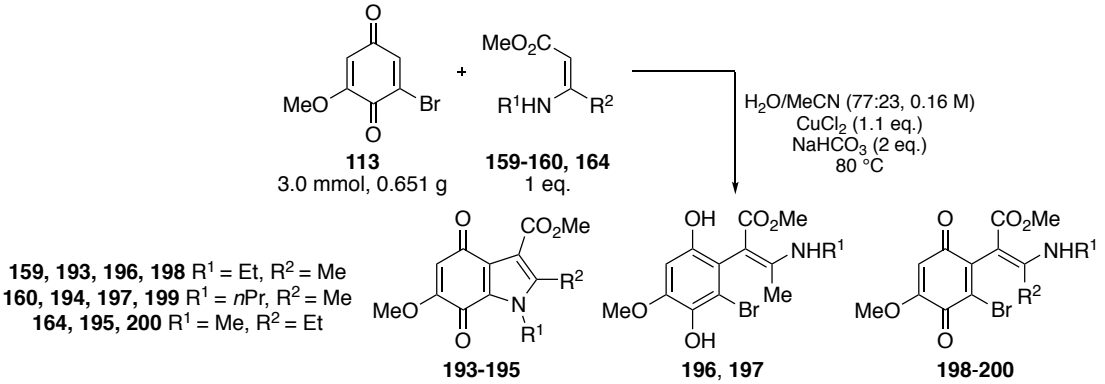
Table 3.22 Comparison between two routes to prepare the indolequinone precursor **168**.

	Previous Method[102]	**This Method**
Enamine 158 (eq.)	4	1.1
Cu(II) (eq.)	$Cu(OAc)_2•H_2O$ (1.5)	$CuCl_2$ (0.5)
Base (eq.)	K_2CO_3 (3)	$NaHCO_3$ (2)
Solvent	MeCN	water/MeCN
Time (min)	300	5 - 15
Purification	Column chromatography	n/a
Yield (%)[a]	49	61

a) 0.50 mmol scale (previous method) vs. 0.25 mmol scale (this method).

3.7.2 FROM 2-BROMO-6-METHOXY-1,4-BENZOQUINONE

The reaction of 2-bromo-6-methoxy-1,4-benzoquinone **113** (3.0 mmol) with three different crotonates **159**, **160** and **164** was carried out in 77:23 ratio water/acetonitrile and in the presence of 1.1 eq. of copper(II) chloride (**Scheme 3.46** and **Table 3.23**).



Scheme 3.46 Initial screening of conditions for the reaction of **113** and enamines in water/MeCN**.**

Table 3.23 Effect of substrate scope on the reaction of **113** and enamines in water/MeCN.

Entry	Enamine	Enamine		Time (min)	Recovery yield (%)[a]	Ratio[b]		
		R^1	R^2			IQ	HQ	Q
1	**159**	Et	Me	15	66	47	35	8
3	**160**	*n*Pr	Me	11	74	63	0	37
4	**160**	*n*Pr	Me	20	64	50	47	3
5	**164**	Me	Et	13	26	71	0	29

a) Based on 100% being 100% recovery of indolequinone;
b) Calculated based on ^{1}H NMR integrations (**IQ** = indolequinone, **HQ** = hydroquinone, **Q** = quinone)

Other reactions were tried between **113** and enamines in water/MeCN and **Table 3.23** only reviews some examples. The presented data suggest that the reaction of 2-bromo-6-methoxy-1,4-benzoquinone **113** with enamines bearing R^1 = Me and $R^2 \neq$ Me behave differently. One reason might be that the enamines are more prone to hydrolysis. From visual experimental evidence, oily reaction mixtures formed when these enamines were reacted. Also, using 1.1 eq. of copper(II) chloride might not be enough to mediate these reactions. The optimisation of this reaction was not further pursued, in the interest of time.

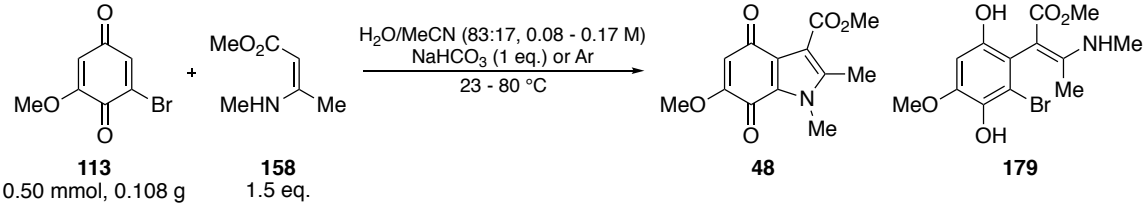
To conclude, the substrates revealed high sensitivity for the reaction conditions, which can be proved based on the several scaffolds generated. On the whole, the lack of selectivity of the reactions demonstrated was the major challenge in the optimisation of the synthetic protocols. With respect to the isolation and purification of the product, the use of laborious and wasteful

purification by chromatography was avoided whenever possible. The products were isolated by filtration of the reaction mixture and washing with water and methanol and, in some situations, acetonitrile. The evaluation of the overall purity degree of the isolated solid relied exclusively on the ^{1}H NMR analysis of the sample solution.

3.8 Optimisation screening for the synthesis of 1,4-hydroquinone 179

Taking into consideration the similar outcomes in the preliminary studies on the substrate scope for the reaction of 2-bromo-6-methoxy-1,4-benzoquinone **113** with different amino-crotonates, we aimed at the isolation of the major by-product **179**. This would contribute to a better understanding of the synthetic pathway for this reaction and might provide an alternative intermediate to get the desired product, at least for this regioisomer.

The selective synthesis of hydroquinone **179** was envisaged. Therefore, the standard conditions were used, in which 2-bromo-6-methoxy-1,4-benzoquinone **113** was reacted with 1.5 eq. of enamine **158** (**Scheme 3.47** and **Table 3.24**).



113 0.50 mmol, 0.108 g — **158** 1.5 eq. — **48** — **179**

Scheme 3.47 Optimisation studies for the synthesis of **179**.

Table 3.24 Optimisation studies for the synthesis of **179**.

Entry	C_{sol} (M)	Additive (eq.)	Temperature (°C)	Time (min)	Recovery yield (%)[a]	Ratio[b] 48	Ratio[b] 179
1	0.17	$NaHCO_3$ (1)	23	2	70	11	89
2	0.17	$NaHCO_3$ (1)	50	1	84	12	88
3[c]	0.08	Ar	23	<1	72	10	90
4	0.08	Ar	23	<1	74	0	100
5	0.12	Ar	23	<1	80	0	100
6	0.12	Ar	23	2	29	0	100
7	0.1	Ar	50	4	73	11	89
8	0.1	-	23	7	77	14	86
9	0.1	-	80	7	81	17	83

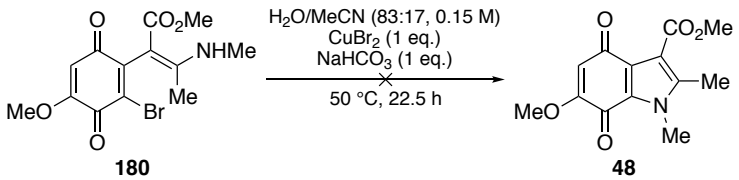
a) Based on 100% being 100% recovery of hydroquinone **179**;
b) Calculated based on ^{1}H NMR integrations; c) After sonication.

The reaction was carried out in the presence of sodium hydrogen carbonate at rt and 50 °C (entries 1 and 2) and gave the product as a mixture of **179**/**48**, in 81:10 and 73:10 ratios, respectively. In entry 3, after combining **113** and **158**, the reaction was briefly sonicated (~ 1 min) and then it was stirred at rt. In this reaction, the hydroquinone **179** was isolated in 72% yield and indolequinone **48** in 8% yield, after sequential filtration of two crops. In the best set of conditions found, the reaction proceeded under an argon atmosphere in less than one minute, at room temperature. The aim was achieved and 1,4-hydroquinone **179** was isolated in good yields when 0.50 mmol (entry 4) and 1.50 mmol (entry 5) of **113** were used. Prolonging the reaction time to 2 min (entry 6) led to a drop of the yield of **179** (29%). When the reaction was performed in 80:20 ratio water/acetonitrile, at 50 °C, under an argon atmosphere led to the isolation of a mixture of **179**/**48** (85:15) (entry 7). Finally, when the reaction took place at rt or at 80 °C, under air, the product was again isolated as mixtures of **179** and **48**, in variable ratios (entries 8 and 9). The best set of conditions to prepare 1,4-hydroquinone **179** is presented in entry 5, where 1.50 mmol of 2-bromo-6-methoxy-1,4-benzoquinone **113** was reacted with 1.5 eq. enamine **158**, in water/acetonitrile (83:17) for less than 1 min, at room temperature, under argon atmosphere.

3.9 Other reactions

With a few by-products in hand, from the condensation of the enamine **158** with the 2-bromo-6-methoxy-1,4-benzoquinone **113**, some reactions were attempted in order to synthesise indolequinone **48** via alternative routes.

One example was the open quinone structure **180**, that resulted from the Michael addition of the aminocrotonate **158** to **113**. The cyclisation of this scaffold to give the corresponding indolequinone **48** was attempted using similar conditions to those from the direct condensation of the amino crotonate **158** and **113** to give **48**. The reaction was carried out in water/acetonitrile system, in the presence of sodium hydrogen carbonate and copper(II) bromide to give a yellow-purple solid, identified as a mixture of **180/48** in 80:20 ratio and a recovery yield of 85% (based on 100% being 100% recovery of product **48**) (**Scheme 3.48**).

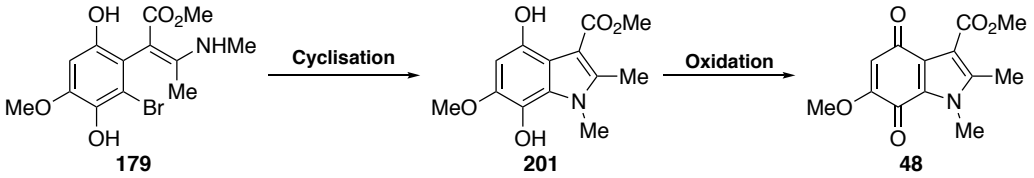


180
0.180 mmol, 62.0 mg

48

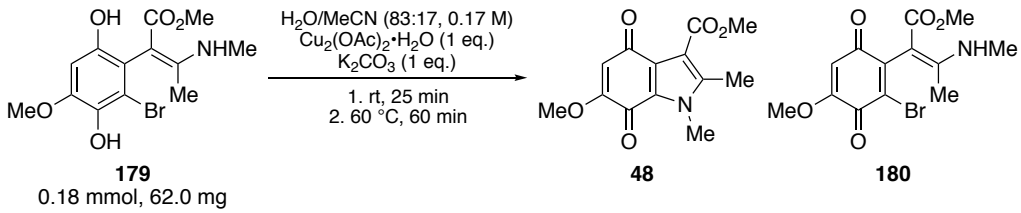
Scheme 3.48 Attempt to cyclise **180** into **48**.

To seize the opportunity of having developed a procedure to synthesise **179** in good yield (**Table 3.24**) it was envisaged the cyclisation of this structure to the corresponding indole **201** as illustrated in **Scheme 3.49**. Then, this intermediate could ultimately be oxidised to give the indolequinone **48**.



Scheme 3.49 Alternative route designed to access **48**.

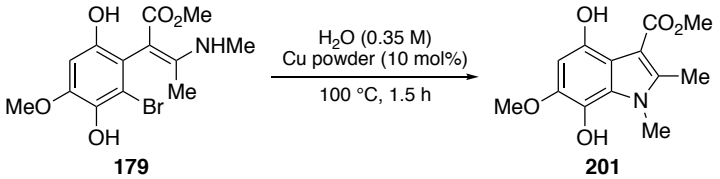
The cyclisation of the hydroquinone **179** was attempted in the presence of copper(II) acetate and base, at rt for 25 min followed by heating at 60 °C for 1 h (**Scheme 3.50**). The product was isolated as a mixture of **180**/**48**, with a 38% recovery yield (based on 100% being 100% recovery of product **48**) and in a 37:10 ratio.



Scheme 3.50 Attempt to cyclise **179** into **48.**

Wei and co-workers described a copper powder mediated procedure of an Ullmann amination of aryl halides with aqueous methylamine in water and under ligand-free conditions at 100 °C to give *N*-arylamines in good to excellent yields.[137] This would be a good approach for the cyclisation of intermediate **179** attending to the use of a catalytic quantity of an inexpensive metal as copper. Furthermore, the reaction would be carried out in water, an environmentally preferred solvent. Based on this assumption, the reaction of 2-bromo-6-methoxy-1,4-hydroquinone **179** was attempted. However, after 1.5 h the reaction did not reach completion (**Scheme 3.51**).

From the ^{1}H NMR spectroscopic analysis of the crude material, it was possible to identify the formation of the indole **201** along with other unidentified compounds, with a recovery yield of 37%, in 18:82 ratio.

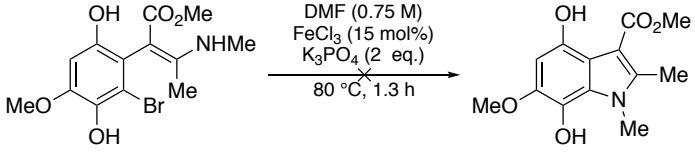


179
0.36 mmol, 0.124 g

201

Scheme 3.51 Attempt to cyclise hydroquinone **179** into the corresponding indole **201**.

Another experiment aiming at the cyclisation of **179** to give **201** or the oxidised quinone form of this structure **48** was carried out. In this reaction, cyclisation of **179** was attempted in the presence of 15 mol% iron(III) chloride and potassium phosphate tribasic (2 eq.) in DMF, at 80 °C (**Scheme 3.52**). The product was isolated as a brown thick oil and a complex mixture of unidentified products by analysis of the ^{1}H NMR spectrum.

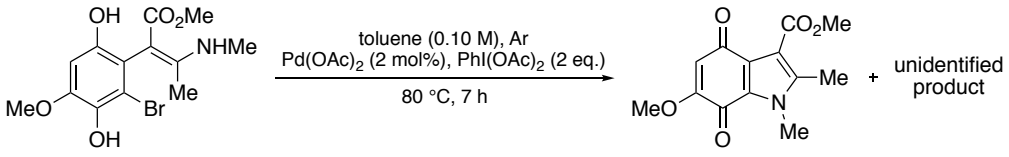


179
0.73 mmol, 0.253 g

201

Scheme 3.52 Attempt to cyclise **179** into the corresponding indole **201**.

A third attempt was carried out following another protocol described in the literature,[138] where the hydroquinone **179** was reacted in the presence of palladium(II) acetate and (diacetoxyiodo)benzene, in toluene, at 80 °C (**Scheme 3.53**). The product was isolated in 127% recovery yield (based on 100% being 100% recovery of product **48**) as an orange solid, that was a mixture of **48**, an unidentified product and (diacetoxyiodo) benzene, in a 40:31:29 ratio following analysis of the ^{1}H NMR spectroscopic data.



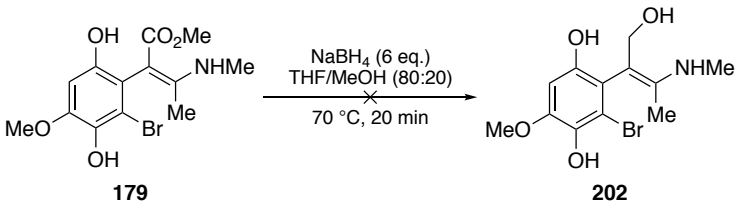
179
0.36 mmol, 0.125 g

48

Scheme 3.53 Attempt to cyclise **179** into the corresponding indolequinone **48**.

From 1,4-hydroquinone **179,** another approach considered was the reduction of the ester functionality in the first place. The result would be the breaking of the hydrogen bonding between the –NH from the enamine and the carbonyl oxygen from the ester, perhaps facilitating the subsequent cyclisation. The reduction of the ester moiety from the open 1,4-

hydroquinone motif **179** to the corresponding alcohol **202** was attempted also using the conditions reported by Costa *et al.*[139] However, the reaction was unsuccessful (**Scheme 3.54**).

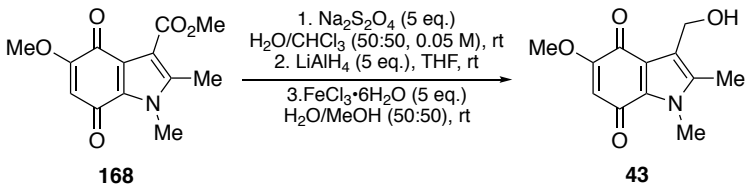


Scheme 3.54 Attempt to reduce ester **179** to the corresponding alcohol **202**.

3.10 Synthesis of alcohols

After the prolonged optimisation studies on the syntheses of indolequinones **168** and **48**, it was time to move further along the synthetic sequence to access the alcohol derivatives. Therefore, the next step was the reduction of the carbonyl functionality (**Scheme 3.12**).

The original route to obtain such scaffolds involved the reduction of the quinone moiety into the corresponding hydroquinone, reduction of the carbonyl functional group, and re-oxidation. Sodium dithionite is a commonly used reagent that facilitates the mild reduction of 1,4-benzoquinones to 1,4-hydroquinones. Reduction of the carbonyl group used classical reducing agents such as lithium aluminium hydride or sodium borohydride. The third step involved re-oxidation of the hydroquinone back to the quinone form. This was traditionally carried out by treatment of the hydroquinone with a large excess of iron(III) chloride (**Scheme 3.55**).



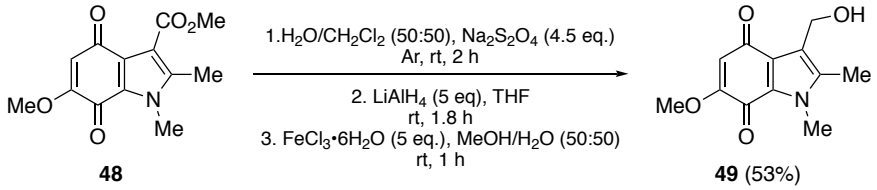
Scheme 3.55 Traditional route to synthesise alcohol **43**.

Unfortunately, these synthetic routes are not ideal from a sustainable point of view. All of the reagents are used in super-stoichiometric quantities. Apart from the use of solvents, the higher E-factors in the fine chemical and pharmaceutical industries are a result of the extensive use of classical stoichiometric reagents instead of catalysts.[140] As a result, a great amount of by-products are generated as waste. It is important to look for routes that use preferably catalytic reducing agents and avoiding the oxidation-reduction-oxidation sequence whenever possible would reduce the number of steps to obtain the indolequinone. Consequently, the overall

efficiency of the reaction should improve. To start with a proof of concept, the reduction of the ester moiety present in the indolequinones was carried following the traditional route.

3.10.1 FROM 6-METHOXY INDOLEQUINONE

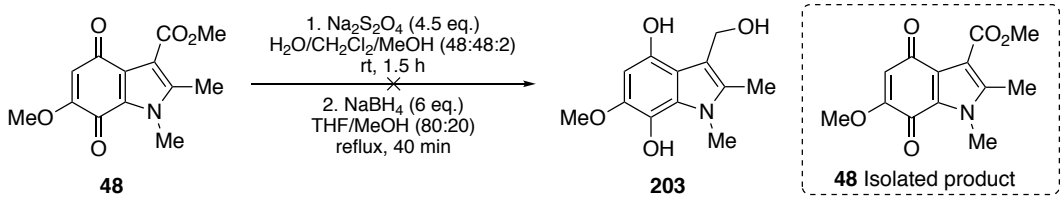
The reaction was carried out without isolation of the hydroquinone and the expected product **49** was obtained in 53% yield and identified from the analysis of ^{1}H NMR spectrum of the crude material (**Scheme 3.56**). Nonetheless, the purification of this product was not successful.



Scheme 3.56 Reduction of the ester **48** to the corresponding alcohol **49**.

Considering the hazards associated with the use of lithium aluminium hydride, the aim was to find a procedure suitable to convert the isolated 6-methoxy hydroquinone-ester into the corresponding 6-methoxy hydroquinone-alcohol making use of less harsh conditions.

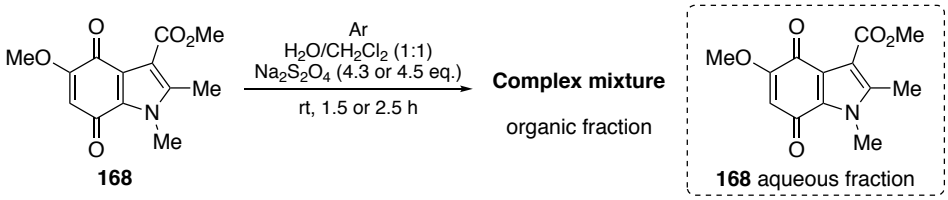
Costa *et al.* reported the reduction of several aromatic esters to their corresponding alcohols in a sodium borohydride-methanol system.[139] The products were isolated in good to excellent yields. This represents a milder and simpler approach for one-pot reduction of aromatic esters and also presents a good perspective for use in reduction of heteroaromatic esters. Following the described protocol, two reactions were tested. The reaction was carried out in the conditions above mentioned for the reduction of the quinone functionality. After that, the crude material was heated under reflux, in the presence of 6 eq. of sodium borohydride and methanol (**Scheme 3.57**). The isolated product was the starting material **48** instead of the hydroquinone-alcohol moiety **203**.



Scheme 3.57 Attempt to convert indolequinone **48** into the corresponding hydroquinone **203**.

3.10.2 FROM 5-METHOXY INDOLEQUINONE

With regards to 5-methoxy indolequinone **168**, the first step did not proceed at all. The reaction was attempted with the same conditions as those used for the synthesis of the 6-methoxy regioisomer **48** (**Scheme 3.58**) but it failed. The ^{1}H NMR analysis of the crude material, after extraction of the reaction mixture with dichloromethane, revealed the presence of a complex mixture of unidentified products in the organic fraction, and the starting material **168**, in the aqueous fraction, as the major component. The reaction was attempted three times, in these conditions, yet with no success.

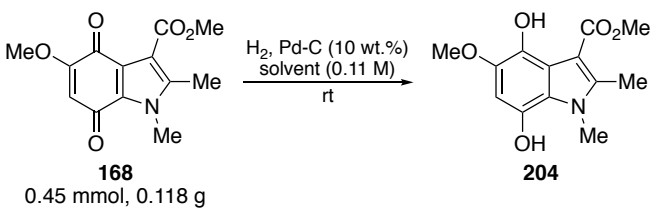


Scheme 3.58 Attempt to convert indolequinone **168** into the corresponding hydroquinone.

The reaction in which the quinone **168** was reduced to the corresponding hydroquinone **138**, was then subjected to a brief optimisation process. The previously reduction method used super-stoichiometric quantities of sodium dithionite to give the hydroquinone, which would, in turn, be reduced to the corresponding alcohol with lithium aluminium hydride and oxidised to the indolequinone, with 50% overall yield. In search of a less wasteful reductive method, a reaction screening was carried out.

Using catalytic hydrogenation to access the hydroquinone from the quinone, instead of the traditional method would represent an advance towards a less wasteful synthetic route to obtain the targeted alcohol derivatives. Therefore, with this objective in mind, a brief solvent screening for catalytic hydrogenation was envisaged. The reactions occurred in a palladium on carbon (10 wt.%) system at rt, and the use of more environmentally benign solvents was studied (**Scheme 3.59** and **Table 2.21**).

Two-step approach



Scheme 3.59 Hydrogenation of **168** into the corresponding hydroquinone **204**.

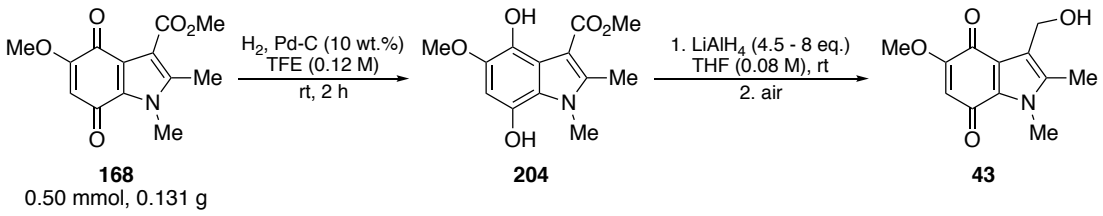
Table 3.25 Solvent screening for the hydrogenation of **168**.

Entry	Solvent	Time (h)	Recovery yield (%)[a]	Ratio[b]	
				168	204
1	TFE	0.9	45	0	100
2	TFE	1.1	99	23	77
3	EtOH	19.5	90	100	0
4	EtOAc	19.5	97	100	0
5	MeOH	0.6	92	23	77

a) Based on 100% being 100% recovery of hydroquinone **204**;
b) Calculated based on ^{1}H NMR integrations.

In summary, from **Table 3.25** it is possible to verify that using TFE as a solvent gave the best results, yielding the hydroquinone **204** in 45% yield. After analysing the effect of starting the reaction with the hydrogenation of the indolequinone **168** into the corresponding hydroquinone **204**, a two-step process was studied.

The catalytic hydrogenation of **168** followed by the reduction of the ester functionality, in a two-step process is described in **Scheme 3.60**. In the hydrogenation both methanol and TFE were tried and for the ester reduction, a range of reductants such as lithium aluminium hydride, lithium borohydride, lithium triethylborohydride and $(EtO)_2MeSiH$ were tested and THF was the selected solvent in most cases (**Table 3.26**).



168
0.50 mmol, 0.131 g

204

43

Scheme 3.60 Ester reduction of **204** to **43**.

Table 3.26 Optimisation studies for the ester reduction for synthesis of **43**.

Entry	Eq.$_{LiAlH4}$	Time (min)	Isolated yield (%)[a]	Ratio[b]	
				168	43
1	4.5	100	30	0	100
2	7.6	115	101	21	79
3	8	180	84	14	86

a) Based on 100% being 100% recovery of alcohol **43**;
b) Calculated based on ^{1}H NMR integrations.

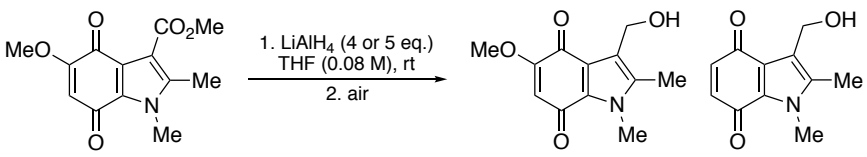
The hydrogenation of **168** into the corresponding hydroquinone **204** is carried out for 2 h, at rt, in the presence of TFE. Followed by reduction of the ester, using 5 eq. of lithium aluminium hydride in THF and oxidation of the hydroquinone to the quinone **43** under air. On the whole, the reactions did not give full conversion. The starting material **168** was usually recovered in this process. The final crude products were normally isolated as mixtures of **168** and the corresponding alcohol **43**. It can be either because, in the isolation of the hydroquinone **204**, part of this intermediate would rapidly re-oxidise back to the corresponding quinone **168**. Elution with ethyl acetate/dichloromethane led to the isolation of the desired alcohol **43**.

Other set of conditions were tried. For instance, the hydrogenation of **168**, was carried out in methanol, for 3 h, followed by the ester reduction with 4.8 eq. lithium triethylborohydride, in THF, for 2 h, to give **168** and trace quantities of unidentified products. Another attempt was conducted following a protocol described in the literature.[141] The hydrogenation of **168**, in TFE for 2 h, followed by the ester reduction with 3 eq. $(EtO)_2MeSiH$ and 10 mol% of $Zn(OAc)_2$ at 65 °C, for more than 18 h, gave a complex mixture of products by TLC analysis. The second attempt to use lithium triethylborohydride as a reducing agent for the ester functionality (following hydrogenation in TFE) also failed and starting material **168** was recovered in 77% yield. Finally, the hydrogenation was tried under pressure (10 bar), for 24 h, but it only gave the alcohol **43**, in 2% yield.

With these results in hand, a single step process to convert indolequinone **168** into the corresponding alcohol **43** will be discussed next.

One-step approach

In this part of the experimental work, the reactions were usually carried out at room temperature, and different reducing agents were studied (**Scheme 3.61** and **Table 3.27**).



168
0.40 mmol, 0.105 g

43 **205**

Scheme 3.61 One-step conversion of **168** into **43**.

Table 3.27 Optimisation studies for the one-step synthesis of alcohol **43**.

Entry	Eq.$_{LiAlH4}$	Time (h)	Recovery yield (%)[a]	Ratio[b]		
				168	43	205
1	4	2	22	0	100	0
2	4	3	19	0	100	0
3	5	3	42	14	72	14
4	5	15	30	13	87	0

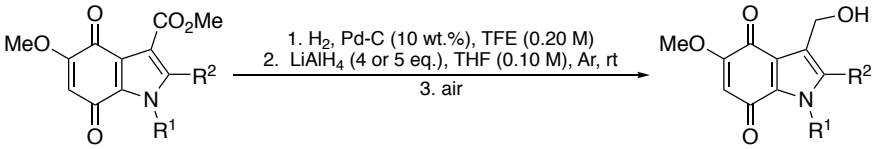
a) Based on 100% being 100% recovery of alcohol **43**;
b) Calculated based on ^{1}H NMR integrations.

The reduction of indolequinone **168** in the presence of lithium aluminium hydride (4 or 5 eq.) in THF (entries 1-4) gave the corresponding alcohol **43** in yields ranging from 19-30%. There was always evidence of the presence of starting material by the end of the reactions, either by isolation of **168** or by TLC analysis of the crude product. Entry 4 gave the best set of reaction conditions, with a comparable yield (30%) to that obtained in a two-step process (**Table 3.26**, entry 11). In this reaction, product **205** was also formed in 6% yield, which results in the loss of the methoxy substituent in the quinone ring. Furthermore, it was possible to recover indolequinone **168** in 6% yield.

A range of other alternatives were also tried. For example, the reaction was also carried out with 4 eq. of lithium aluminium hydride in diethyl ether, at reflux, and there was evidence of trace quantities of the alcohol **43** and the ester **168** as the major component of the reaction mixture, after 5.3 h. One reaction was also attempted in the presence of lithium borohydride (4 eq.) and led to the isolation of **43** (25%) and recovery of **168** (27%). Using the reduction system sodium borohydride in THF/methanol (80:20), at 65 °C, previously reported in the literature, allowed the recovery of **168** in 54% yield. Performing the reduction of indolequinone **168** with 5.2 eq. of lithium triethylborohydride, in THF, for 30 min, gave a complex mixture of products by ^{1}H NMR spectrum analysis of the crude material. DIBAL was also selected as the reducing agent for two reactions. A large excess (88 eq.) of the reducing agent were used and the reactions was carried out in dichloromethane. One of the reactions was carried for 3.2 h under reflux and another one at rt, for 1.2 h. In both cases, they yielded complex mixtures by TLC, with multiple product formation. Another reaction was also attempted with 1.1 eq. of phenylsilane and KOH (12 mol%), in acetonitrile, following a procedure from the literature,[142] and gave an insoluble product in deuterated solvents that was not possible to identify. Finally, the reduction of **168** was carried out in the presence of 1.6 eq. of ammonium formate, based on work developed by Pande.[143] The reaction took place in a palladium-carbon system and methanol, under an argon atmosphere, for almost 26 h and the starting material **168** was isolated in 66% yield.

3.10.3 SUBSTRATE SCOPE

With the optimised set of conditions for the conversion of the indolequinone **168** into the corresponding alcohol **43** as a guide, the aim at this stage was to extend and adapt the conditions to broaden the substrate scope of the reaction. TFE was chosen as the solvent to be used for the hydrogenation step and lithium aluminium hydride as the reducing agent for the ester transformation into the alcohol. The reactions were carried out at room temperature and in the ester reduction under an argon atmosphere (**Scheme 3.62** and **Table 3.28**).



186 R^1 = Et, R^2 = Me
177 R^1 = Me, R^2 = Et
1.0 mmol, 0.277 g

206 R^1 = Et, R^2 = Me
207 R^1 = Me, R^2 = Et

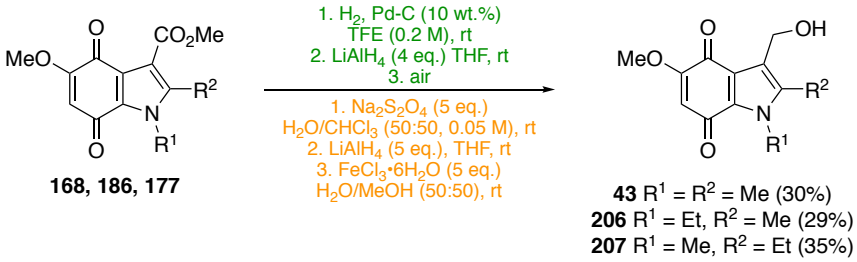
Scheme 3.62 Substrate scope for the two-step conversion of indolequinones into the corresponding alcohols.

Table 3.28 Substrate scope for the two-step synthesis of alcohols.

Entry	IQ	IQ		Hydrogenation	Ester reduction		Isolated yield (%)	
		R^1	R^2	Time (h)	$Eq._{reductant}$	Time (h)	Ester	Alcohol
1	**186**	Et	Me	4	4	19	0	29
2	**186**	Et	Me	4	5	17	4	30
3	**177**	Me	Et	6	4	17	0	35
4	**177**	Me	Et	4	5	17	0	28

A range of other reactions were attempted for substrates **177** and **186** nonetheless, in **Table 3.28** only two examples for each substrate are presented. Succinctly, alcohol derivatives **206** and **207** were accessed through catalytic hydrogenation of esters **177** and **186** followed by reduction with $LiAlH_4$. In addition, the previously reported re-oxidation step stoichiometric iron(III) chloride was eliminated from both reactions. The work-up conditions for the quenching of the lithium aluminium hydride reaction allowed the re-oxidation of the hydroquinone to the corresponding quinone in the presence of air.

Below is presented a brief comparison of the protocol used in this project and the process used previously (**Scheme 3.63** and **Table 3.29**).



Scheme 3.63 Conversion of esters into the corresponding primary alcohols.

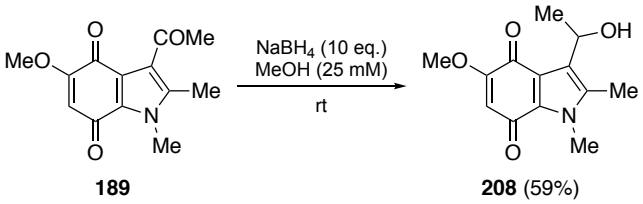
Table 3.29 Comparison between two routes to obtaining alcohols from indolequinones.

		Previous Method[a]	This Method
Q-HQ Red.	**Reducing system**	$Na_2S_2O_4$ (5 eq.)	H_2, Pd-C (10 wt.%)
	Solvent	$H_2O/CHCl_3$	TFE
Ester red.	**$LiAlH_4$ (eq.)**	5	4 or 5
	Solvent	THF	THF
HQ-Q oxid.	**Oxidative system**	$FeCl_3•6H_2O$ (5 eq.)	air
	Solvent	H_2O/MeOH	n/a

a) Used to prepare 3-hydroxymethyl-6-methoxy-1,2-dimethyl-1*H*-indole-4,7-dione **49** in 49% yield.

Despite not using stoichiometric quantities of sodium dithionite as reducing agent, the use of catalytic hydrogenation has also intrinsic disadvantages. First, it requires a metal catalyst. In this case, palladium, an expensive and rare metal was used. Second, hydrogenation involves the hazards of the use of hydrogen under pressure. Besides, the catalyst can be rendered more pyrophoric by the adsorption of hydrogen.

For ketone **189**, the reduction was carried out, in one-step, in the presence of sodium borohydride in methanol at rt, to give the secondary alcohol **208** in 59% yield (**Scheme 3.64**).



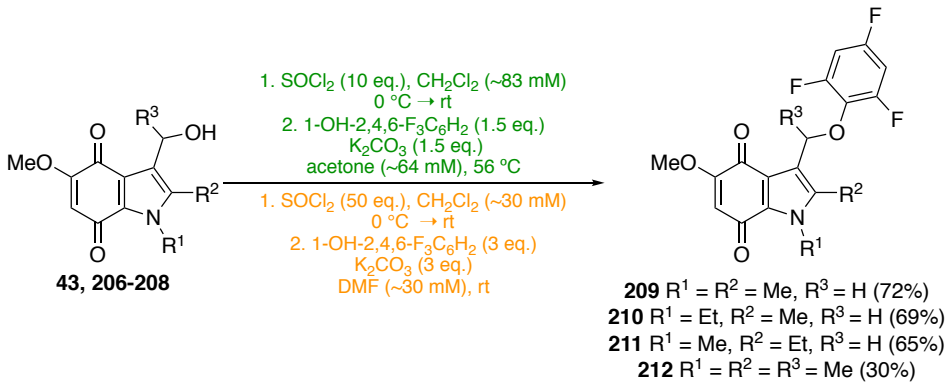
Scheme 3.64 Conversion of the ketone **189** into the corresponding secondary alcohol **208**.

The reaction was also attempted with 6 eq. of sodium borohydride, however the starting material **189** was recovered in 18% yield and the secondary alcohol **208** was formed in 38% yield. It should be noted that this reaction was not further optimised.

3.11 Synthesis of 2,4,6-trifluoro phenyl ethers

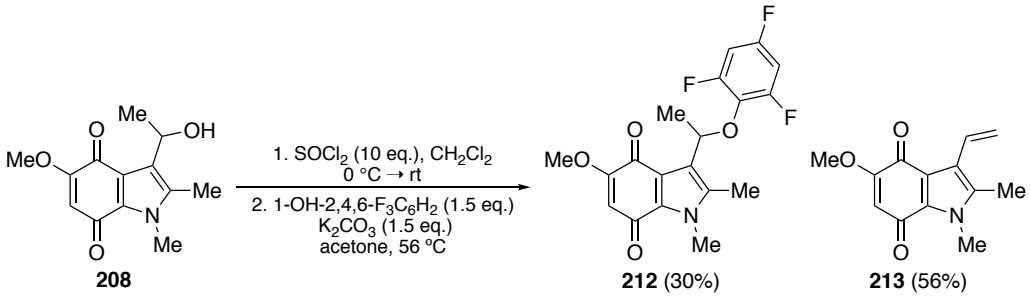
The next step in the synthetic sequence to obtain 5-aminoindolequinones (**Scheme 3.12**) was the transformation of the alcohol derivatives **43** and **206-208** into the corresponding 2,4,6-trifluoro phenyl ethers **209-212**

Initially, the alcohol precursors were transformed into their alkyl chlorides in the presence of 10 eq. of thionyl chloride (**Scheme 3.65**).This represents a reduced number of eq. in comparison to the previously used protocol that used 50 eq. of thionyl chloride.[144] The next reaction was the introduction of the 2,4,6-trifluorophenol that was carried out in acetone and in the presence of 1.5 eq. of potassium carbonate and generated the ether substituted compounds **209-212** in moderate to good yields (30-72%). The former method used DMF a less preferred solvent in several solvent guides,[145,146] and 3 eq. of the base. Again, no further optimisation attempts were carried out.



Scheme 3.65 Conversion of alcohols into the corresponding aryl ethers **209-212**.

When the reaction was carried out for the secondary alcohol, a side product **232** was also isolated (**Scheme 3.66**).

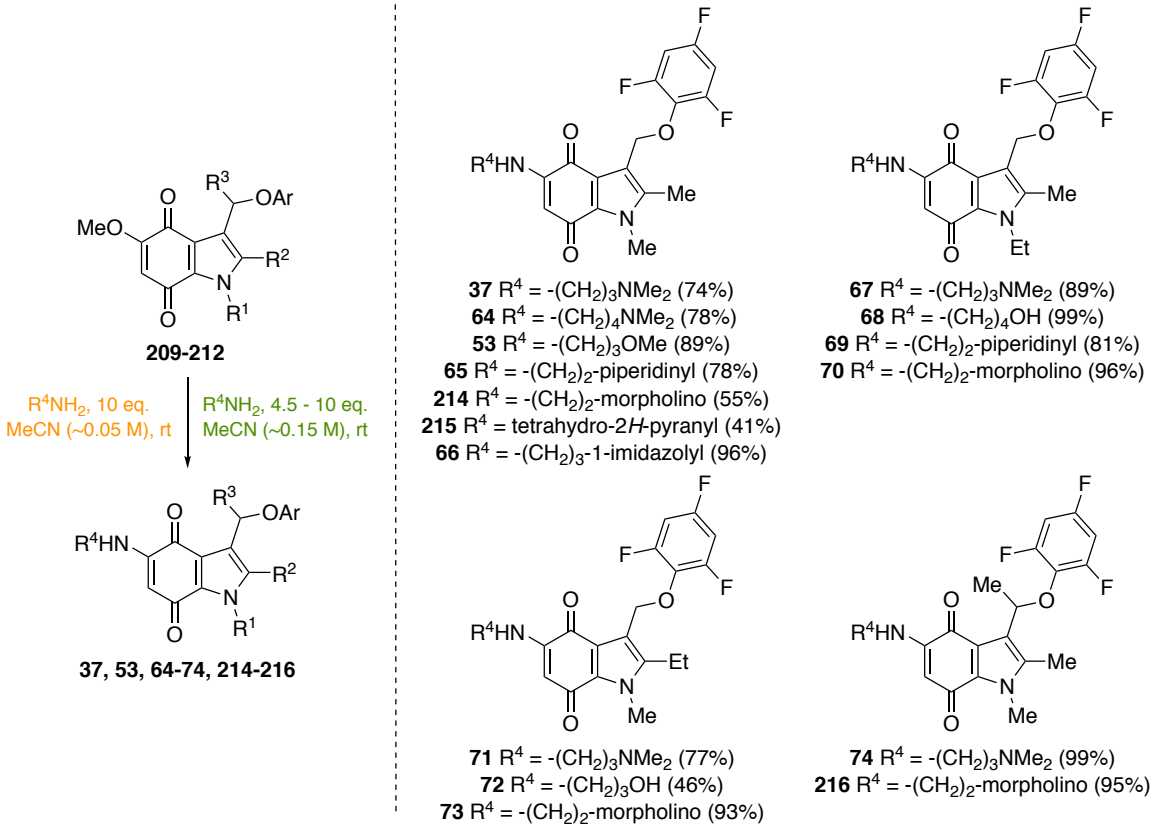


Scheme 3.66 Synthesis of **212** and alkene **213**.

This results from the elimination of either water or hydrochloric acid from the corresponding alcohol or alkyl chloride intermediate species to give the terminal alkene and this might explain the lower yield for the aryl ether **212** formation, for this derivative. On a closing note, compound **213** proved to be unstable in deuterated chloroform solution.

3.12 Synthesis of 5-aminoindolequinones

The last step in the reaction sequence to prepare 5-aminoindolequinones (**Scheme 3.12**) consisted of the functionalisation of the C5 position. This was achieved through the reaction of aryl ethers **209-212,** with primary amines (**Scheme 3.67**).



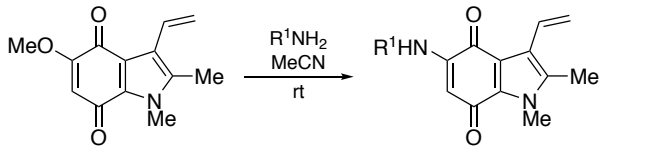
Scheme 3.67 Synthesis of the 5-aminoindolequinones.

The displacement of the methoxy group by the amine nucleophiles gave a series of 5-amino-substituted indolequinones **37,53, 64-74** and **214-216**.

Overall, the reactions were performed using a smaller excess of the amine and under more concentrated solutions and therefore less solvent (acetonitrile) was required. No further attempts to increase the concentration the reaction were made.

The improved approach was used to prepare a range of new compounds, in moderate to excellent yields, to further investigate the biological activity of 5-aminoindolequinones against NQO2.

Having the by-product **213** in hand, it was also reacted with three different primary amines to access the 5-amino substituted alkene derivatives **217-219**, in excellent yields (**Scheme 3.68**). As mentioned beforehand, these compounds were also quite unstable in deuterated chloroform solution.



213

217 $R^1 = (CH_2)_3NMe_2$ (88%)
218 $R^1 = (CH_2)_4NH_2$ (99%)
219 $R^1 = (CH_2)_2$morpholino (95%)

Scheme 3.68 Synthesis of the 5-amino alkenes **217-219**.

3.13 Conclusions

This Chapter has discussed the efforts towards a more sustainable synthesis of 5-aminoindolequinones, through the optimisation of each synthetic step in the reaction sequence.

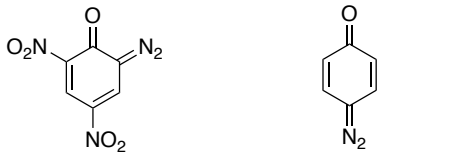
Section 3.5 presented the successful preparation of indolequinone precursors, enamines and 2-bromo-1,4-benzoquinones in good to excellent yields. In **Section 3.6** a range of indolequinones was accessed from the indolequinone precursors, through a copper(II)-mediated process in the presence of a more environmentally benign solvent system in which water was used as the main solvent. There are no precedents in the literature for the synthesis of these derivatives in water. A brief substrate scope combining the 2-bromo-methoxy-1,4-benzoquinones with a range of enamines was carried out in **Section 3.7**. When 2-bromo-6-methoxy-1,4-benzoquinone **113** was used, the products were in most cases isolated as mixtures or with a high degree of impurities. The reaction with 2-bromo-5-methoxy-1,4-benzoquinone **156** led to the isolation of seven indolequinones, in low to moderate yields. In **Section 3.8**, the brief optimisation study for the preparation of hydroquinone **179** allowed the isolation of this derivative in good yield. The preparation of alcohols was also investigated and attempts to optimise the conversion of the ester functionality into the corresponding primary alcohol were carried out, mostly by moving away from the use of super-stoichiometric reagents, such as sodium dithionite and iron(III) chloride, and replacing them with hydrogen in

a catalytic palladium-carbon system and air, respectively. Four alcohol derivatives were prepared in low to moderate yields (**Section 3.9**). The next stage was the transformation of these derivatives into the corresponding aryl ethers. Here, the reaction was carried out in the presence of a reduced number of equivalents of thionyl chloride and dimethylformamide was replaced by acetone, a more environmentally benign solvent (**Section 3.10**). Sixteen 5-amino substituted indolequinones were prepared in **Section 3.11** from the aryl ethers precursors, in a more concentrated reaction mixture and in the presence of a reduced excess of the primary amines. Finally, in **Section 3.12** the reactivity of some by-products obtained from the reaction between 2-bromo-6-methoxy-1,4-benzoquinone **113** and enamine **158** in Section 3.6 was briefly analysed however the attempts to cyclise the open structures proved unsuccessful.

4 Application of quinone diazides in synthesis

4.1 Quinone diazides

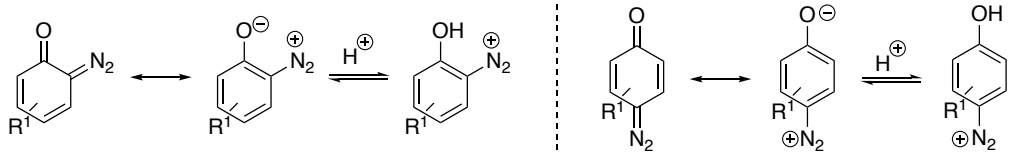
Diazoquinones, often referred to as quinone diazides or arene diazo oxides, are more correctly named as diazocyclohexadienones, although this nomenclature is seldom used. Historically, quinone diazides are among the longest-known compounds and were reviewed in 2006.[147] The first example described was dinitrobenzo-1,2-quinone diazide (DDNP, diazol, or dinol) **220**, an initiating explosive, in 1858.[147–149] These scaffolds are also formed in the gastrointestinal tract by the action of phenol with nitrite [150] and have also been detected in biological materials, for instance, the parent compound, 4-diazocyclohexa-2,5-dien-1-one **221** has been detected in the fruit bodies of the mushroom genus *Agaricus* (**Scheme 4.1**).[151,152]



220 Diazodinitrophenol **221** 4-Diazocyclohexa-2,5-dien-1-one

Scheme 4.1. Some examples of synthetic and natural quinone diazides.

Quinone diazides are compounds that can be classified as diazo carbonyl compounds bearing two electron-acceptor groups,[153–157] and they have intermediate reactivity between diazonium salts and aliphatic diazoketones.[158] Quinone diazides benefit from modest resonance stabilisation as indicated for both *ortho*- and *para*-isomers, **222a** and **224a**, and they are in equilibrium with the hydroxy diazonium species, **223** and **225**, respectively, if a source of protons is present (**Scheme 4.2**).[159] The resonance energy values have been estimated on the basis of thermochemical data and increment methods. For example, for the parent *para* compound **224a**, a resonance energy of 15 kJ/mol is reported, indicating the predominance of the quinoid structure.[160]



222a **222b** **223** **224a** **224b** **225**

Scheme 4.2 Electronic structures of *o*- and *p*-quinone diazides, **222a** and **224a**.

Typical spectral characteristics of quinone diazides are the IR stretching frequencies of the C=O (1620 ± 30 cm^{-1}) and the typical diazo valence vibrational band N=N (2080 ± 30 cm^{-1}) and the ^{13}C NMR shifts for the C=O (δ 180 ± 5 ppm) and the C=N groups (δ 72 ± 5 ppm).[161]

4.2 Natural products

Quinone diazides are probably best known for their involvement as a versatile intermediate in synthetic organic chemistry and only a small number occur naturally. Below are presented three examples of natural quinone diazides: SQ 30957 **226**, cremeomycin **227** and lagunamycin **228**.

4.2.1 SQ 30957

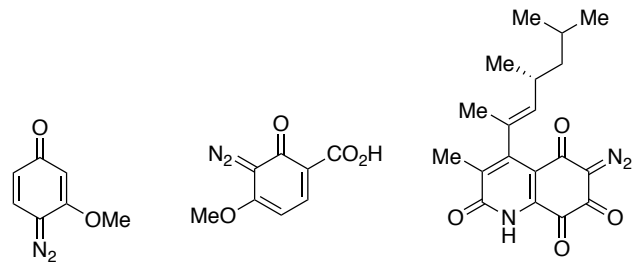
A new antibiotic isolated from *Penicillium funiculosum* was identified as 4-diazo-3-methoxy-2,5-cyclohexadien-1-one (SQ 30957) **226** (**Figure 4.1**).[162] The position of the methoxy group was confirmed by reduction of the compound with hypophosphorous acid to methoxyphenol, and also by reaction of 2-methoxybenzo-1,4-quinone with 4-toluenesulfonyl hydrazide.

4.2.2 CREMEOMYCIN

The ortho-diazoquinone, cremeomycin **227** (**Figure 4.1**) was originally described in 1967 as a photodegradable antibiotic, isolated from *Streptomyces cremeus*.[163] However, no structure was reported for the natural product, and the diazoquinone structure **227** was finally assigned by Rinehart and co-workers using X-ray crystallography almost three decades later.[164] In 2008, this was further confirmed after synthetic cremeomycin being isolated as a bright yellow solid with identical spectroscopic properties to the natural product.[165]

4.2.3 LAGUNAMYCIN

Lagunamycin **228** (**Figure 4.1**) was isolated in 1993 from a *Streptomyces* culture following a screen for potential inhibitors of the 5-lipoxygenase enzyme. This compound is a potent inhibitor of 5-lipoxygenase but shows no effect on lipid peroxidation besides it displays modest activity against Gram-positive bacteria.[166] The structure was determined as the uncommon diazo-tetraoxoquinoline **228** by a combination of chemical degradation and NMR spectroscopy.[167] Only in 2006 was the stereocentre in the side chain specified later after structure confirmation, and assignment of the stereochemistry, by total synthesis.[168]



226 SQ 30957 **227** cremeomycin **228** lagunamycin

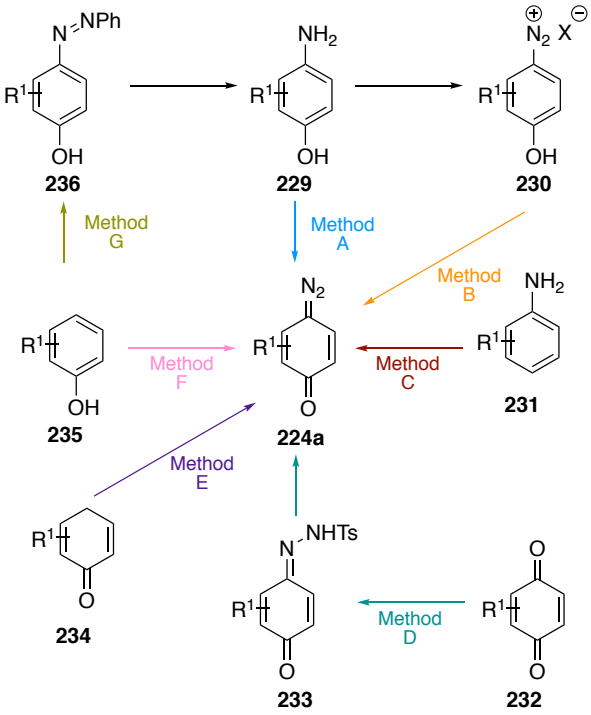
Figure 4.1 Natural quinones diazides with biological application.

4.3 General synthesis

Numerous methods have been developed, and comprehensively reviewed,[169–173] for the preparation of individual classes of diazo compounds. In this section, the synthesis of quinone diazides derived from benzoquinones as well as fused quinones such as naphthoquinones, with the diazo and the carbonyl groups in the 1,2- and 1,4-positions, respectively, are described. Typically, the resulting quinone diazides can be isolated or used *in situ* and the synthesis of quinone diazides follows six fundamental routes: 1) diazotisation of anilines (method a), which are often obtained from the corresponding nitro compound, with or without the isolation of the intermediate 4-hydroxyarenediazonium salt (method b or a, respectively), that are converted into quinone diazides by HX elimination; 2) from monofunctionalised aromatic compounds such as anilines or phenols with appropriate leaving groups (method c or f, respectively); 3) from cyclohexadienones by diazo group transfer (method e); 4) from quinones by way of intermediate hydrazone formation and base-induced elimination (method d); 5) from aromatic phenols (method g) and subsequently converted into quinone diazides via reduction to the anilines or 6) introduction of new substituents into a quinone diazide already synthesised. It should be mentioned that each of these methods requires an acid medium at one stage, and therefore the synthesis of quinone diazides is restricted to the preparation of the corresponding hydroxyaryl-diazonium salt with subsequent elimination of a molecule of acid from this salt (**Scheme 4.3**).

4.3.1 Diazotisation of amino-substituted phenols or naphthols

The most frequently used method of preparing quinone diazides is the direct introduction of the diazonium function by nitrosation of a suitable substrate, namely 2- or 4-aminophenols and the analogous benzoannulated substrates. Difficulties can be encountered in the diazotisation of aminophenols by the usual methods, because of the readiness with which these compounds are oxidised, or because of their low solubility. Different solvents can be used depending on



Scheme 4.3 General synthetic routes to access 1,4-quinone diazides.

the solubility, stability of the substrates and products towards acid, oxidation and hydrolysis, and on the convenience of product isolation. Under aqueous conditions, dilute mineral acids, such as hydrochloric or sulfuric acid, are conveniently used. As nonaqueous media for the preparation of quinone diazides, glacial acetic acid, trifluoroacetic acid, solutions of gaseous hydrochloric acid in alcohols, as well as nitrous gases have been reported. Diazotisation of aminophenols with sodium nitrite in a medium of concentrated organic acid proved to be a very convenient method, with the corresponding diazonium salts being obtained in high yields.[174–176] Nevertheless, when aminophenols are diazotised in the usual way with nitrite in hydrochloric acid solution, large amounts of oxidation products can also be obtained, depending on the substrate. Thus, diazotisation of aminonaphthols in aqueous acid gives the corresponding quinones and azo-dyes.[177] Aminophenols with electron-donating substituents in the nucleus are very strongly oxidised.[178] However, the diazotisation of unsubstituted *o*- and *p*-aminophenols in dilute sulfuric acid proceeds without complications.[179] The presence of electron-withdrawing substituents in the nucleus involves another limitation: diazotisation does not occur because of the poor solubility of these aminophenols in weakly acid solution. Regardless, aminophenols with electron-withdrawing substituents are easily diazotised by nitrosylsulfuric acid in concentrated sulfuric acid.[178,180–183]

4.3.2 MONOFUNCTIONALISED AROMATIC COMPOUNDS

From phenols

In this method, phenols or nitrosophenols are treated with nitrous acid in aqueous solution. Hydroxy aryldiazonium salts are first formed, and these are next converted into quinone diazides. Although this method has not been so widely studied, it is very promising, since it allows the preparation of quinone diazides directly from phenols, and it avoids the synthesis of aminophenols, which are not always accessible and are sometimes difficult to diazotise. This procedure applies particularly to phenols with electron donating substituents (**Scheme 4.3**, methods F and G).[184,185]

4.3.3 DIAZO GROUP TRANSFER REACTIONS

One of the best methods for preparing diazo compounds consists of the direct introduction of both nitrogen atoms in a single reaction step.[186,187] The entire azo group is transferred by a donor, generally an azide, to an acceptor (CH-acidic compound). Analogous to the azo coupling, phenolates can be treated with tosyl azide and quinone diazides are produced after elimination of tosylamide. Commonly used *p*-Tosyl azide is a shock sensitive reagent;[188,189] as a result modified reagents have been developed as alternatives (**Scheme 4.3**, method E).[190,191]

4.3.4 FORMATION OF TOSYLHYDRAZONES FROM QUINONES

The direct Bamford-Stevens approach to quinone diazides from the corresponding quinones includes the formation of monotosylhydrazones by treatment of the dicarbonyl substrates with tosylhydrazine and subsequent basic hydrolysis. Depending on the structure of the tosylhydrazone, the elimination of tolylsulfinate can occur already under the acidic conditions of the first step. In the case of unsymmetrically substituted quinones, the regioselectivity of hydrazone formation depends on the degree of steric shielding of the carbonyl groups (**Scheme 4.3**, method D).[192–196]

4.3.5 ELECTROPHILIC SUBSTITUTION OF QUINONE DIAZIDES

Due to the thermal and photochemical instability of quinone diazides, this is not a usual protocol to access these structures. One of first most promising methods reported was bromination and nitration with conservation of the quinone diazide structures. In 1881, Bohmer showed that treating *o*- and *p*-benzoquinone diazides with bromine water, gave the corresponding 2-diazo-4,6-dibromo-*o*-benzoquinone and 4-diazo-2,6-dibromo-*p*-benzo-

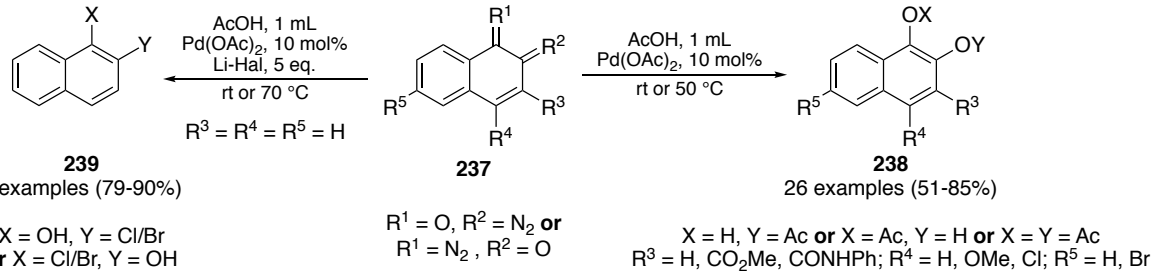
quinone.[197] The reaction with hydroxyphenyldiazonium nitrate and hydrobromic acid proceeded in the same way; a nitrate ion formed in the first stage of the process oxidises the acid to bromine, which then brominates the diazide (**Scheme 4.3**).

4.4 Reactivity

Quinone diazides possesses higher stability than the other diazo compounds, thus making them less susceptible towards metal-mediated decomposition. Consequently, forcing conditions, such as elevated temperature or reactive catalysts, are often required for the formation of metal carbenoids.[198,199] Furthermore, these highly electrophilic carbenoids are prone to side reactions such as carbene dimerisation and hydride transfer to form zwitterionic intermediates and they can react by either loss or retention of molecular nitrogen, depending on reaction conditions.[200,201] Reactions with retention of nitrogen in azo-coupling reactions are widely used in industry for the synthesis of hydroxyazo dyes.[202,203] Owing to their high reactivity, a range of applications has arisen in dissimilar fields such as polymer chemistry,[204,205] photolithography,[206] photoaffinity labelling,[207,208] and electronic and optical materials.[209–211] The 1960s to 1980s epitomised the “golden age” of quinone diazide chemistry, with a great emphasis on azo coupling, thermolysis and photolysis-promoted Wolff-type rearrangements, and decomposition of the diazo moiety to the corresponding cyclohexadienone carbene.[148,200,212–216] This final mode of reactivity presents an untapped opportunity for synthesis since these carbenes render a phenol electrophilic at its normally nucleophilic *para* or *ortho* position.

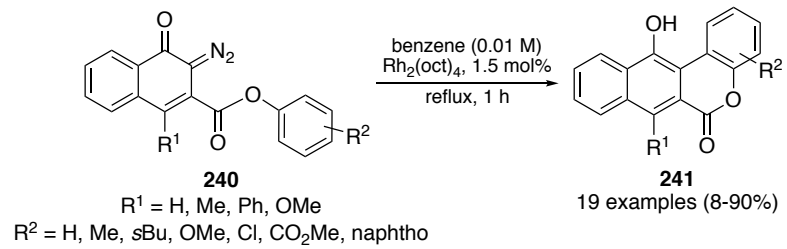
Today the catalytic decomposition of diazo compounds is of fundamental interest because it affords a reliable entry to carbene[217,218] and carbenoid chemistry.[219,220] Cyclopropanation [221] reactions that are preferentially performed in a stereospecific manner, insertion reactions into various X-H bonds, and the formation of ylides [222–224] that undergo subsequent reactions are also aspects of carbene and carbenoid chemistry that are worth mentioning. In addition, there has also been substantial progress with 1,3-dipolar cycloaddition chemistry of the intact diazo moiety with π-bonds of alkenes or alkynes.[225–227] A brief selection of examples focusing on the reactivity of quinone diazides or naphthoquinone diazides will be presented next.

Kitamura *et al.* reported a palladium(II)-mediated -OH insertion of diazonaphthoquinones **237** to acetic acid to afford 1,2-naphthalenediol monoacetates **238**, in moderate to good yields (**Scheme 4.4**).[228] The reaction proceeded at rt or at 50 °C. Carrying out the reaction with lithium halides, as chloride and bromide, gave halonaphthols **239** in good yields.



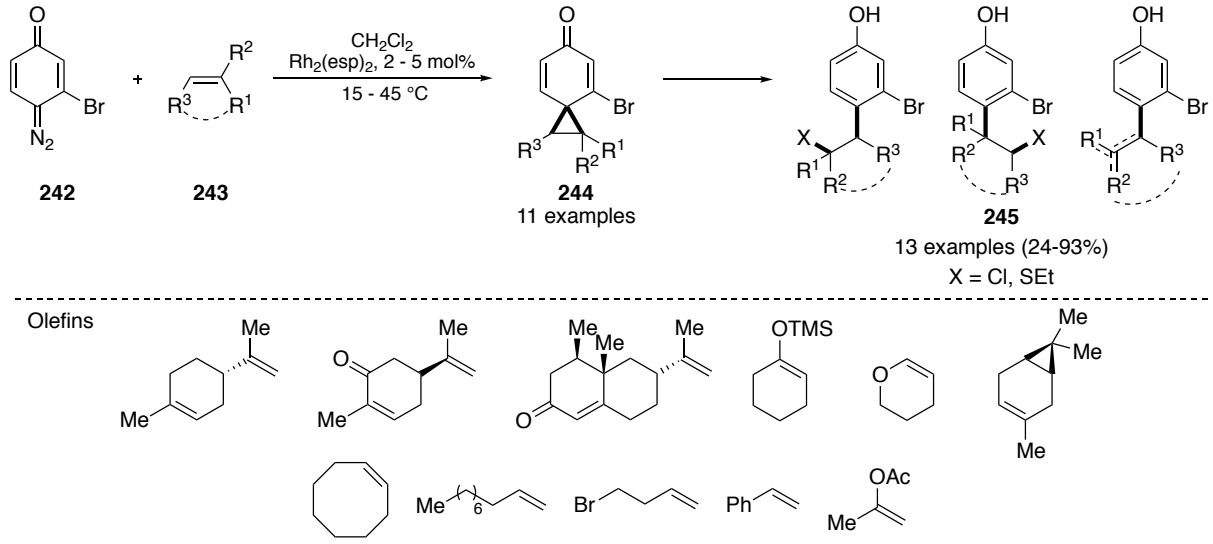
Scheme 4.4 Palladium(II)-mediated acetylation and halogenation of diazonaphthoquinones **237**.

The same authors also developed a method for the synthesis of β-phenylnaphthalene lactones **241**.[229] 3-aryloxycarbonyldiazonaphthoquinones **240** were converted to the lactones **241**, in low to excellent yields, through a Rh(II)-mediated intramolecular formal C–H insertion reaction (**Scheme 4.5**). Furthermore, this method of lactone formation was applied to the formal total synthesis of pradimicinone.



Scheme 4.5 Rh(II)-mediated synthesis of β-phenylnaphthalene lactones **241**.

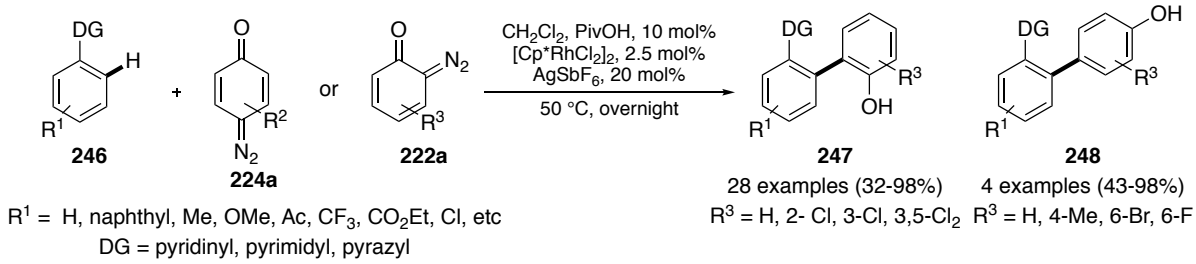
Dao and Baran reported the use of the quinone diazide **242** for olefin functionalisation, through a rhodium(II)-mediated intermolecular cyclopropanation of these diazo compounds with a variety of olefin classes (**Scheme 4.6**).[230]



Scheme 4.6 Rhodium(II)-mediated intermolecular cyclopropanation of quinone diazides.

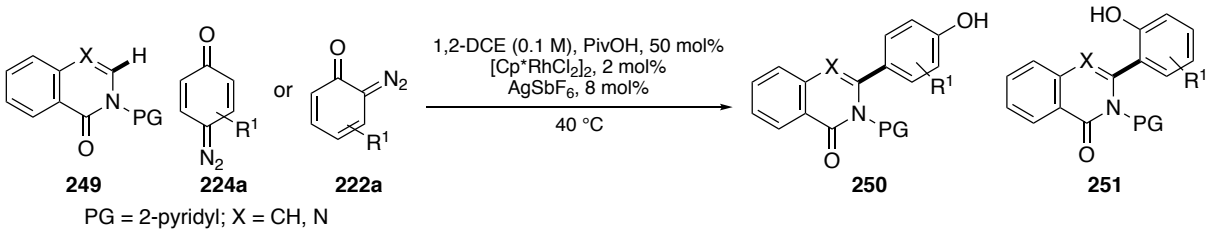
These cyclohexadienone adducts **244** were not isolated and were immediately subjected to a divergent strategy to access different steroid scaffolds.

A Cp*Rh(III)- and Cp*Ir(III)-mediated direct C–H arylation with quinone diazides has been described in the literature.[231] This redox-neutral protocol offers a facile, quick and environmentally benign access to arylated phenols using these diazo compounds as efficient coupling partners (**Scheme 4.7**). A range of 1,2 and 1,4-quinone diazides **222a** and **224a** have been used and overall 32 phenols **247** and **248** were prepared in moderate to excellent yields.



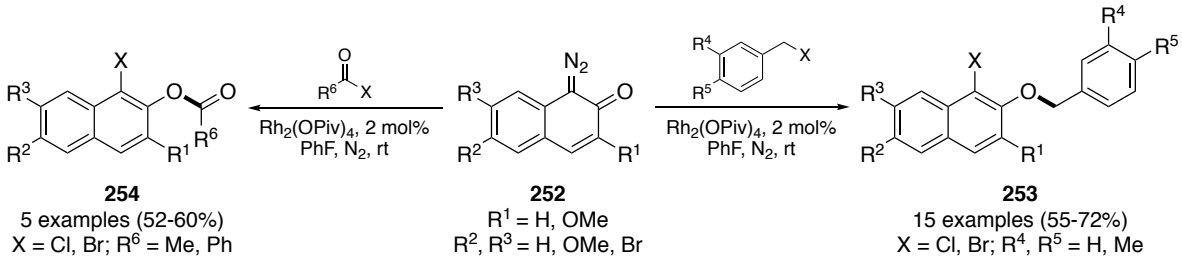
Scheme 4.7 Cp*Rh(III)- and Cp*Ir(III)-mediated direct C–H arylation with quinone diazides.

Das *et al.* developed a direct C6-arylation of 2-pyridones with quinone diazides **222a** and **224a** under Rh(III)-mediated redox-neutral conditions (**Scheme 4.8**).[232] The strict regioselectivity is guided by the pyridyl substituent attached to the nitrogen of the pyridone ring. This represents a highly regioselective, mild, and simple method especially considering that this directing group can easily be removed at any suitable stage after functionalisation, giving rapid access to complex heteroarylated phenol functionalities by wide-ranging heterocyclic scaffolds.



Scheme 4.8 Rhodium(III)-mediated C6 selective arylation of 2-pyridones and related heterocycles using quinone diazides.

Another example is the rhodium(II)-mediated reaction of readily available 1-diazonaphthalen-2(1*H*)-one derivatives **252** and 2-diazonaphthalen-1(2*H*)-one **255** with benzyl halides or acid halides (**Scheme 4.9** and **Scheme 4.10**).[233] Various functionalised halonaphthalenyl ethers **253** and **256** and esters **254** and **257** were prepared through this efficient and simple synthetic approach in moderate to good yields.

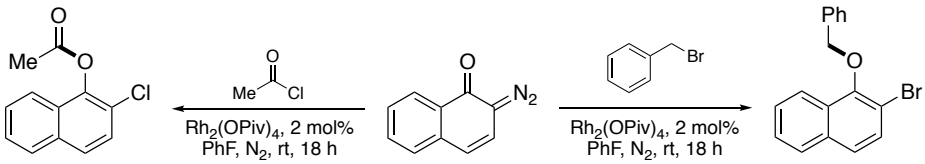


254
5 examples (52-60%)
X = Cl, Br; R^6 = Me, Ph

252
R^1 = H, OMe
R^2, R^3 = H, OMe, Br

253
15 examples (55-72%)
X = Cl, Br; R^4, R^5 = H, Me

Scheme 4.9 Rhodium(II)-mediated synthesis of halonaphthalenyl ethers **253** and esters **254** from 1-diazonaphthalen-2(1*H*)-one **252**.



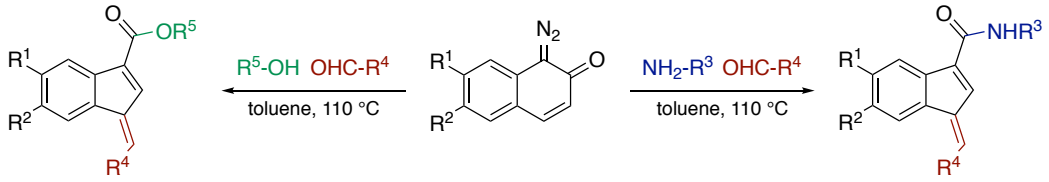
257 (52%)

255

256 (55%)

Scheme 4.10 Rhodium(II)-mediated synthesis of halonaphthalenyl ethers **256** and esters **257** from 2-diazonaphthalen-1(2*H*)-one **255**.

Magar and Lee reported a sequential Wolff rearrangement of 1-diazonaphthalen-2(1*H*)-ones **258** followed by trapping of the ketene intermediate with primary and aromatic amines or alcohols and phenols in the presence of various aldehydes to access 1*H*-indene-3-carboxamides **259** or 1*H*-indene-3-carboxylates **260** (**Scheme 4.11**).[234] This reaction allows for the synthesis of functionalised indene derivatives under catalyst-free thermal conditions.



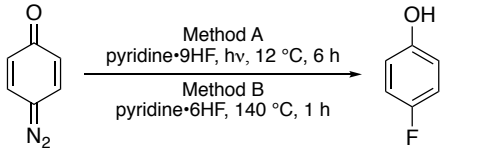
260
15 examples (39-65%)

258
R^1, R^2 = H, OMe; R^3 = Bu, Bn, tryptyl
R^4 = furyl, thienyl, imidazolyl, naphthyl, aryl

259
38 examples (45-94%)

Scheme 4.11 Synthesis of 1*H*-indene-3-carboxamides **259** and 1*H*-indene-3-carboxylates **260** through a Wolff rearrangement of 1-diazonaphthalen-2(1*H*)-ones **258**.

Sawaguchi and co-workers described the thermal and photochemical fluorinative decomposition of 1,4-benzoquinone diazide **221** in pyridine•*n*HF (*n* = 6 or 9) (**Scheme 4.12**).[235] This transformation took place readily and cleanly to give the corresponding 4-fuorophenol **264** in good yields without the formation of undesirable by-products.



221 **261** (A: 85%, B: 80%)

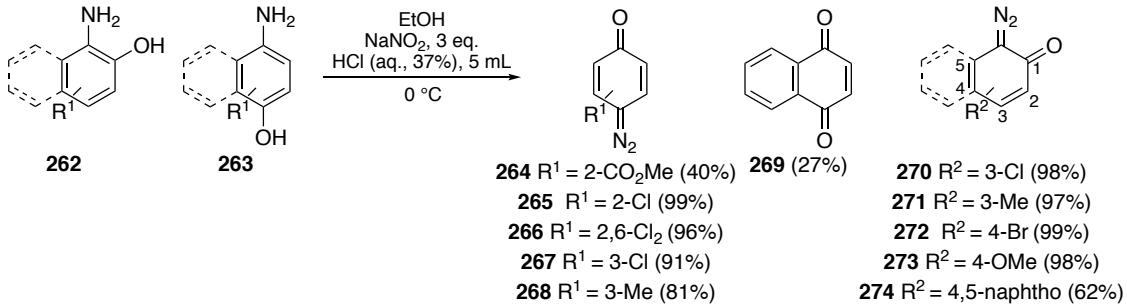
Scheme 4.12 Thermal and photochemical fluorinative decomposition of 1,4-benzoquinone diazide **221** in pyridine•nHF.

4.5 Aims & Objectives

Despite the extensive number of reports on the synthesis and reactivity of quinone diazides, the lack of efficient and mild synthetic methods to manipulate quinone diazides likely contributed to the paucity of applications for the construction of complex molecules. Therefore, the aim of this work is to further study the potential of this compounds to act as electrophiles. In **Section 4.6** the preparation of quinone diazides from the diazotization of 2- or 4-amino phenols or naphthols will be described. These compounds will then be used next to evaluate their reactivity towards nucleophilic substitution (**Section 4.7**) where the optimisation studies to obtain 4-bromo-2,6-dichlorophenol will be presented. Followed by the nucleophile scope the same substrate, in the presence of alcohols, pseudo-halides, triphenyl phosphite, etc. in **Section 4.8**. A range of control reactions is presented in **Section 4.9**. The final part will comprise the substrate scope of the reactions towards halogenation (**Section 4.10**) in which the reactivity of range of quinone diazides in the presence of lithium halides will be analysed and discussed.

4.6 Synthesis of quinone diazides

Quinone diazides **264-268** and **270-274** were readily synthesised by the diazotization of 2- or 4-amino phenols or naphthols **262** and **263** (**Scheme 4.13**). The products were obtained in moderate to good yields and were used in the next stage without further purification. However, when 4-amino-1-naphthol was used, in the form of hydrochloride salt, the reaction led to the isolation of 1,4-naphthoquinone **269**, instead, in 27% yield.

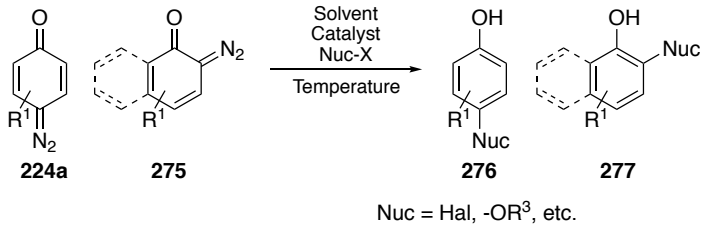


Scheme 4.13 Preparation of quinone diazides from the corresponding aminophenols.

In the ^{13}C NMR spectrum of most of the diazo compounds, the signal for the C=N group was not observed. However, data from the literature reports the typical chemical shift for the corresponding group to be δ 72 ± 5 ppm,[161] besides the signal for the C=N should be very faint due to relaxation times, which might suggest that the signal may be masked under the signals of the deuterated solvent used - chloroform. It is noteworthy to mention that different deuterated solvents or relaxation times were not tried. In addition, the data from IR spectrum of each compound displayed the typical diazo valence vibrational band N=N at ~2100 cm^{-1}.

4.7 Reactivity of quinone diazides towards nucleophilic substitution

In this project, the reactivity of quinone diazides towards nucleophilic substitution has been studied aiming to prepare, in the first instance, halogenated phenols (**Scheme 4.14**). A range of reaction conditions were analysed, such as solvent, catalyst, nucleophile, temperature and substrate based on the reaction from the literature applied to similar scaffolds, as mentioned beforehand (**Scheme 4.4**). With this objective, a series of optimisation experiments were performed and these conditions are discussed below.

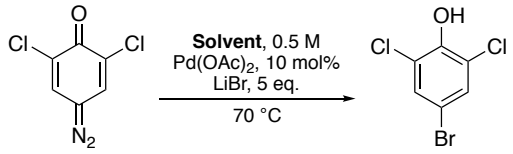


Scheme 4.14 General reaction conditions to assess the reactivity of quinone diazides towards nucleophilic substitution.

4.7.1 Solvent screening

The optimisation studies started with the screening of some more environmentally benign solvents, such as water, ethanol, *t*-butanol, dimethyl carbonate, acetone and acetic acid.

2,6-Dichloro-4-diazocyclohexa-2,5-dien-1-one diazide **269** was selected for the optimisation studies due to being easy to prepare from the corresponding 4-amino-2,6-dichlorophenol, and quite stable towards handling. This quinone diazide **269** was reacted with an excess of lithium bromide (5 eq.) as nucleophile, in the presence of 10 mol% of palladium(II) acetate at 70 °C (**Scheme 4.15** and **Table 4.1**).



266
0.50 mmol, 94.5 mg

278

Scheme 4.15 Effect of the solvent in the reactivity of **266** towards bromination.

Table 4.1 Solvent screening for the reactivity of **266** towards bromination.

Entry	Solvent	Time (min)	Recovery yield (%)[a]	Ratio[b]	
				266	278
1	H_2O	90	40	80	20
2	*t*-BuOH	100	85	100	0
3	DMC	100	13	100	0
4	DMSO	220	86	100	0
5	AcOH	55	91	0	100

a) Based on 100% being 100% recovery of brominated phenol **278**;
b) Calculated based on ^{1}H NMR integrations.

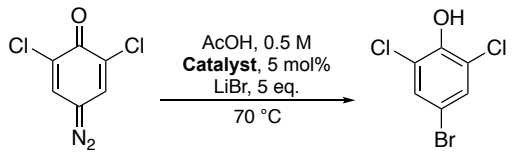
The reaction in the presence of water gave the product as a mixture of the brominated phenol **278** and starting material **266**, in 80:20 ratio (entry 1). When the reaction was performed using *t*-butanol, dimethyl carbonate and DMSO (entries 5 and 6) the quinone diazide **266** was recovered in 85%, 13% and 86% yield, respectively. Ethanol and acetone were also tried but the reaction gave either a complex mixture of products and/or no evidence of product formation (^{1}H NMR and TLC).

After this brief solvent screening, acetic acid proved to be the best solvent for this reaction, leading to the isolation of the product **278** in 91% yield (entry 7). The following parameter to be assessed was the effect of the catalyst used in the reaction.

4.7.2 METAL MEDIATOR SCREENING

The performance of a wide array of metal mediators was evaluated for the template reaction. The aim was to move away from the use of a rare and expensive metal catalyst, such as palladium, and use a cheaper and more earth-abundant metal, such as copper, zinc, iron or

nickel. A range of acetate complexes - with the exception being for copper(II) triflate - were tried and the results are summarised in **Table 4.2**.



266
0.50 mmol, 94.5 mg

278

Scheme 4.16 Effect of the metal mediator in the reactivity of **266** towards bromination.

Table 4.2 Catalyst screening for the reactivity of 266 towards bromination.

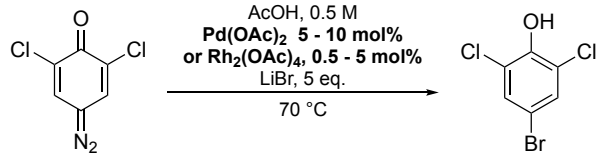
Entry	Catalyst	Time (min)	Recovery yield (%)[a]	Ratio[b]		
				266	278	Unidentified product
1	$Pd(OAc)_2$	135	77	0	100	0
2	$Rh_2(OAc)_4$	12	80	0	100	0
3	$Cu(OAc)_2 \cdot H_2O$	60	69	6	83	11
4	Cu(OAc)	60	36	8	77	15
5	$Cu(OSO_2CF_3)_2$	60	79	6	83	11
6	AgOAc	120	100	2	97	≤1

a) Based on 100% being 100% recovery of brominated phenol **278**;
b) Calculated based on ^{1}H NMR integrations.

Unfortunately, only palladium(II) and rhodium(II) acetate (entries 1-4) gave exclusively the brominated phenol **278**, in good yields, 71-80%. Both copper(I) and copper(II) catalysts (entries 5-7) gave a mixture of the targeted product **278** plus two other unidentified compounds. Using silver(I) acetate gave the unreacted quinone diazide **266** as the major product and traces of the phenol **278** besides another product by TLC analysis of the crude material (entry 8). Furthermore, the use of nickel(II), for more than 20 h, gave a dark polymeric material. The reactions in the presence of zinc(II) and iron(II), for more than 19 h, led to complex mixtures of products, from TLC analysis of the crude product. Overall, the best set of conditions used rhodium(II) acetate (entries 2), despite the yields being comparable to that obtained when the reaction was carried out with palladium(II) acetate (entry 1) the reaction times was significantly shorter. Also, following quenching of the reaction mixture with a solution of sodium hydrogen carbonate, two alternatives approaches were tried for the isolation the product **278**. The first one was the extraction of the aqueous phase with dichloromethane and the other one was filtration of the precipitated product and washing with water. The latter protocol led to the isolation of the phenol **278** in lower yields, in general.

4.7.3 CATALYST LOADING

After failure of pursuing the reaction using a more earth-abundant metal catalyst, the objective was to reduce the loading of rhodium(II) and palladium(II) acetate. Next, the effect of variable loadings of both catalysts will be studied. 1,4-Benzoquinone diazide **266** was reacted with an excess lithium bromide in the presence of 10 and 5 mol% of $Pd(OAc)_2$ and with 5 to 0.5 mol% $Rh_2(OAc)_4$ (**Scheme 4.17** and **Table 4.3**).



266
0.50 mmol, 94.5 mg

278

Scheme 4.17 Effect of the catalyst loading in the reactivity of **266** towards bromination.

Table 4.3 Catalyst loading in the reactivity of **266** towards bromination.

Entry	Catalyst	Mol cat (%)	Time (min)	Isolated yield 278 (%)
1	$Pd(OAc)_2$	10	55	91
2	$Pd(OAc)_2$	5	57	76
3	$Rh_2(OAc)_4$	5	6	71
4	$Rh_2(OAc)_4$	2.5	7	75
5	$Rh_2(OAc)_4$	1	7	98
6	$Rh_2(OAc)_4$	0.5	28	97

When 10 and 5 mol% of palladium(II) acetate were used (entries 1 and 2), the reaction time was approximately 1 h and the phenol **278** was isolated in good yields. The reaction was not attempted with lower loadings of this catalyst. Carrying out the reaction with 5-0.5 mol% of rhodium(II) acetate, took between 6 to 28 min (entries 3-6) to reach completion and led to the isolation of the brominated product **278** in 76-91% yield.

Palladium ranks about 71st in natural abundance among the elements in crustal rocks, with an 0.0006 ppm abundance in the Earth's Crust. The metal occurs in the pure state in platinum ores and in the combined state in Canadian nickel ore.[236] Rhodium is the rarest of all non-radioactive metals with a 0.0002 ppm abundance in Earth's Crust. It occurs non-combined in nature, in river sands in North and South America. It is also found in the copper-nickel sulfide ores of Ontario, Canada. Commercially, rhodium is obtained as a by-product of copper and nickel refining and its world production is approximately 30 tonnes per year.[237]

The standard reaction in the optimisation studies was carried out in the presence of 0.50 mmol of quinone diazide **266**. **Table 4.4** below gives a summary of the quantities and prices of both palladium(II) and rhodium(II) acetate catalysts used in the previous screening reactions with the lowest loadings (5 and 0.5 mol%, respectively).

Table 4.4 Comparison between the price per reaction when using Rh(II) and Pd(II) acetate.

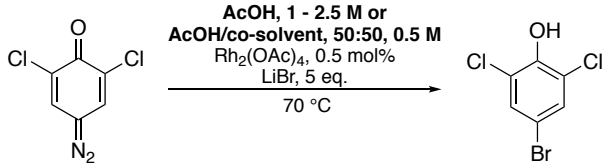
Catalyst	Loading (mol%)	Mass (mg)	Price 1 g (£)[a]	Price/reaction (p)
Pd	5	5.6	143.0	8.03
Rh	0.5	1	828.0	8.28

a) Sigma Aldrich price for 1 g of 99.98% $Pd(OAc)_2$ and 1 g of 99.99% $Rh_2(OAc)_4$.

When comparing both catalysts, palladium would still represent the best alternative, because it is less expensive and more abundant than rhodium. Nonetheless, despite the somewhat higher cost of using rhodium(II) acetate (+0.25 p/reaction) when compared to the use of palladium(II) acetate, the ability of performing the reaction with a much lower loading of catalyst and faster reaction times made us choose the former to be used in the subsequent optimisation studies. Furthermore, the scale-up of this reaction was not pursued at this stage which means that the use of a lower loading of the rhodium catalyst was not attempted due to the limitation of the weighing scale.

4.7.4 Acetic acid as solvent

Considering the previous experiments and also literature precedents, acetic acid proved to be the best solvent for this reaction. However, concerns due to the competing nucleophilic nature of the acetate (from the solvent) vs. the bromide (from the nucleophile) led us to attempt to reduce the equivalents of acetic acid used. Carrying the reaction at 0.5 M, means using 35 eq. of acetic acid in relation to the quinone diazide **266**. At this stage, we considered to either reduce the overall volume of the reaction or dilute the acid with other solvents, such as water or DMSO (**Scheme 4.18** and **Table 4.5**).



266
0.50 mmol, 94.5 mg

278

Scheme 4.18 Analysis of concentration and co-solvent effect in the reactivity of **266**.

Table 4.5 Effect of concentration and co-solvent in the reactivity of **266**.

Entry	Solvent	C_{sol} (M)	Time (min)	Isolated yield 278 (%)
1	AcOH	1	10	85
2	AcOH	1.25	16	89
3	AcOH	2.5[a] (1.25)	11 (+26)	86
4[b]	H_2O/AcOH	0.5	89	87

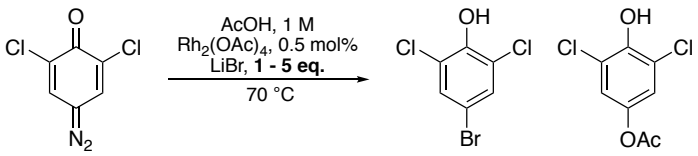
a) Inefficient stirring; b) Trace quantities of impurities.

In entries 1-3, the reaction was carried out using more concentrated reactions. At 1.25 M (14 eq.) and 1 M (17 eq.), the reaction led to the isolation of the brominated product **278** in excellent yields. When the reaction was performed at 2.5 M (7 eq.) of acetic acid, the reagents would not stir and solubilise. After 11 min of stirring at 70 °C, extra 7 eq. of solvent was added and the reaction proceeded as normal yielding **278** in 86% yield. A 50:50 ratio mixture of water/acetic acid was also attempted however, the reaction was much slower, taking approximately 1.5 h, to give **278** in 87% yield (entry 4). Finally, the reactivity of **266** towards bromination was tested a 50:50 ratio mixture of DMSO/acetic acid and this led to identification of starting material **266** and, at least, three different products by TLC analysis of the crude material.

In view of the slower rate when the reaction was carried in a more dilute aqueous acetic acid solution (entry 4), the best option appeared to be reducing the overall volume of solvent used (entries 1 and 2).

4.7.5 Nucleophile loading

Next, the role of the nucleophile loading in this reaction was evaluated. In the pursuit of a more sustainable synthetic transformation of the quinone diazide **266** into the corresponding brominated phenol **278**, the goal at this stage was to reduce the number of equivalents of the lithium salt used. The starting material **266** (0.50 mmol) was reacted with 1-5 equivalents of lithium bromide, in acetic acid, and in the presence of rhodium(II) acetate (**Scheme 4.19**).



266
0.50 mmol, 94.5 mg

278 **279**

Scheme 4.19 Effect of the lithium bromide loading in the reactivity of **266** towards bromination.

Table 4.6 Lithium bromide loading in the reactivity of **266** towards bromination.

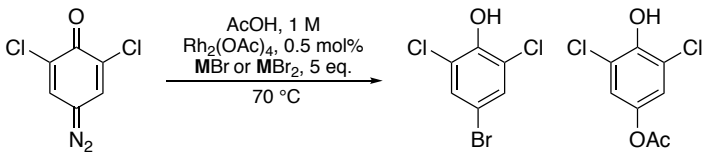
Entry	LiBr (eq.)	Time (min)	Recovery yield (%)[a]	Ratio[b]	
				278	279
1	1	50	80	90	10
2	1.2	40	80	95	5
3	1.5	26	86	97	3
4	2	37	87	>99	<1
5	3	30	85	>99	<1
6	4	26	82	>99	<1
7	5	28	98	>99	<1

a) Based on 100% being 100% recovery of brominated phenol **278**;
b) Calculated based on ^{1}H NMR integrations.

Unfortunately, reducing the number of equivalents resulted in an increase in the formation of the acetylated by-product **279**, as it is possible to verify from the analysis of **Table 4.6**.

4.7.6 CATION EFFECT

The next step was the evaluation of the influence of the cation for the bromination reaction to happen. The reactivity of the starting material **266** against different sources of bromide was assessed, in the presence of acetic acid and 0.5 mol% of rhodium(II) acetate. A range of bromide salts comprising one organic and eight inorganic derivatives were tested as well as hydrobromic acid (**Scheme 4.20** and **Table 4.7**).



266
0.50 mmol, 94.5 mg

278 **279**

Scheme 4.20 Cation of the bromide source screening in the reactivity of **266** towards bromination.

Table 4.7 Effect of the cation of the bromide source in the reactivity of **266** towards bromination.

Entry	X^{n+}	Time (min)	Recovery yield (%)[a]	Ratio[b]		
				266	278	279
1	Li^{+}	28	97	0	100	0
2	Na^{+}	87	78	0	70	30
3	K^{+}	86	58	0	87	13
4	Mg^{2+}	90	76	0	100	0

5	Cu^{+}	120	72	0	81	19
6	Cu^{2+}	120	68	0	80	20
7	H^{+}	17	71	20	80	0
8	H^{+}	90	70	20	80	0

a) Based on 100% being 100% recovery of brominated phenol **278**;
b) Calculated based on ^{1}H NMR integrations.

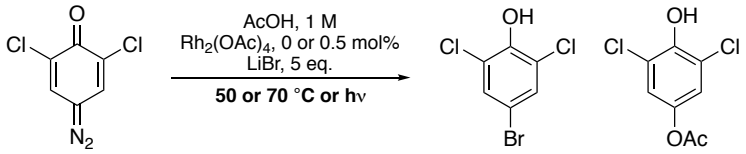
The reactions in which lithium and magnesium bromide were employed as nucleophiles gave the best results, with the exclusive formation of the brominated product **278** (entries 1 and 4). However, the latter gave **278** in lower yield, 76% vs 97% for lithium bromide. Both sodium and potassium bromide displayed a good profile as alternative sources of bromide for the bromination of quinone diazide **266**. Nevertheless, the products were isolated as a mixture of brominated and acetylated products, **278** and **279**, in 70:30 and 87:13 ratios, respectively (entries 2 and 3). The reactions in the presence of Cu(I) and Cu(II) bromide gave a mixture of brominated and acetylated products, **278** and **279**, besides trace impurities by analysis of the ^{1}H NMR spectrum of the crude materials (entries 5 and 6). The use of hydrobromic acid (**48** wt% in water) gave a mixture of the brominated product, **278**, and starting material, **266,** in a 80:20 ratio. The reaction time did not seem to make a difference on this ratio, according to entries 7 and 8. This may be due to a protonation effect of the starting material **266**, in the presence of acid, and re-oxidation in the workup process.

When the reaction was carried out using zinc and caesium bromide, for 6 h, it afforded a mixture of starting material **266**, plus at least 3 other products as suggested by TLC analysis of the crude material. Also, the reaction was tried in the presence of tetrabutylammonium bromide, which gave a complex mixture of products, with the major product being the salt used in excess.

Both lithium and magnesium bromide are the best cations for this reaction, giving exclusively the brominated product **278** but the rationale for this behaviour is not clear. The similarity between lithium and magnesium may arise because of their similar sizes: atomic radii, Li = 152 pm, Mg = 160 pm and ionic radii: Li^{+} = 76 pm, Mg^{2+} = 72 pm. Furthermore, some metals used in the study, namely hydrogen, lithium, magnesium, copper and zinc have their oxophilicity values reported in the literature (H = 0.4, Li = 0.3, Mg = 0.6, Cu = 0.2, Zn = 0.2).[238] But, considering these values, there is not a correlation between their oxophilicity and the ability to mediate the reaction.

4.7.7 TEMPERATURE AND LIGHT-INDUCED BROMINATION

The next parameter to be assessed was temperature. Ideally, less energy intensive reactions are preferred. Therefore, the aim was to assess whether the nucleophilic substitution of **266**, in the presence of 0.5 mol% rhodium(II) acetate, would occur at lower temperatures. Besides this, the opportunity to use a light source as a more sustainable alternative in relation to the metal catalyst to promote the decomposition of the diazo compound was envisaged (**Scheme 4.21** and **Table 4.8**).



266
0.50 mmol, 94.5 mg

278 **279**

Scheme 4.21 Effect of temperature and light in the reactivity of **266** towards bromination.

Table 4.8 Effect of temperature and light in the reactivity of **266** towards bromination.

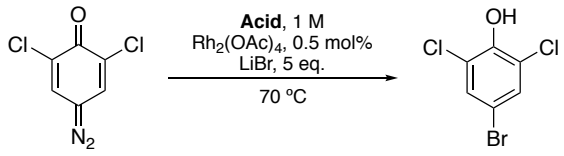
Entry	Temperature (°C) / hν	Time (min)	Recovery yield (%)[a]	Ratio[b]		
				266	278	279
1	70	28	97	0	100	0
2	50	50	99	100	0	0
3[c]	hν	1340	39	0	77	23
4	hν	120	63	5	86	9
5	hν	170	80	0	90	10

a) Based on 100% being 100% recovery of brominated phenol **278**;
b) Calculated based on ^{1}H NMR integrations; c) $Rh_2(OAc)_4$ was not added in this reaction.

In the usual temperature conditions used, performing the reaction at 70 °C, for 28 min, gave the brominated phenol **278**, in 97% yield (entry 1). Carrying out the reaction at 50 °C, for 50 min, gave the unreacted quinone diazide **266**, in quantitative yield (entry 2). This reaction was also tried photochemically, both in the presence and absence of catalyst however it did show any improvements. The reaction in the absence of rhodium catalyst took much longer, and the product was isolated as a mixture of brominated phenol **278** and acetylated product **279** (entry 3). Two reactions were carried out in which the catalyst has been added (entries 4 and 5). The difference between these two was the reaction time, 2 h and, approximately, 3 h. In both cases, products **278** and **279** was formed, in a similar ratio 9:1, however, in the shorter reaction, there was still starting material **266** present following the analysis of ^{1}H NMR spectrum of the crude material. No further attempts to optimise these reactions were conducted.

4.7.8 ACID SCREENING

Due to the competing nature of nucleophilic substitution by the anion of the solvent (acetate) and the salt used as nucleophile (bromide), leading to mixtures of acetylated and brominated product, it was hypothesised that the use of an alternative acid could be advantageous in this reaction. Therefore, TFA (CF_3CO_2H) and pivalic acid ($(CH_3)_3CCO_2H$) were selected (**Scheme 4.22**). Whereas acetic and pivalic acid have similar pKas 4.76 and 5.01, respectively, TFA's pKa is 0.23, making it a much stronger acid due to the electron withdrawing effect of the fluorine atoms. But the lower boiling point (72 °C) in comparison to the two other alternatives (118 °C and 164 °C, for acetic and pivalic acid, respectively) would facilitate product isolation due to the fact that it can be removed under reduced pressure. Besides, any of these acids should reduce the competitive nucleophilic substitution from the anion of the solvent, either by increasing the steric hindrance or by reducing the nucleophilicity of the acid.



266
0.50 mmol, 94.5 mg

278

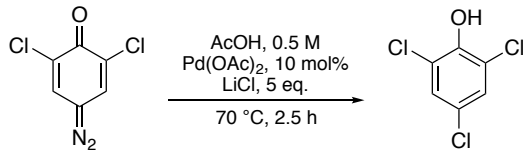
Scheme 4.22 Acid screening in the reactivity of **266** towards bromination.

The reaction in the presence of TFA was carried out for more than 2 h, and the product was isolated predominantly as the starting material **266**, with traces of brominated phenol **278**. Using pivalic acid led to the isolation of pivalic acid as the major product with traces of an unidentified product. These outcomes resulted from the analysis of the ^{1}H NMR spectrum of the crude products and no other acids were attempted, neither further optimisation of these reactions. As a result, acetic acid was used in all the following studies.

4.8 Nucleophile scope

4.8.1 CHLORIDE

The reactivity of quinone diazide **266** was initially analysed following the conditions described in the literature. The palladium(II) acetate mediated reaction of **266** with an excess of lithium chloride was carried out at 70 °C to give the corresponding chlorinated phenol **280** in very good yield (**Scheme 4.23**).



266
0.50 mmol, 94.5 mg

280 (86%)

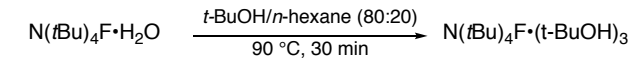
Scheme **4.23** Reactivity of **266** towards chlorination**.**

4.8.2 FLUORIDE

With the optimised set of conditions from the reaction of **266** with lithium bromide in hand and early evidence of reactivity towards chloride (**Scheme 4.23**), which will be further discussed later, we decided to move towards the use of fluoride. Fluorinated compounds have dominated a large percentage of agrochemicals and pharmaceuticals as well as a number of functional materials.[239–246] The incorporation of fluorine atoms into organic molecules through many traditional methods usually cannot satisfy the simplicity and cost efficiency for industrial production and laboratory synthesis.[247–250] This reaction would be an easy way to prepare fluorinated phenols, preferably not relying on the use of metal catalysts, hazardous solvents or reagents and using a mild source of fluoride.

Metal-free mediated fluorination

In the first set of experiments, the metal-free reaction between quinone diazide **266** and a range of different fluoride sources in four different solvents was attempted, at 70 °C. The fluoride sources ranged from caesium fluoride to hydrogen fluoride pyridine, boron trifluoride diethyl etherate, tetrafluoroboric acid diethyl ether complex and tetrabutylammonium tri(*t*-butyl alcohol) coordinated fluoride, both adsorbed in silica gel or as a *t*-butanol complex prepared from a reported protocol in the literature. The latter has been described as a facile fluoride source.[251] TBAF(*t*-BuOH)$_3$ was prepared using the procedure represented in **Scheme 4.24**. Commercially available TBAF hydrate **281** was heated in *t*-BuOH/hexane to 90 °C for 30 min, and recrystallised at room temperature to give TBAF(*t*-BuOH)$_3$ **282** as a colourless, crystalline solid in 92% yield. Despite the ^{1}H, ^{19}F and ^{13}C NMR data being consistent with that reported in the literature, the integration of the proton signals does not integrate for a tetra but for a tri(*t*-butylalcohol) coordinated fluoride.

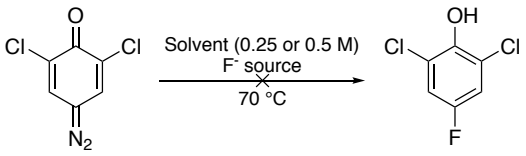


281
3.83 mmol, 1.09 g

282 (92%)

Scheme 4.24 Preparation of tetrabutylammonium tri(t-butyl alcohol) coordinated fluoride **282**.

The selected solvents for this study were acetonitrile, 1,2-dichloroethane, *t*-butanol and a water/acetonitrile system (**Scheme 4.25**).



266
0.50 mmol, 94.5 mg

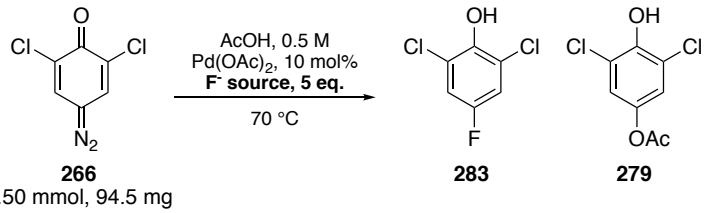
283

Scheme 4.25 Attempts towards a non-metal mediated fluorination.

The reaction carried out in acetonitrile, in the presence of 2 eq. of caesium fluoride, at 0 °C followed by 3 days at rt and finally, 3.5 h at 90 °C, led to the formation of a product that did not give any ^{1}H NMR signals. When 1 and 2 eq. of tetrabutylammonium tri(*t*-butyl alcohol) coordinated fluoride **282** was reacted with quinone diazide **266**, at 0 °C, for 30 min, the product was isolated as a complex mixture by TLC. In the reactions attempted in 1,2-dichloroethane, the quinone diazide **266** was combined with 2 eq. of tetrafluoroboric acid diethyl ether (2 h), 2 eq. of boron triflate etherate (24 min) and 2.6 eq. of hydrogen fluoride pyridine (4 h followed by the addition of extra 6 eq. of hydrogen fluoride pyridine and 2 h at 80 °C). These reactions were not successful leading to the recovery of starting material **266**, which was confirmed by ^{1}H NMR, TLC and IR data, in 54 to 71% yields. Also, using 1.5 eq. tetrabutylammonium fluoride on silica gel at 70 °C, for 1.5 h, gave a complex mixture of products by ^{1}H NMR analysis. The reaction in *t*-butanol was initially carried out at 30 °C for 3 days, without evidence of reaction. Then, the temperature was set at 80 °C for 3.8 h to give a complex mixture of products by TLC analysis. Lastly, combining **266** with 2 eq. of caesium fluoride in a water/acetonitrile system, at rt, for 3 days, gave unreacted quinone diazide **266** by TLC analysis of the reaction mixture.

Pd mediated fluorination

The lack of promising results in the non-metal mediated process led to a reconsideration of the synthetic strategy, and a return to the protocol previously described in the literature.[228] Both organic and inorganic sources of fluoride were attempted, and the reaction was carried out in the presence of palladium(II) acetate in acetic acid (**Scheme 4.26** and **Table 4.9**).



266
0.50 mmol, 94.5 mg

283 **279**

Scheme 4.26 Fluoride source screening of palladium(II) acetate mediated fluorination of **266**.

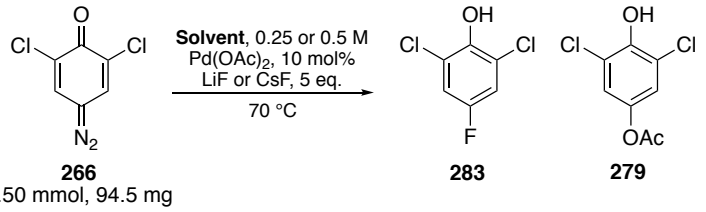
Table 4.9 Effect of fluoride source in the palladium(II) acetate mediated fluorination of **266**.

Entry	F^- source	Time (h)	Isolated yield 279 (%)
1	LiF	1.7	97
2	NaF	2.6	99
3	KF	2.5	94
4	CsF	1.5	88
5	KHF_2	1.3	94
6	TBAF on SiO_2[a]	1.8	n/a[b]

a) 1.5 eq.; b) **279**/**TBAF** (69:31 and 196% recovery yield, based on 100% being 100% recovery of fluorinated phenol **283**).

Using inorganic fluorides, such as lithium, sodium, potassium, caesium and potassium dihydrogen fluoride gave the acetylated product **279** in excellent yields (entries 1-5). When tetrabutylammonium fluoride on silica gel (1.5 instead of 5 eq.) was employed, the product was isolated as a mixture of the acetylated phenol **279** and the organic salt, in a 69:31 ratio (entry 6). To sum up, fluorination of quinone diazide **266** was unsuccessful in all the reactions attempted.

Next, a solvent screening for the same reaction was carried out for the palladium(II) acetate mediated reaction of the quinone diazide **266** and fluorides (**Scheme 4.27**). Lithium fluoride was chosen as the preferred source of fluoride, but one reaction was taken in the presence of caesium fluoride The solvent selection for this study included acetonitrile, ethanol, *t*-butanol, DMSO, DMC, acetone, TFE and 1,2-dichloroethane.



266
0.50 mmol, 94.5 mg

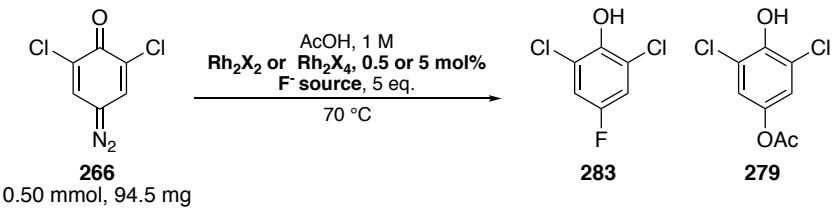
283 **279**

Scheme 4.27 Solvent screening of palladium(II) acetate mediated fluorination of **266**.

Solvents as acetonitrile and ethanol and acetone and TFE gave complex mixture of products from the analysis of the ^{1}H NMR spectrum of the crude material. The reactions in the presence of *t*-butanol, DMSO, DMC and 1,2-dichloroethane led to the recovery of starting material **266** besides the formation of other products than the fluorinated phenol **283** both from TLC or ^{1}H NMR analysis. The reaction in 1,2-dichloroethane was also tried in the presence of caesium fluoride however the reaction was not successful, leading to a complex mixture of products from the ^{1}H NMR data collected for the crude material. To recapitulate, fluorination of quinone diazide **266** was unsuccessful in all the reactions attempted.

Rh mediated fluorination

At this stage, the previous optimised set of conditions from the reaction of **266** with lithium bromide, in the presence of 0.5 mol% of rhodium(II) acetate and acetic acid, were used as a guide. The effect of three rhodium(II) catalysts were analysed, namely rhodium(II) acetate, rhodium(II) trimethylacetate and bis[rhodium(α,α,α′,α′-tetramethyl-1,3-benzenedipropionic acid)] in 0.5 or 5 mol% loadings. Also, different sources of fluoride, both inorganic and organic, were examined such as lithium and caesium fluoride, potassium dihydrogen fluoride, triethylamine hydrofluoride, hydrogen fluoride pyridine and tetrafluoroboric acid diethyl ether complex (**Scheme 4.28**).



266
0.50 mmol, 94.5 mg

283 **279**

Scheme 4.28 Fluoride source and catalyst screening of rhodium(II) mediated fluorination.

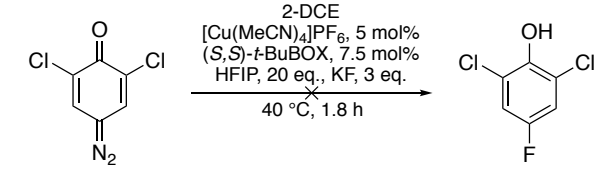
Eight reactions were carried out in the presence of 0.5 mol% of rhodium(II) acetate, with a range of fluoride sources and the products were usually isolated as mixtures of starting material **266**, acetylated phenol **279** and/or unidentified products. Quinone diazide **266** was reacted with potassium dihydrogen fluoride and caesium fluoride in two 5 mol% rhodium(II) trimethylacetate mediated reactions. This led to the isolation of **279** as the major product however, by analysis of ^{19}F NMR there was evidence of the fluorinated product **283** formation in both reactions. In three other situations, the reaction was carried out in the presence of 5 mol% bis[rhodium(α,α,α′,α′-tetramethyl-1,3-benzene-dipropionic acid)] and three different fluoride sources. Again, the major product of these reactions was phenol **279** but there was also evidence of fluorinated phenol **283** by analysis of ^{19}F NMR spectrum of the crude material.

In one case, the product was isolated as a mixture of starting material **266,** fluorinated phenol **282** and acetylated phenol **279** in 7:10:83 ratio, which may represent the most promising set of conditions so far. Overall, despite the efforts to optimise the reaction conditions it was not possible to obtain more than trace quantities of the fluorinated product **282**, as suggested by ^{19}F NMR analysis of the crude material from some reactions.

Copper mediated fluorination

Gray *et al.* reported a copper-mediated H– F insertion into α-diazocarbonyl compounds using a combination of potassium fluoride and hexafluoroisopropanol (HFIP) as a reactive fluoride source.[252] Applying these conditions, complex α-fluorocarbonyl derivatives were assessed under mild conditions.

The copper(II) mediated reaction of **266** with 3 eq. potassium fluoride and 20 eq. of HFIP, in 1,2-dichloroethane, at 40 °C, was unsuccessful (**Scheme 4.29**). The starting material **266** was recovered and there was no evidence of any product formation by analysis of the ^{1}H NMR of the crude material.



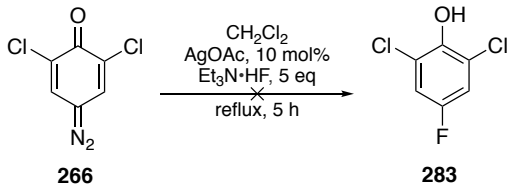
266
0.50 mmol, 94.5 mg

283

Scheme 4.29 Attempt towards a copper(II) mediated fluorination of quinone diazide **266**.

Silver mediated fluorination

Quin and Davies presented a silver-mediated vinylogous fluorination of vinyl diazoacetates to generate γ-fluoro-α,β-unsaturated carbonyls and this approach was applied to substrate **266**.[253] The silver(I) mediated reaction of **266** with 5 eq. of triethylamine hydrofluoride, in dichloromethane, under reflux, proved to be unsuccessful (**Scheme 4.30**). The starting material **266** was recovered and no evidence of any product formation by analysis of the ^{1}H NMR of the crude material.



266
0.50 mmol, 94.5 mg

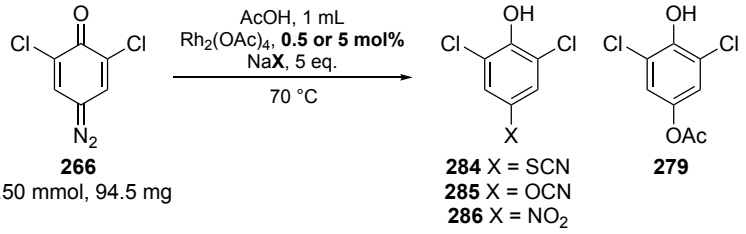
283

Scheme 4.30 Attempt towards a silver(I) mediated fluorination of quinone diazide **266**.

4.1.1 PSEUDO-HALIDES

The reactivity of **266** towards nucleophilic substitution with pseudo halides, in the presence of 0.5 and 5 mol% of rhodium(II) acetate was next explored (**Scheme 4.31**).

Classical linear pseudo-halides, for example cyanide, cyanate, thiocyanate and azide anions, show chemical properties analogous to single halogen atoms and they are well known to do similar chemistry.[254]



Scheme 4.31 Rhodium(II) acetate mediated pseudo-halogenation.

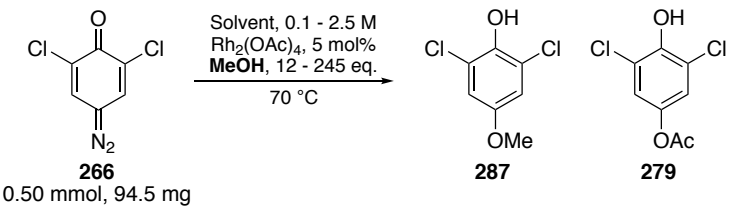
In this study, the selection was limited to sodium thiocyanate, sodium cyanate and sodium nitrite and, in general, the results were not promising after 4.5 – 6.2 h, yielding complex mixture of unreacted starting material **266** and acetylated phenol **279** and/or other unidentified products, in variable ratios, according to ^{1}H NMR spectrum analysis of the crude products.

4.8.3 ALCOHOLS

The reactivity of quinone diazide **266** towards nucleophilic substitution was also attempted with alcohols. Methanol and pentan-1ol were selected to carry most of the studies and the results for both will be discussed separately next. Likewise, one reaction was attempted with cyclohexanol.

Methanol

The reaction of starting material **266** with methanol, mediated by 5 mol% of rhodium(II)acetate, in acetic acid was attempted in three different solvents: methanol, acetic acid and 1,2-dichloroethane (**Scheme 4.32** and **Table 4.10**).



Scheme 4.32 Solvent screening of rhodium(II) mediated etherification of **266** with methanol.

Table 4.10 Effect of the solvent in the rhodium(II) mediated etherification of **266** with methanol.

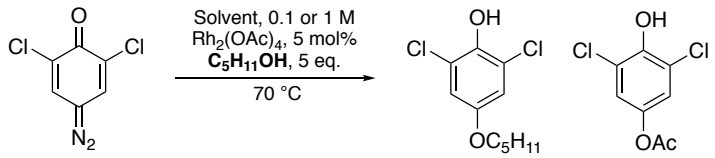
Entry	Eq. MeOH	Solvent	C_{sol} (M)	Time (min)	Recovery yield (%)[a]	Ratio[b]		
						278	279	287
1[c]	24	AcOH	2.5	310	51	0	50	50
2[c]	24	AcOH	1	277	83	0	50	50
3[d]	24	AcOH	1	270	87	3	0	81
4[d]	12	AcOH	1	270	86	0	33	67
5[d]	245	MeOH	0.1	275	100	0	0	100
6[d]	245	MeOH (dry)	0.1	270	100	0	0	100
7[d]	24	DCE	0.1	270	100	0	0	86

a) Based on 100% being 100% recovery of phenol ether **287**; b) Calculated based on ^{1}H NMR integrations; c) Unsuccessful separation of the products; d) Isolated with trace quantities of impurities.

When acetic acid was used as the solvent, both the phenol ether **287** and the acetylated phenol **279** were formed, with variable ratios, depending on the excess of methanol used (entries 1-4). Carrying out the reaction just in methanol led to the isolation of the phenol ether **287** with minor impurities (entries 5 and 6). One reaction in dry methanol (entry 6) was performed aimed to understand if the impurities could be due to the competing reaction of the quinone diazide **266** with water present in the solvent. Nevertheless, the reaction did not show a significant improvement. Besides, the inherent problem of using methanol as both solvent and nucleophile is that this would restrain the substrate scope for the reaction. Unfortunately, purification of the crude materials was not successful and it was not possible to isolate and fully characterise **287**.

Pentan-1-ol

The rhodium(II) acetate mediated reaction of quinone diazide **266** with 5 eq. of pentan-1-ol was attempted in three different solvents: acetic acid, 1,2-dichloroethane and acetonitrile (**Scheme 4.33**).



266
0.50 mmol, 94.5 mg

288 **279**

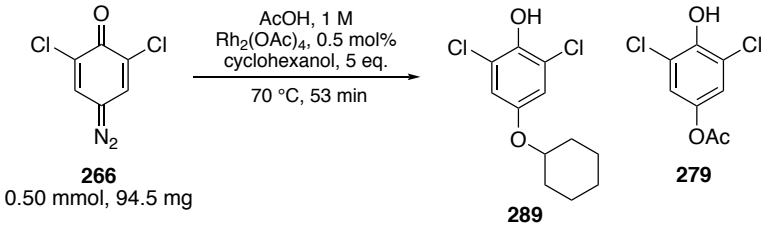
Scheme 4.33 Solvent screening of rhodium(II) mediated etherification of **266** with pentan-1-ol.

Three reactions were carried out in 1,2-dichloroethane and gave the aryl ether **288** along with other products. In the first one, the product was separated but the purification was not effective.

In the other reactions, the analysis of the crude material was done after concentration of the solvent under reduced pressure. Also, two reactions were tried where 4Å molecular sieves were added aiming to trap any water that might be competing as nucleophile for this reaction; however, it did not improve the final outcome. Performing the reaction in acetic acid afforded the product usually as a mixture of phenols **288** and **279**, and a third unidentified product. In one case, hydrolysis of the ester by-product **279** was attempted aiming to making the separation of these compounds easier. The crude material was stirred at rt, with NaOH (aq., 1M) in methanol but unfortunately, this was unsuccessful. Finally, when acetonitrile was also used and the starting material **266** was recovered with a complex mixture of product.
Overall, the reaction led to the formation of the phenol ether **288** along with other products and minor contaminants considering the ^{1}H NMR data collected. Regrettably, the purification of the crude material was not effective and it was not possible to isolate and fully characterise **288**.

Cyclohexanol

The rhodium(II) acetate mediated reaction of quinone diazide **266** with 5 eq. of cyclohexanol was carried out in 1,2-dichloroethane, for 53 min (**Scheme 4.34**).



266
0.50 mmol, 94.5 mg

289

279

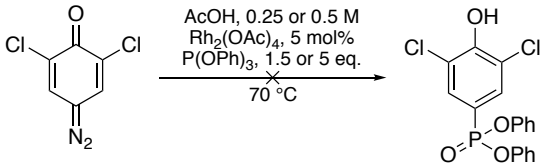
Scheme 4.34 Rhodium(II) mediated etherification of **266** with cyclohexanol in AcOH.

The product was recovered in 81% yield (based on 100% being 100% recovery of the desired product **289**) as a mixture of the phenol ether **289**, **279** and starting material **266** in 23:37:40 ratio besides trace quantities of other unidentified contaminants. Nonetheless, the purification of the crude material was unsuccessful and it was not possible to fully characterise **289**.

To summarise, the reaction of quinone diazide **266** with alcohols yielded the targeted phenol ether derivative besides competing by-products. When the reaction was performed in acetic acid, the acetate derivative was also isolated and in 1,2-dichloroethane, the by-products have not been identified. Inefficient separation and purification of these products constrained further nucleophile scope and characterisation of the products.

4.8.4 TRIPHENYL PHOSPHITE

At this stage, a Sandmeyer-type phosphorylation based on a recently published protocol was attempted.[255] Quinone diazide **266** was combined with 1.5 or 5 eq. of triphenyl phosphite, in 5 mol% rhodium(II) acetate mediated reactions, in acetic acid (**Scheme 4.35**). The separation and identification of the products was unsuccessful. Nevertheless, it was not possible to detect the phosphorylated product **290**, by analysis of ^{1}H and ^{31}P NMR spectra of the crude material.



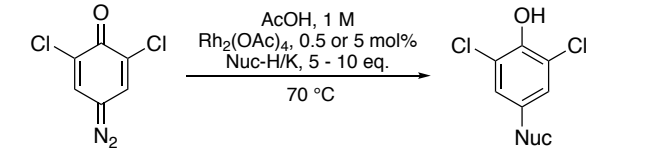
266
0.50 mmol, 94.5 mg

290

Scheme 4.35 Rhodium(II) mediated nucleophilic substitution of **266** with triphenyl phosphite.

4.8.5 MISCELLANEOUS NUCLEOPHILES

The rhodium(II) acetate mediated reaction of quinone diazide **266** with a range of miscellaneous nucleophiles was also attempted (**Scheme 4.36**).



269
0.50 mmol, 94.5 mg

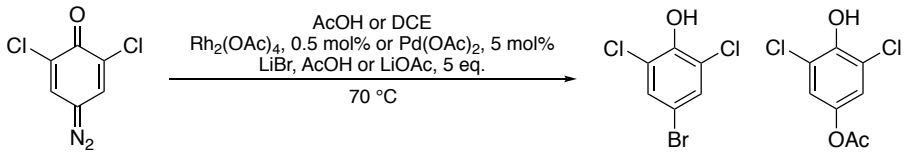
291 Nuc = -NH$(CH_2)_4CH_3$
292 Nuc = -NHC_6H_5
293 Nuc = - S(SOEt)
294 Nuc = -O-(4-Br-C_6H_4)

Scheme 4.36 Rhodium(II) mediated nucleophilic substitution of **266** with different nucleophiles.

Carrying out the reaction with excess of 1-pentylamine and aniline gave complex mixtures of products. When *O*-ethyl xanthate was used, the starting material **266** was recovered and there was evidence of the formation of trace quantities of unidentified products. An excess of 4-bromo-phenol was also reacted with quinone diazide **266**, in the presence of 5 mol% of rhodium(II) acetate and in 1,2-dichloroethane which led to the isolation of 4-bromo-phenol and two unidentified products. It is noteworthy to point out that, the product of all the reactions was identified from the analysis of ^{1}H NMR data of the crude material.

4.9 Control reactions

A set of control reactions was also carried out for the conversion of quinone diazide **266** at 70 °C (**Scheme 4.37** and **Table 4.11**). The solvents used included acetic acid and 1,2-dichloroethane, the metal catalysts were palladium(II) and rhodium(II) acetate and the nucleophiles tested were acetic acid, lithium bromide and lithium acetate.



266
0.50 mmol, 94.5 mg

278 **279**

Scheme 4.37 Control reactions for the transformation of **266**.

Table 4.11 Effect of some of the individual components of the reaction conditions.

Entry	Nuc	Solvent	Catalyst	Time (h)	Recovery yield (%)[a]	Ratio[b]			
						266	278	279	Unidentified products
1	-	AcOH	-	17.5	26	35	0	52	13
3	-	AcOH	$Pd(OAc)_2$	4.7	81	0	0	100	0
4	-	AcOH	$Rh_2(OAc)_4$	2.5	67	40	0	60	0
5	-	AcOH	$Rh_2(OAc)_4$	17.5	80	0	0	98	2
6	LiBr	AcOH	-	0.6	89	100	0	0	0
7[b]	LiBr	AcOH	-	17.5	55	11	54	34	1
8[a]	AcOH	DCE	$Pd(OAc)_2$	4.7	92	0	0	100	0
9	LiOAc	AcOH	$Rh_2(OAc)_4$	3.2	74	91	0	9	0

a) Based on 100% being 100% recovery of brominated phenol **278** (entries 6-7) or acetylated phenol **279** (entries 1-5 and 8-9); b) Calculated based on ^{1}H NMR integrations.

The reaction performed solely in acetic acid (entry 1) gave the product as a mixture of phenol **279**, starting material **266** and a minor unidentified product in 52:35:13 ratio. The quinone diazide **266** was also stirred at 70 °C, for 4.5 h, in 1,2-dichloroethane, without any additives. The aim of this specific experiment was to ensure that none of the minor products/contaminants in the reactions carried out in this solvent would come from the reaction of the hypothesised carbene intermediate - formed after the diazo decomposition - and the solvent. The outcome of this reaction was a complex mixture of products, with no evidence of formation of the previously observed products. In entries 3-5, the effect of using the metal catalyst (rhodium(II) or palladium(II) acetate) in acetic acid, was tested. The acetylated phenol **279** was obtained in 81% yield, after 4.7 h, when palladium(II) acetate was employed (entry

3). The use of rhodium(II) acetate led to the isolation of a mixture of phenol **279** and starting material **266** in 60:40 ratio, after 2.5 h (entry 4) or **279** and trace quantities of unidentified products, after 17.5 h (entry 5). In entries 6 and 7, the bromination of quinone diazide **266** was attempted without the addition of metal catalyst. After 35 min, the recovery of **266** in 89% yield may suggest no conversion at this point (entry 6). Prolonging the reaction time to 17.5 h gave the product as a mixture of **278, 266** and **279** besides trace quantities of two minor unidentified products (entry 7). Carrying out the palladium(II) mediated acetylation of **266**, with acetic acid in 1,2-dichloroethane gave **279** in 92% yield, after 4.7 h (entry 8). Considering entry 5 and previous evidence that acetylation of **266** would occur faster in the presence of a range of fluoride salts, especially lithium fluoride, the effect of using lithium acetate in the reaction was also evaluated. The product was isolated as a mixture of starting material **266** and acetylated phenol **279** in 91:9 ratio (entry 9).

Considering all the above-mentioned studies and with a set of optimised conditions in hand as a guide, the next step was the analysis of the substrate scope for this reaction.

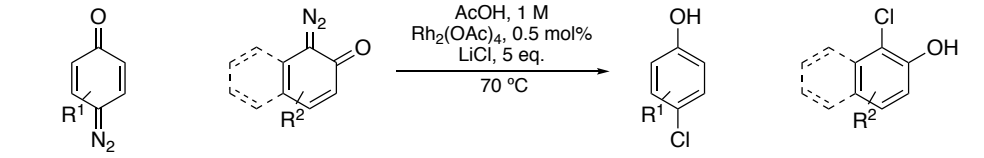
4.10 Substrate scope

Halogenated aromatic molecules provide important chemical precursors for the synthesis of valuable pharmaceuticals, agrochemicals, bioactive natural products, and photographic materials.[256–258] In addition, they have been broadly used as key intermediates in the preparation of organometallic reagents for the synthesis of a wide range of biologically active molecules and functional materials.[259–261] They have also found extensive utility as important starting materials in transition metal-mediated coupling reactions for the preparation of new molecules.[262–264] The Sandmeyer reaction is a classical and important organic transformation that converts an arylamine to an aryl halide using Cu(I) halide via a diazonium salt intermediate.[265–267] The reaction was first reported in 1884 and it is an example of a radical-nucleophilic aromatic substitution, with a number of named reactions closely related to it being developed and widely applied in organic synthesis throughout the 20th century.[268] Besides, the procedure that has been the subject of the above described optimisation studies can also be deemed similar to the Sandmeyer reaction.

To close the experimental optimisation studies the substrate scope for this reaction in the presence of three lithium halides (chloride, bromide and iodide) was analysed, with substrates ranging from 1,2-diazonaphthoquinone to 1,4-diazo-benzoquinones and 1,2-diazobenzo-quinones.

4.10.1 CHLORINATION

The reaction of five 1,2 and 1,4-benzoquinone diazides and the 1,2-naphthoquinone diazide with 5 equivalents of lithium chloride, in the presence of 0.5 mol% of rhodium(II) acetate is summarised in **Scheme 4.38** and **Table 4.12** below.



264 R^1 = 2-CO_2Me **273** R^2 = 4-OMe
265 R^1 = 2-Cl **274** R^2 = 4,5-naphtho
266 R^1 = 2,6-Cl_2
295 R^1 = 2-Br

0.50 mmol, 75.1 - 99.5 mg

296 R^1 = 2-CO_2Me **299** R^2 = 4-OMe
297 R^1 = 2-Cl **300** R^2 = 4,5-naphtho
280 R^1 = 2,6-Cl_2
298 R^1 = 2-Br

Scheme 4.38 Rhodium(II) mediated chlorination of quinone diazides.

Table 4.12 Effect of the substrate in the rhodium(II) mediated chlorination of quinone diazides.

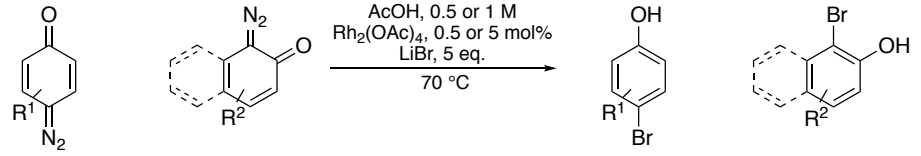
Entry	QD	R^1 or R^2	Time (min)	Phenol	Isolated yield (%)
1	**266**	2,6-Cl_2	127	**280**	89
2	**274**	4,5-naphtho	48	**300**	36
3	**265**	2-Cl	70	**297**	50
4	**295**	2-Br	21	**298**	97[a]

a) Unsuccessful purification of the crude material by column chromatography.

The reaction of substrates **266**, **268, 274** and **295** gave the corresponding chlorinated phenol or naphthol in low to good yields (36 - 89%). Unfortunately, purification of the phenol **298** was unsuccessful. When the reaction was attempted for substrates **264** and **273**, it led to a complex mixture of products, with no evidence of product formation from the ^{1}H NMR analysis of the crude material.

4.10.2 BROMINATION

The reaction of six 1,2 and 1,4-benzoquinone diazides and 1,2-naphthoquinone diazide **274** with lithium bromide, in the presence of 0.5 and 5 mol% of rhodium(II) acetate is summarised in **Scheme 4.39** and **Table 4.13** below.



264 R^1 = 2-CO_2Me **273** R^2 = 4-OMe
265 R^1 = 2-Cl **274** R^2 = 4,5-naphtho
267 R^1 = 3-Cl
268 R^1 = 3-Me
295 R^1 = 2-Br
0.50 mmol, 67.1 - 99.5 mg

301 R^1 = 2-CO_2Me **306** R^2 = 4-OMe
302 R^1 = 2-Cl **307** R^2 = 4,5-naphtho
303 R^1 = 3-Cl
304 R^1 = 3-Me
305 R^1 = 2-Br

Scheme 4.39 Rhodium(II) mediated bromination of quinone diazides.

Table 4.13 Effect of the substrate in the rhodium(II) mediated bromination of quinone diazides.

Entry	QD	R^1 or R^2	C_{sol} (M)	$Rh_2(OAc)_4$ (mol%)	Time (min)	Phenol	Isolated yield (%)
1	**268**	3-Me	1	0.5	23	**304**	71
2	**274**	4,5-naphtho	1	0.5	47	**307**	48
3	**274**	4,5-naphtho	0.5	5	41	**307**	84[a]
4	**265**	2-Cl	1	0.5	12	**302**	72
5	**265**	2-Cl	0.5	5	14	**302**	58
6	**264**	2-CO_2Me	1	0.5	110	**301**	83
7	**295**	2-Br	1	0.5	17	**305**	116[a]

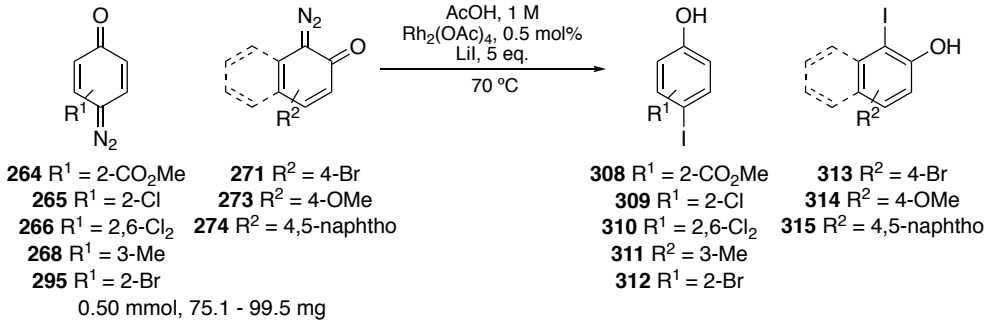
a) Unsuccessful purification of the crude material by column chromatography and isolated as **305/307**+acetylated phenol (95:5).

The reaction of substrate **268** in the presence of 0.5 mol% of rhodium(II) acetate led to the isolated of the corresponding brominated phenol **304**, in 71% yield (entry 1). When the reaction was attempted with 5 mol% of the catalyst and for a prolonged period of time, the product was isolated as an unidentified product or a complex mixture. Two reactions were attempted with substrate **274**, both in the presence of 0.5 and 5 mol% of the catalyst. Using a lower loading of the catalyst led to the isolation of the brominated naphthol **307** in 48% yield (entry 2). In the other reaction (entry 3) the crude material was isolated, in 84% recovery yield (based on 100% being 100% recovery of the brominated naphthol **307**) as a mixture of the naphthol **307** and the acetylated by-product, in a 95:5 ratio. Nonetheless, purification of the crude material failed and the same happened for the phenol **305** (entry 7). Quinone diazide **265** was also reacted in the presence of 0.5 and 5 mol% of the rhodium(II) catalyst (entries 4 and 5) to give the brominated phenol **302** in 72 and 58% yield, respectively. The phenol **301** was obtained in good yield, from the reaction of **264** in the presence of 0.5 mol% of the metal catalyst (entry 6). The reaction of substrate **295** yielded the phenol **305** (entry 7) however, purification of this product was unsuccessful.

One reaction using **273** was attempted however, from the analysis of the ^{1}H NMR data of the crude material it was not possible to identify the formation of the phenol **304**. Reacting quinone diazide **267** for an extended period of time gave a mixture of at least two unidentified products or recovery of starting material and a complex mixture of unidentified products (entries 4 and 6). The reaction of substrate **267** in the presence 5 mol% of rhodium(II) acetate for 6 min gave the final product as a mixture of starting material **270** and the phenol **303**, in 82:18 ratio and a recovery yield of 61% (based on 100% being 100% recovery of the phenol **303**).

4.10.3 IODINATION

The reaction of five 1,2 and 1,4-benzoquinone diazides and 1,2-naphthoquinone diazide with lithium iodide, in the presence of 0.5 mol% of rhodium(II) acetate is summarised in **Scheme 4.40** and **Table 4.14** below.



Scheme 4.40 Rhodium(II) mediated iodination of quinone diazides.

Table 4.14 Effect of the substrate in the rhodium(II) mediated iodination of quinone diazides.

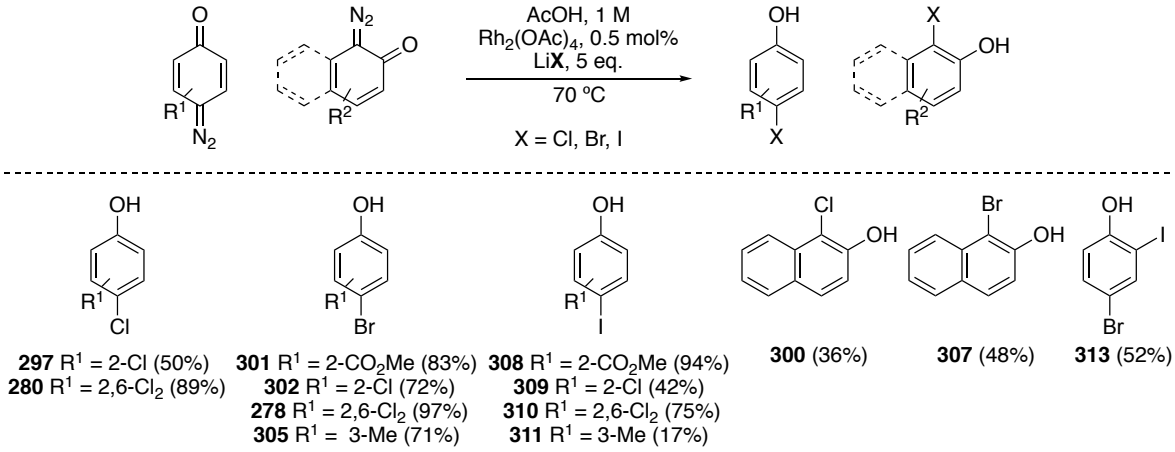
Entry	QD	R^1 or R^2	Time (min)	Product	Isolated yield (%)
1	**266**	2,6-Cl_2	35	**310**	75
2	**264**	2-CO_2Me	195	**308**	94
3	**274**	4,5-naphtho	46	**315**	110[a]
4	**274**	4,5-naphtho	215	**315**	77[a]
5	**265**	2-Cl	24	**309**	42
6	**295**	2-Br	17	**312**	89[a]
7	**268**	3-Me	78	**311**	17
8	**271**	4-Br	26	**313**	52

a) Unsuccessful purification of the crude material by column chromatography.

The reaction of **264-266, 265** and **271** in the above mentioned conditions led to the isolation of the corresponding iodinated phenols **308-311** and **313**, in low to excellent yields. When the same conditions were applied to substrates **271** and **295** the phenols **314** and **312** were also obtained. Unfortunately, purification of the crude material by column chromatography and recrystallisation proved to be unsuccessful. Finally, quinone diazide **273** was also tested but the product was isolated as a complex mixture from the ^{1}H NMR spectrum analysis of the crude material.

4.10.4 HALOGENATION SUMMARY

Below is a summary of the substrate scope conducted for this reaction (**Scheme 4.41**). On the whole, halogenated phenols were isolated in moderate to excellent yields. Unfortunately, some of the quinone diazides proved to be unreactive and other gave complex mixture of products. When 1,2-diazonaphthoquinone **274** was used as the substrate the yields were lower. Besides, when lithium iodide was used as used as iodide source, the reaction tended to be less selective and clean. Purification of these products by column chromatography was required for most derivatives and no further attempts to optimise the yields and broaden the substrate scope were taken.



Scheme 4.41 Rhodium(II) mediated halogenation of quinone diazides.

4.11 Conclusions

In general, efforts towards a more sustainable application of quinones diazides to the synthesis of phenols were made. In **Section 4.6** the successful preparation of quinone diazides from the diazotization of 2- or 4-amino phenols or 2-naphthol, usually in good yields, was presented. When 4-amino-1-naphthol was used, the product was isolated as the 1,4-naphthoquinone. In **Section 4.7** optimisation studies focusing in the reactivity of a quinone diazide towards

bromination were performed. Carrying out the reaction of quinone diazide with 5 eq. of nucleophile in acetic acid, and mediated by rhodium(II) acetate gave the best set of conditions, leading to faster reaction times than the reactions mediated by palladium(II) acetate and the product was isolated in excellent yield. Also, the concentration of the solution was increased from 0.5 to 1 M and the catalyst loading was reduced from 10 to 0.5 mol%. The nucleophile scope for the same starting material was then analysed (**Section 4.8**) and a range of alcohols, pseudo-halides, triphenyl phosphite, amines, etc. were tested. In general, only the reaction with alcohols showed promising results nevertheless, it was not possible to purify the final products. A few control reactions for this synthetic protocol were described in **Section 4.9** and finally, the substrate scope of the reaction towards halogenation (**Section 4.10**) was performed. At this stage, the reactivity of range of quinone diazides in the presence of lithium halides was evaluated and some problems arose again from the ineffective purification of some of the final products. Thirteen halogenated phenols were successfully prepared, typically in low to moderate yields, using the optimised set of conditions developed previously.

To sum up, this work can potentially serve as basis for further substrate and nucleophile optimisation studies. Nonetheless, alternative methods of purification may be required. Also, it might be worth to consider the scale up/out of the reaction and reduce the loading of metal catalyst used.

5 Experimental Procedures

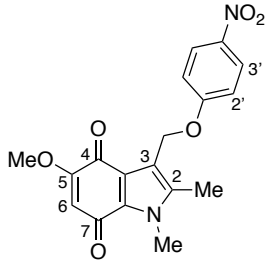
5.1 General experimental methods

Commercially available reagents were used throughout without purification. Melting points are uncorrected and were measured using an Electrothermal Digital IA9200 apparatus. Thin-layer chromatography was carried out with aluminium foil backed plates and visualised under UV light (at 254 nm) and/or by using permanganate stain. UV/Vis absorption spectra were measured with a Perkin-Elmer Lambda 25 UV/vis spectrometer. Infrared spectra were recorded on a Perkin Elmer 1600 series FT-IR spectrometer. Electrospray ionisation (ESI) mass spectra were measured on a Bruker MicroTOF spectrometer. NMR spectra were recorded at 400 or 500 MHz for ^{1}H NMR and at 100 or 125 MHz for ^{13}C NMR, and deuterated chloroform, dimethyl sulfoxide and acetone were used as solvents. Chemical shifts are quoted in parts per million (ppm) and are referenced to residual H in the deuterated solvent as the internal standard. The multiplicity of each signal is given as the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet and br, broad. Assignments were made with the use of correlation techniques such as NOESY, HSQC and HMBC spectra. *J* values are recorded in Hertz (Hz).

FAD, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), menadione were obtained from Sigma (St. Louis, MO). Recombinant human NQO2 (rhNQO2) was purchased from Sigma, dissolved in 250 mM sucrose, and stored at -80 °C. Dihydronicotinamide riboside (NRH) was prepared from NADH using previously reported methods.[20,269]

5.2 Syntheses and Biological Assays Chapter 2

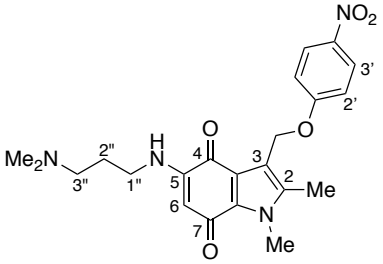
5-Methoxy-1,2-dimethyl-3-((4-nitrophenoxy)methyl)-1H-indole-4,7-dione 44



Thionyl chloride (0.398 g, 3.34 mmol) was added dropwise to a stirred solution of the 5-methoxy-hydroxymethyl-1,2-dimethylindole-4,7-dione **43** (70.0 mg, 0.297 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was stirred at rt for 2.5 h. The solvent and excess thionyl chloride were removed under reduced pressure and the crude product was used directly in the next step without further purification. A suspension of the crude 3-chloromethylindolequinone in dimethylformamide (3 mL), 4-nitrophenol (62.4 mg, 0.448 mmol) and potassium carbonate (70.3 mg, 0.509 mmol) was

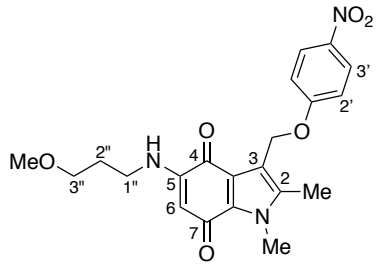
stirred at rt for 17.7 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (30 mL). The dichloromethane solution was washed with sodium hydroxide (2 M, 15 mL), hydrochloric acid (2 M, 15 mL) and water (15 mL), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography eluting with ethyl acetate/light petroleum (50:50) to give the *title compound* as an orange solid (47.8 mg, 45%); mp 234-235 °C (lit.,[270] mp 227-288 °C); (Found: M+Na^+, 379.0901. $C_{18}H_{16}N_2O_6Na^+$ requires 379.0901); ν_{max} (solid)/cm^{-1} 3046, 2940, 2845, 1731, 1666, 1633, 1591, 1505, 1494, 1459, 1442, 1414, 1378, 1333; δ_H (500 MHz; $CDCl_3$) 8.19 (2H, d, *J* 7.0, H-3'), 7.05 (2H, d, *J* 7.0, H-2'), 5.64 (1H, s, H-6), 5.40 (2H, s, -OC$\underline{H}_2$), 3.91 (3H, s, -NC$\underline{H}_3$), 3.82 (3H, s, -OC$\underline{H}_3$), 2.32 (3H, s, -C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 179.1 (C1 or C4), 178.7 (C1 or C4), 164.0 (C1'), 156.0 (C5), 142.0 (C4'), 138.5 (C2), 129.4 (C7a), 126.3 (C3'), 121.6 (C3), 116.1 (C3a), 115.3 (C2'), 107.2 (C6), 61.4 (-O$\underline{C}H_2$), 56.9 (-O$\underline{C}H_3$), 32.9 (-N$\underline{C}H_3$), 10.3 (-$\underline{C}H_3$).

5-((3-(Dimethylamino)propyl)amino)-1,2-dimethyl-3-((4-nitrophenoxy)methyl)-*1H*-indole-4,7-dione 45



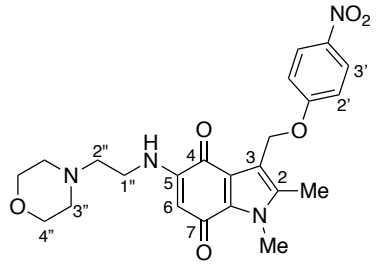
A solution of 5-methoxy-1,2-dimethyl-3-(1-(4-nitrophenoxy)-ethyl)-*1H*-indole-4,7-dione **204** (20.0 mg, 56.1 μmol) in acetonitrile (0.5 mL) was treated with N^1,N^1-dimethylpropane-1,3-diamine (100 μL, 779 μmol). The mixture was stirred at rt for 98 h. The solvent and excess amine were removed under reduced pressure and the residue was extracted with dichloromethane (20 mL), washed with water (3 x 15 mL), dried (Na_2SO_4), filtered and the filtrate concentrated under reduced pressure to give the *title compound* as a purple solid (21.2 mg, 89%); mp 67-68 °C; (Found: M+H^+, 427.1995. $C_{22}H_{27}N_4O_5^+$ requires 427.1976); λ_{max} (MeOH)/nm 201 (log ε 4.65), 241 (log ε 4.31), 311 (log ε 4.29), 525 (log ε 3.41); ν_{max} (solid)/cm^{-1} 3212, 2933, 2858, 2821, 2770, 1667, 1589, 1494, 1457, 1412, 1380, 1334; δ_H (500 MHz; $CDCl_3$) 8.19 (2H, d, *J* 7.5, H-3'), 7.06 (2H, d, *J* 7.5, H-2'), 6.87 (1H, t, *J* 5.5, -N$\underline{H}$), 5.35 (2H, s, -OC$\underline{H}_2$), 5.14 (1H, s, H-6), 3.93 (3H, s, -NC$\underline{H}_3$), 3.19 (2H, q, *J* 6.5, H-3"), 2.39 (2H, t, *J* 6.5, H-1"), 2.27 (3H, s, -C$\underline{H}_3$), 2.25 (6H, s, N(C$\underline{H}_3$)$_2$), 1.80 (2H, quint, *J* 6.5, H-2"); δ_C (125 MHz; $CDCl_3$) 179.5 (C1 or C4), 179.2 (C1 or C4), 164.2 (C1'), 149.0 (C5), 141.9 (C4'), 136.4 (C2), 131.8 (C7a), 126.3 (C3'), 119.7 (C3), 115.2 (C2'), 114.9 (C3a), 96.8 (C6), 61.8 (-O$\underline{C}H_2$), 58.3 (C3"), 45.8 (-N($\underline{C}H_3$)$_2$), 42.6 (C1"), 32.8 (-N$\underline{C}H_3$), 25.8 (C2"), 10.2 (-$\underline{C}H_3$).

5-((3-Methoxypropyl)amino)-1,2-dimethyl-3-((4-nitrophenoxy)methyl)-*1H*-indole-4,7-dione 46



A solution of 5-methoxy-1,2-dimethyl-3-(1-(4-nitrophenoxy)-ethyl)-*1H*-indole-4,7-dione **204** (14.1 mg, 39.6 μmol) in acetonitrile (0.4 mL) was treated with 3-methoxypropan-1-amine (100 μL, 971 μmol). The mixture was stirred at rt for 5 days. The solvent and excess amine were removed under reduced pressure and the residue was extracted with dichloromethane (30 mL), washed with water (4 x 20 mL), dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography, eluting with a 50-60% gradient ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (14.6 mg, 89%); mp 155-157 °C; (Found: M+H$^+$, 414.1655. $C_{21}H_{24}N_3O_6^+$ requires 414.1660); λ_{max} (MeOH)/nm 219 (log ε 4.23), 242 (log ε 4.22), 311 (log ε 4.26), 528 (log ε 3.19); ν_{max} (solid)/cm^{-1} 3342, 2926, 2884, 2830, 1738, 1661, 1581, 1495, 1459, 1382, 1342 ; δ_H (500 MHz; $CDCl_3$) 8.20 (2H, d, *J* 9.0, H-3'), 7.05 (2H, d, *J* 9.0, H-2'), 5.34 (2H, s, -OC$\underline{H}_2$), 5.16 (1H, s, H-6), 3.96 (3H, s, -NC$\underline{H}_3$), 3.49 (2H, t, *J* 6.0, H-3"), 3.38 (3H, s, -OC$\underline{H}_3$), 3.23 (2H, q, *J* 6.0, H-1"), 2.28 (3H, s, -C$\underline{H}_3$), 1.91 (2H, quint, *J* 6.0, H-2"); δ_C (125 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.2 (C1 or C4), 164.2 (C1'), 148.7 (C5), 141.9 (C4'), 136.5 (C2), 131.8 (C7a), 126.3 (C3'), 119.7 (C3), 115.2 (C2'), 114.9 (C3a), 97.0 (C6), 71.1 (-O$\underline{C}H_3$), 61.8 (-O$\underline{C}H_2$), 59.3 (C3"), 41.3 (C1"), 32.8 (-N$\underline{C}H_3$), 28.5 (C2"), 10.2 (-$\underline{C}H_3$).

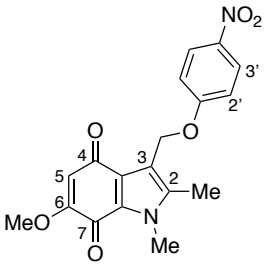
1,2-Dimethyl-5-((2-morpholinoethyl)amino)-3-((4-nitrophenoxy)methyl)-*1H*-indole-4,7-dione 47



A solution of 5-methoxy-1,2-dimethyl-3-((4-nitrophenoxy)-methyl)-*1H*-indole-4,7-dione **44** (15.8 mg, 42.1 μmol) in acetonitrile (0.7 mL) was treated with 2-morpholinoethan-1-amine (100 μL, 754 μmol). The mixture was stirred at rt for 4 days. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with a 50-60% gradient ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (13.7 mg, 72%); (Found: M+H$^+$, 455.1932. $C_{23}H_{27}N_4O_6^+$ requires 455.1925); δ_H (500 MHz; $CDCl_3$) 8.20 (2H, d, *J* 9.0, H-3'), 7.06 (2H, d, *J* 9.0, H-2'), 6.37 (1H, br s, -N$\underline{H}$), 5.35 (2H, s, -OC$\underline{H}_2$), 5.14 (1H, s, H-6), 3.93 (3H, s, -NC$\underline{H}_3$), 3.72 (4H, t, *J* 4.5, H-4"), 3.15 (2H, q, *J* 6.0, H-1"), 2.65 (2H, t, *J* 6.0, H-2"), 2.47 (4H, t, *J* 4.5, H-3"), 2.28 (3H, s, -C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.2 (C1 or C4), 164.1

(C1'), 148.5 (C5), 142.0 (C4'), 136.6 (C2), 131.7 (C7a), 126.4 (C3'), 119.8 (C3), 115.2 (C2'), 115.0 (C3a), 97.5 (C6), 67.3 (-O<u>C</u>H$_2$), 61.7 (C4"), 56.1 (C2"), 53.6 (C3"), 39.1 (C1"), 32.8 (-N<u>C</u>H$_3$), 10.2 (-<u>C</u>H$_3$).

6-Methoxy-1,2-dimethyl-3-((4-nitrophenoxy)methyl)-*1H*-indole-4,7-dione 50

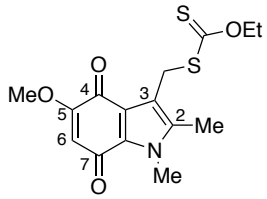


<u>Ester reduction</u>: A solution of sodium dithionite (0.419 g, 2.04 mmol) in water (3.5 mL) was added to a solution of methyl 6-methoxy-1,2-dimethyl-4,7-dioxo-4,7-dihydro-*1H*-indole-3-carboxylate **48** (0.108 g, 0.409 mmol) in chloroform (3.5 mL). The resulting mixture was stirred at rt for 1.2 h. The reaction was diluted with dichloromethane (10 mL), extracted with dichloromethane (2 x 10 mL). The combined organic phases were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a beige residue. Lithium aluminium hydride (78.6 mg, 2.04 mmol) was added portion wise to a suspension of the crude material in THF (6 mL). The resulting mixture was stirred at rt for 1 h and sequentially quenched with ethyl acetate (10 mL), water (10 mL), NaOH (aq., 2 M, 5 mL) and SiO_2 (100 mg). The mixture was filtered, concentrated and the residue was purified by column chromatography, eluting with dichloromethane/ethyl acetate (83:17) to give 6-methoxy-hydroxymethyl-1,2-dimethylindole-4,7-dione **49** as an orange solid (49.2 mg, 51%); mp 196-198 °C (lit.,[13] mp 197-199 °C (from dichloromethane/light petroleum)); (Found: M+Na$^+$, 258.0741. $C_{12}H_{13}NO_4Na^+$ requires 258.0737); ν_{max} (solid)/cm^{-1} 3358, 3054, 2942, 2842, 1737, 1656, 1636, 1613, 1596, 1543, 1499, 1485, 1455, 1380, 1336; δ_H (500 MHz; $CDCl_3$) 5.70 (1H, s, H-5), 4.60 (2H, d, *J* 7.0, -C<u>H</u>$_2$), 4.51 (1H, t, *J* 7.0, -O<u>H</u>), 3.90 (3H, s, -NC<u>H</u>$_3$), 3.84 (3H, s, -OC<u>H</u>$_3$), 2.24 (3H, s, -C<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 186.0 (C1 or C4), 171.4 (C1 or C4), 160.9 (C6), 137.1 (C2), 127.9 (C7a), 125.2 (C3), 123.8 (C3a), 106.5 (C5), 57.1 (-O<u>C</u>H$_3$), 56.4 (-<u>C</u>H$_2$OH), 33.1 (-N<u>C</u>H$_3$), 10.3 (-<u>C</u>H$_3$).

<u>4-nitrophenoxide attachment</u>: Thionyl chloride (0.207 g, 1.74 mmol) was added dropwise to a stirred solution of the 6-methoxy-hydroxymethyl-1,2-dimethylindole-4,7-dione **49** (23.5 mg, 0.100 mmol) in dichloromethane (3 mL) at 0 °C. The reaction mixture was stirred at rt for 2 h. The solvent and excess thionyl chloride were removed under reduced pressure and the crude product was used directly in the next step without further purification. A suspension of the crude 3-chloromethylindolequinone in dimethylformamide (2 mL), 4-nitrophenol (20.6 mg, 0.148 mmol) and potassium carbonate (23.9 mg, 0.173 mmol) was stirred at rt for 17.3 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (20 mL). The dichloromethane was washed with sodium hydroxide (2 M, 10 mL), hydrochloric acid (2 M, 10 mL), and water (10 mL), dried ($MgSO_4$), and concentrated

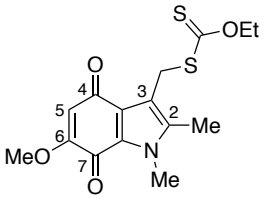
under reduced pressure. The residue was purified by chromatography eluting with ethyl acetate/light petroleum (50:50) to give the *title compound* as a yellow-orange solid (23.7 mg, 66%); mp 205-208 °C (lit.,[271] mp 213-215 °C (dec. from methanol)); (Found: M+Na$^+$, 379.0898. $C_{18}H_{16}N_2O_6Na^+$ requires 379.0901); ν_{max} (solid)/cm^{-1} 3116, 2920, 2848, 1715, 1666, 1649, 1591, 1548, 1507, 1492, 1463, 1438, 1377, 1341, 1332; δ_H (500 MHz; $CDCl_3$) 8.18 (2H, d, *J* 9.0, H-3'), 7.06 (2H, d, *J* 9.0, H-2'), 5.68 (1H, s, H-5), 5.43 (2H, s, -OC$\underline{H}_2$), 3.92 (3H, s, -NC$\underline{H}_3$), 3.83 (3H, s, -OC$\underline{H}_3$), 2.33 (3H, s, -C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 184.9 (C1 or C4), 171.8 (C1 or C4), 163.9 (C1'), 160.4 (C6), 142.0 (C4'), 140.7 (C2), 127.6 (C7a), 126.3 (C3'), 124.3 (C3), 116.4 (C3a), 115.3 (C2'), 107.1 (C5), 61.4 (-O$\underline{C}H_2$), 57.1 (-O$\underline{C}H_3$), 33.1(-N$\underline{C}H_3$), 10.6 (-$\underline{C}H_3$).

_O_-Ethyl _S_-((5-methoxy-1,2-dimethyl-4,7-dioxo-4,7-dihydro-_1H_-indol-3-yl)methyl)carbonodithioate 51



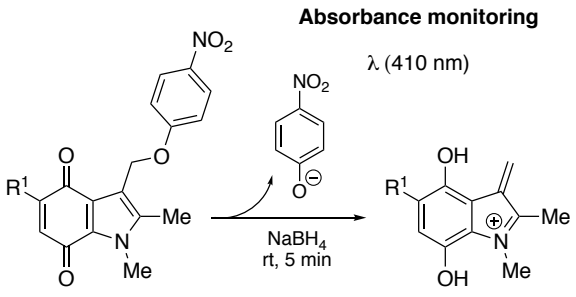
A suspension of 5-methoxy-1,2-dimethyl-3-((4-nitrophenoxy)methyl)-*1H*-indole-4,7-dione **44** (14.8 mg, 41.5 μmol) in THF/water (3:1, 10 mL) was stirred at rt and degassed by bubbling argon directly into the stirring solution. After 5 min, potassium *O*-ethyl xanthate (35.4 mg, 212 μmol) was added and the resulting mixture stirred at rt. After 5 min, sodium dithionite (44.4 mg, 217 μmol) was added and the reaction mixture was stirred under Ar, at rt for 30 min. The mixture was concentrated under reduced pressure and the residue dissolved in dichloromethane (20 mL) and washed with NH_4Cl (aq. sat., 2 x 10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure and the residue was purified by column chromatography, eluting with dichloromethane/ethyl acetate (50:50) to give the *title compound* as an orange solid (6.6 mg, 47%); mp 205-207 °C (lit.,[58] mp 205-207 °C); (Found: M+Na$^+$, 362.0489. $C_{15}H_{17}NO_4S_2Na^+$ requires 362.0491); δ_H (500 MHz; $CDCl_3$) 5.61(1H, s, H-6), 4.66 (2H, q, *J* 7.0, -OC$\underline{H}_2$), 4.58 (2H, s, -SC$\underline{H}_2$), 3.88 (3H, s, -OC$\underline{H}_3$), 3.80 (3H, s, -NC$\underline{H}_3$), 2.29 (3H, s, -C$\underline{H}_3$), 1.41 (3H, t, *J* 7.0, -CH$_2$C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 215.3 (CS), 179.0 (C1 or C4), 178.1 (C1 or C4), 160.0 (C5), 137.0 (C2), 129.3 (C7a), 121.6 (C3), 116.4 (C3a), 107.2 (C6), 70.4 (-O$\underline{C}$H$_2$CH$_3$), 56.8 (-O$\underline{C}H_3$), 32.9 (-N$\underline{C}H_3$), 30.9 (-S$\underline{C}H_2$), 14.3 (-CH$_2\underline{C}H_3$), 10.4 (-$\underline{C}H_3$).

_O_-Ethyl _S_-((6-methoxy-1,2-dimethyl-4,7-dioxo-4,7-dihydro-_1H_-indol-3-yl)methyl)carbonodithioate 52



A suspension of 6-methoxy-1,2-dimethyl-3-((4-nitrophenoxy)methyl)-*1H*-indole-4,7-dione **50** (20.7 mg, 58.1 μmol) in THF/water (75:25, 10 mL) was stirred at rt and degassed by bubbling argon directly into the stirring solution. After 5 min, potassium *O*-ethyl xanthate (49.0 mg, 290 μmol) was added and the resulting mixture stirred at rt. After 5 min, sodium dithionite (59.7 mg, 290 μmol) was added and the reaction mixture was stirred under Ar, at rt for 30 min. The mixture was concentrated under reduced pressure and the residue dissolved in dichloromethane (20 mL) and washed with NH_4Cl (aq. sat., 2 x 10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure and the residue was purified by column chromatography, eluting with dichloromethane/ethyl acetate (50:50) to give the *title compound* as an orange solid (17.0 mg, 86%); mp 159-162 °C; (Found: M+Na^+, 362.0484. $C_{15}H_{17}NO_4S_2Na^+$ requires 362.0491); δ_H (500 MHz; $CDCl_3$) 5.64 (1H, s, H-5), 4.66 (2H, q, *J* 7.0, -OC<u>H</u>$_2$), 4.59 (2H, s, -SC<u>H</u>$_2$), 3.90 (3H, s, -OC<u>H</u>$_3$), 3.80 (3H, s, -NC<u>H</u>$_3$), 2.31 (3H, s, -C<u>H</u>$_3$), 1.42 (3H, t, *J* 7.0, -CH_2C<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 215.3 (CS), 184.3 (C1 or C4), 171.6 (C1 or C4), 160.3 (C6), 139.4 (C2), 127.6 (C7a), 124.2 (C3), 116.5 (C3a), 107.0 (C6), 70.3 (-O<u>C</u>H_2CH_3), 57.0 (-O<u>C</u>H_3), 33.1 (-N<u>C</u>H_3), 30.8 (-S<u>C</u>H_2), 14.2 (-CH_2<u>C</u>H_3), 10.6 (-<u>C</u>H_3).

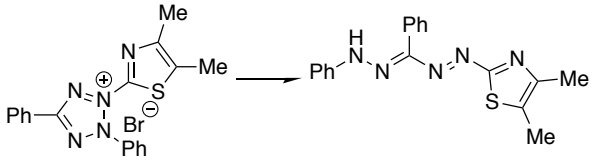
5.2.1 4-NITROPHENOL RELEASING FROM IQS FOLLOWING REDUCTION BY $NaBH_4$



44 R^1 = OMe
46 R^1 = NH$(CH_2)_3$OMe

Potassium phosphate buffer (pH 7.4, 50 mM, 990 μL) and 4-(nitrophenoxy)methyl-*1H*-indole-4,7-dione solution (5 mM in DMSO, 10 μL) were added to a quartz cuvette, mixed and used as a reference solution. Potassium phosphate buffer (pH 7.4, 50 mM, 965 μL), 4-(nitrophenoxymethyl)-*1H*-indole-4,7-dione **44** and 5-((3-methoxypropyl)amino)-1,2-dimethyl-3-((4-nitrophenoxy)methyl)-1*H*-indole-4,7-dione **46** solutions (5 mM in DMSO, 10 μL) were added to another quartz cuvette and mixed. Then, $NaBH_4$ (12.5 mg/mL in H_2O, 25 μL) was added, mixed and used as reaction solution. The increase in absorbance was monitored spectrophotometrically at 410 nm, over 5 min at rt.

5.2.2 CATALYTIC ACTIVITY



60 MTT **61** Formazan

A mixture of human recombinant NQO2 (rhNQO2) (4 µg/mL, 1 µL), potassium phosphate buffer (pH 7.4, 50 mM, containing 250 mM sucrose, 946 µL), FAD (5 mM in H_2O, 1 µL), Tween 20 (0.1% v/v, 1 µL), MTT (5 mg/mL in H_2O, 50 µL) and menadione (10 mM in DMSO, 1 µL) were added to a quartz cuvette, mixed and used as a reference solution. A mixture of human recombinant NQO2 (rhNQO2) (4 µg/mL, 1 µL), potassium phosphate buffer (pH 7.4, 50 mM, containing 250 mM sucrose, 906 µL), FAD (5 mM in H_2O, 1 µL), Tween 20 (0.1% v/v, 1 µL), MTT (5 mg/mL in H_2O, 50 µL), menadione (10 mM in DMSO, 1 µL) and NRH (5 mM, 40 µL) were added to another quartz cuvette, mixed and used as reaction solution. The linear increase in absorbance was monitored spectrophotometrically at 550 nm, over 2 min at rt.

5.2.3 HPLC AND MASS SPECTROMETRY ANALYSIS OF FAD ADDUCTS

Indolequinone-inactivated human recombinant NQO2 protein for HPLC and MS analysis was prepared by incubating rhNQO2 (25 µg) with a 10-fold molar excess of indolequinones in potassium phosphate buffer (pH 7.4, 25 mM, final volume of 40 µL) in the presence or absence of NRH (0.5 mM). Reactions were stopped after 10 min by the addition of ice-cold methanol (60 µL) to denature NQO2 and release FAD. Precipitated protein was removed by centrifugation (13000 rpm for 5 min at 4 °C), and the supernatant (100 µL) was then transferred to LC-MS vials for analysis. Part of this solution (10 µL) was loaded onto a C18 reverse-phase HPLC column [X-Select HSS T3 3.5 µm, 2.1 x 50 mm] and eluted at a rate of 0.5 mL/min with a mobile phase of 10 mM ammonium acetate (A) and 95% acetonitrile (B) with 100% A (0 - 1 min), a linear gradient from 100% A to 100% B (1 - 16.5 min) and 100% A (16.5 - 17 min) using a detection wavelength of 220 nm and the reference set at 500 nm. The conditions are compiled in **Table 5.1** and **Table 5.2**.Ion chromatograms of the appropriate MS $[M+H]^+$ ion for each component were generated and integrated using MassLynx 4.1 software (Waters).

Table 5.1 HPLC -MS conditions for the analysis of the reaction between NQO2 and **37**.

HPLC Apparatus	Agilent 1100 Series - LC
Column	[X-Select HSS T3 3.5 μm, 2.1 x 50 mm]
Column temperature	25
Mobile phase A	10 mM ammonium acetate
Mobile phase B	95% acetonitrile
Flow rate (mL/min)	0.5
Injection volume (μL)	10
MS Apparatus	Waters Synapt HDMS
Capillary voltage (kV)	2.8
Sampling cone (V)	25.0
Polarity	+ve
Source Temperature (°C)	150
Desolvation Gas Flow (mL/h)	600
Desolvation Temperature (°C)	500
Acquisition range (m/z)	300-1200

Table 5.2 Gradient for HPLC -MS analysis of the reaction between NQO2 and **37**.

Time (min)	Gradient A (%)	Gradient B (%)
0	100	0
1	100	0
16.5	0	100
17	100	0

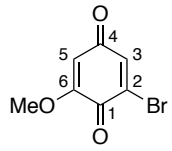
5.2.4 INHIBITOR SCREENING FOR NQO2

Mechanism-based inactivation of NQO2 by the indolequinone series was assayed using purified human recombinant NQO2 (rhNQO2). In these reactions (final volume of 0.5 mL), rhNQO2 (4 μg/mL) was incubated with indolequinone (0.1 - 10.0 μM) in the absence and presence of NRH (0.2 mM) in potassium phosphate buffer (pH 7.4, 50 mM) containing FAD (5 μM), NaCl (125 mM), and bovine serum albumin (1 mg/mL) at room temperature. After 5 min, an aliquot (50 μL) was removed and diluted 50-fold in stop buffer [50 mM potassium phosphate buffer (pH 7.4) containing sucrose (250 mM), FAD (5 μM), Tween 20 (0.1% (v/v)) and MTT (0.25 mg/mL)]. The linear increase in absorbance was monitored spectrophotometrically at 550 nm, over 2 min at rt. NQO2 activity was measured as NRH-dependent menadione reductase activity using MTT as the final electron acceptor.[37]

5.3 Syntheses Chapter 3

5.3.1 Synthesis of 2-bromo-1,4-benzoquinones

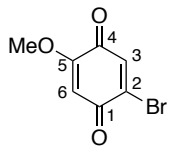
2-Bromo-6-methoxy-1,4-benzoquinone 113



a) Sodium percarbonate (8.24 g, 52.5 mmol) was added as a single portion to a stirred solution of 5-bromovanillin **89** (11.60 g, 50.2 mmol) in THF (150 mL) and water (60 mL). The resulting mixture was stirred at rt for 5 h, concentrated under reduced pressure to approximately one-third of its volume. The resulting mixture was filtered, washed with water (30 mL) and methanol (10 mL) to give 2-bromo-6-methoxy-1,4-hydroquinone[272] as a pale grey solid (9.44 g, 86%); δ_H (400 MHz; acetone-d_6) 8.19 (1H, br s, OH), 7.54 (1H, br s, -O<u>H</u>), 6.59 (1H, d, *J* 2.4, H-3), 6.50 (1H, d, J 2.4, H-5), 3.82 (3H, s, -OC<u>H</u>$_3$); δ_C (100 MHz; acetone-d_6) 150.7 (C4), 148.7 (C6), 137.3 (C1), 110.0 (C3), 108.1 (C2), 99.7 (C5), 55.6 (-O<u>C</u>H$_3$), which was used directly in the next step.

(b) A solution of iron(III) chloride hexahydrate (57.2 g, 216 mmol) in water (270 mL) was added as a single portion to a stirred solution of 2-bromo-6-methoxy-1,4-hydroquinone (9.44 g, 43.1 mmol) in methanol (35 mL). The resulting mixture was stirred at rt for 5 h, and the precipitate was filtered, washed with water to give the *title compound* as an orange solid (9.11 g, 97%); mp 154-156 °C (lit.,[273] mp 162-163 °C); ν_{max} (solid)/cm^{-1} 2987, 1688, 1636, 1581, 1450, 1352, 1311; δ_H (400 MHz; $CDCl_3$) 7.22 (1H, d, *J* 2.4, H-3), 5.98 (1H, d, *J* 2.4, H-5), 3.88 (3H, s, OC<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 184.7 (C4), 174.6 (C1), 158.3 (C6), 138.5 (C3), 134.1 (C2), 107.6 (C5), 56.8 (-O<u>C</u>H$_3$).

2-Bromo-5-methoxy-1,4-benzoquinone 156



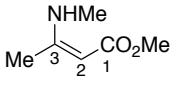
a) A solution of bromine (3.24 mL, 63.2 mmol) in dichloromethane (50 mL) was added dropwise at 0 ºC to a stirred solution of 1,2,4-trimethoxybenzene **87** (10.10 g, 60.0 mmol) in dichloromethane (200 mL) over 40 min. The resulting mixture was stirred at 0 ºC for 5 min, washed sequentially with saturated aqueous sodium thiosulfate solution (400 mL), saturated aqueous sodium hydrogen carbonate solution (100 mL) and brine (200 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give 1-bromo-2,4,5-trimethyoxybenzene as a pale-yellow solid (14.07 g, 95%), which was used directly in the next step.

b) A solution of cerium(IV) ammonium nitrate (78.11 g, 142 mmol) in water (380 mL) was added to a stirred solution of 1-bromo-2,4,5-trimethyoxybenzene (14.05 g, 56.9 mmol) at rt. The resulting mixture was stirred at rt for 15 h, filtered and washed with water (40 mL) to give the *title compound* as a yellow solid (12.03 g, 98%); mp 183-184 ºC (lit.,[273] mp 192-194 °C);

(Found: M+H$^+$, 216.9490. $C_7H_6{}^{79}BrO_3^+$ requires 216.9495); ν_{max} (solid)/cm^{-1} 3043, 1668, 1649, 1622, 1583, 1461, 1437, 1366, 1324, 1266; δ_H (400 MHz; $CDCl_3$) 7.28 (1H, s, H-3), 6.15 (1H, s, H-6), 3.88 (3H, s, OC$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 180.0 (C1 or C4), 179.2 (C1 or C4), 159.0 (C5), 139.2 (C2), 135.8 (C3), 106.9 (C6), 56.7 (-O$\underline{C}H_3$).

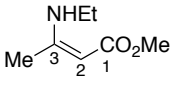
5.3.2 SYNTHESIS OF ENAMINES

Methyl (*Z*)-3-(methylamino)but-2-enoate 158



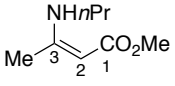
Silica gel (1.1 g) was added in a single portion to a stirred solution of methylamine (25% w/v in water, 15 mL, 120 mmol) and methyl acetoacetate (11.5 g, 100 mmol) at rt, and the resulting mixture was stirred at rt for 17 h. The mixture was filtered and washed with water and methanol to give the *title compound* as a colourless solid (11.97 g, 92%); mp 61-63 ºC (lit.,[274] mp 65-67 °C); (Found: M+H$^+$, 130.0876. $C_6H_{12}NO_2^+$ requires 130.0863); ν_{max} (solid)/cm^{-1} 3310, 2949, 2080, 1640, 1594, 1509, 1438, 1333, 1267; δ_H (400 MHz; $CDCl_3$) 8.49 (1H, br s, -N$\underline{H}$), 4.46 (1H, s, H-2), 3.61 (3H, s, -OC$\underline{H}_3$), 2.90 (3H, d, *J* 5.2, -NHC$\underline{H}_3$), 1.91 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 170.9 (C1), 162.9 (C3), 81.4 (C2), 49.9 (-O$\underline{C}H_3$), 29.5 (-NH$\underline{C}H_3$), 19.2 (-$\underline{C}H_3$).

Methyl (*Z*)-3-(ethylamino)but-2-enoate 159



A solution of ethylamine (70% w/w in water, 8.5 mL, 107 mmol) was added in a single portion to a stirred suspension of methyl acetoacetate (11.5 g, 100 mmol) and silica gel (1 g) at rt. The resulting mixture was stirred at rt for 18.5 h. The mixture was extracted with ethyl acetate (3 x 10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* as a yellow liquid (14.1 g, 99%); (Found: M+Na$^+$, 166.0834. $C_7H_{13}NO_2Na^+$ requires 166.0838); ν_{max} (thin film)/cm^{-1} 3286, 2974, 2947, 2877, 2327, 2097, 1689, 1650, 1599, 1537, 1502, 1476, 1440, 1382, 1306, 1255; δ_H (400 MHz; $CDCl_3$) 8.45 (1H, br s, -N$\underline{H}$), 4.41 (1H, s, H-2), 3.60 (3H, s, -OC$\underline{H}_3$), 3.23 (2H, dq, *J* 7.2, -NHC$\underline{H}_2$), 1.90 (3H, s, -C$\underline{H}_3$), 1.19 (3H, q, *J* 7.2, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 170.9 (C1), 162.9 (C3), 81.4 (C2), 49.9 (-O$\underline{C}H_3$), 38.0 (-NH$\underline{C}H_2$), 29.5 (-$CH_2\underline{C}H_3$), 19.2 (-$\underline{C}H_3$).[275]

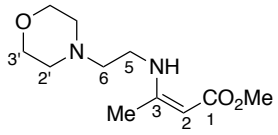
Methyl (*Z*)-3-(propylamino)but-2-enoate 160



Silica gel (1 g) was added in a single portion to a stirred solution of *n*-propylamine (10 mL, 122 mmol) and methyl acetoacetate (11.5 g, 100 mmol) at rt. The resulting mixture was stirred at rt for 24 h after the addition of water (1 mL) then at 40 ºC for 6 days and 60 ºC for 24 h. The mixture was extracted with dichloromethane (2 x 20

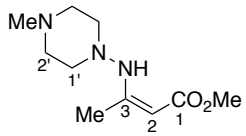
mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* as an orange liquid (9.26 g, 65%); (Found: M+H$^+$, 158.1181. $C_8H_{16}NO_2^+$ requires 158.1176); ν_{max} (solid)/cm^{-1} 2963, 1650, 1600, 1500, 1440, 1266; δ_H (400 MHz; $CDCl_3$) 8.54 (1H, br s, -NH), 4.43 (1H, s, H-2), 3.61 (3H, s, -OC$\underline{H}_3$), 3.17 (2H, dt, *J* 6.8, 5.6, H-5), 1.91 (3H, s, -C$\underline{H}_3$), 1.58 (2H, tq, *J* 7.6, 6.8, H-6), 0.97 (3H, t, *J* 7.6, H-7); δ_C (100 MHz; $CDCl_3$) 170.9 (C1), 162.1 (C3), 81.3 (C2), 49.8 (-O$\underline{C}H_3$), 44.7 (-NH$\underline{C}H_2$), 23.3 (-$\underline{C}H_2$), 19.3 (-$\underline{C}H_3$), 11.3 (-$\underline{C}H_3$).[275]

Methyl (*Z*)-3-((2-morpholinoethyl)amino)but-2-enoate 161



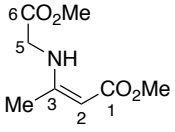
A solution of 2-morpholinoethan-1-amine (1.40 mL, 10.0 mmol) was added in a single portion to a stirred suspension of methyl acetoacetate (1.16 g, 10.0 mmol) and silica gel (0.203 g) at rt. The resulting mixture was stirred at rt for 18.8 h. The mixture was extracted with ethyl acetate (3 x 10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* as a yellow liquid (2.24 g, 98%); (Found M+H$^+$, 229.1551. $C_{11}H_{21}N_2O_3^+$ requires 229.1547); ν_{max} (thin film)/cm^{-1} 2946, 2855, 2809, 1652, 1593, 1500, 1442, 1385, 1358, 1273, 1252, 1230; δ_H (400 MHz; $CDCl_3$) 8.61 (1H, br s, -NH), 4.44 (1H, s, H-2), 3.71 (4H, t, *J* 4.8, H-3'), 3.30 (2H, q, *J* 6.4, H-5), 2.52 (4H, d, *J* 6.4, H-6), 2.46 (2H, t, *J* 4.8, H-2'), 1.91 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 170.8 (C1), 161.4 (C3), 888 (C2), 66.9 (C3'), 58.2 (C6), 53.7 (C2'), 50.0 (-O$\underline{C}H_3$), 40.1 (C5), 19.7 (-$\underline{C}H_3$).[276]

Methyl (*Z*)-3-((4-methylpiperazin-1-yl)amino)but-2-enoate 162



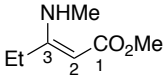
1-Amino-4-methylpiperazine (1.4 mL, 11.5 mmol) was added to a stirred suspension of methyl acetoacetate (1.16 g, 10.0 mmol) and silica gel (100 mg). The resulting mixture was stirred at rt for 19 h, diluted with dichloromethane (10 mL), filtered and washed with dichloromethane (20 mL). The organic phase was concentrated under reduced pressure to give the *title compound* as a yellow oil (1.49 g, 69%); (Found: M+H$^+$, 215.1559. $C_{10}H_{20}N_3O_2^+$ requires 215.1550); ν_{max} (thin film)/cm^{-1} 2944, 2796, 1739, 1657, 1602, 1433, 1283; δ_H (400 MHz; $CDCl_3$) 8.97 (1H, br s, -NH), 4.38 (1H, s, H-2), 3.64 (3H, s, -OC$\underline{H}_3$), 2.73 (4H, t, *J* 4.4, H-1'), 2.33 (3H, s, -NC$\underline{H}_3$), 2.30 (4H, t, *J* 4.4, H-2'), 1.97 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 171.0 (C1), 162.5 (C3), 81.3 (C2), 57.1 (C1'), 55.4 (C2'), 50.9 (-O$\underline{C}H_3$), 46.6 (-N$\underline{C}H_3$), 19.8 (-$\underline{C}H_3$).

Methyl (*Z*)-3-((2-methoxy-2-oxoethyl)amino)but-2-enoate 163



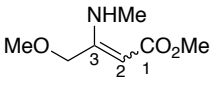
Methyl acetoacetate (1.16 g, 10.0 mmol) was added to a stirred suspension of glycine methyl ester hydrochloride (1.25 g, 9.97 mmol) and triethylamine (1.4 mL, 10.0 mL) in dichloromethane (5 mL). The resulting mixture was stirred at rt for 19 h, washed with water (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give *the title compound* as a yellow oil (1.63 g, 87%); (Found: M+Na^+, 188.0992. $C_8H_{13}NO_4Na^+$ requires 188.0917); ν_{max} (thin film)/cm^{-1} 3297, 2951, 1746, 1651, 1594, 1503, 1436, 1368; δ_H (400 MHz; $CDCl_3$) 8.71 (1H, br s, -NH), 4.49 (1H, s, H-2), 3.93 (2H, d, *J* 6.0, -CH_2), 3.68 (3H, s, -OCH_3), 3.54 (3H, s, -OCH_3), 1.81 (s, 3H, -CH_3); δ_C (100 MHz; $CDCl_3$) 170.5 (C1 or C4), 170.2 (C1 or C4), 160.6 (C3), 84.1 (C2), 52.3 (-OCH_3), 50.0 (-OCH_3), 44.5 (-CH_2), 19.1 (-CH_3).

Methyl (*Z*)-3-(methylamino)pent-2-enoate 164



A solution of methylamine (25% w/v in water, 1.7 mL, 12 mmol) was added in a single portion to a stirred suspension of methylpropionyl acetate (1.30 g, 10 mmol) and silica gel (100 mg) at rt. The resulting mixture was stirred at rt for 16 h after the addition of water (1 mL). The mixture was extracted with dichloromethane (3 x 10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* as a yellow liquid (0.994 g, 69%); (Found: M+Na^+, 166.0834. $C_7H_{13}NO_2Na^+$ requires 166.0838); ν_{max} (thin film)/ cm^{-1} 3303, 2945, 1717, 1649, 1592, 1436, 1414, 1266; δ_H (400 MHz; $CDCl_3$) 8.35 (1H, br s, -NH), 4.46 (1H, s, H-2), 3.60 (3H, s, -OCH_3), 2.90 (3H, d, *J* 5.6, -NHCH_3), 881 (2H, q, *J* 7.6, -CH_2), 1.12 (3H, t, *J* 7.6, -CH_3); δ_C (100 MHz; $CDCl_3$) 171.31 (C1 or C4), 168.0 (C3), 79.6 (C2), 50.0 (-OCH_3), 29.0 (-NHCH_3), 23.6 (-CH_2), 11.9 (-CH_3).[277]

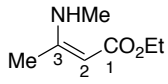
Methyl 4-methoxy-3-(methylamino)but-2-enoate (*E/Z* mixture) 165



A solution of methylamine (25% w/v in water, 8 mL, 57.8 mmol) was added in a single portion to a stirred suspension of methyl 4-methoxyacetoacetate (7.19 g, 49.2 mmol) and silica gel (0.50 g) at rt. The resulting mixture was stirred at rt for 18h 15min. The mixture was extracted with ethyl acetate (3 x 10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* as a yellow-orange liquid (7.39 g, 94%, 1.8:1 *E:Z*); (Found: M+H^+, 160.1080. $C_7H_{14}NO_3^+$ requires 160.0974); ν_{max} (thin film)/cm^{-1} 3383, 2986, 2939, 2904, 2824, 1677, 1657, 1583, 1525, 1436, 1380, 1261; δ_H (400 MHz; $CDCl_3$) *E* isomer: 5.69 (1H, br s, -NH), 4.68 (2H, s, -CH_2), 4.49, (1H, s, H-2), 3.60 (3H, s, -OCH_3), 3.43 (3H, s, -OCH_3), 2.73 (3H, d, *J* 5.2, -NHCH_3); *Z* isomer: 8.19 (1H, br s, -NH), 4.62 (2H, s, -CH_2), 3.99 (1H, s, -CH), 3.61 (3H, s, -OCH_3), 3.35 (3H, s, -OCH_3), 2.93 (3H, d, *J*

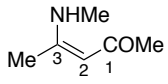
5.2, -NHC$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) *E* isomer: 169.4 (C1 or C4), 161.1 (C3), 79.0 (C2), 70.9 (-$\underline{C}H_2$), 59.4 (-$CO_2\underline{C}H_3$), 50.5 (-O$\underline{C}H_3$), 30.0 (-NH$\underline{C}H_3$); *Z* isomer: 171.9 (C1 or C4), 160.6 (C3), 82.9 (C2), 71.7 (-$\underline{C}H_2$), 58.5 (-$CO_2\underline{C}H_3$), 50.6 (-O$\underline{C}H_3$), 29.9 (-NH$\underline{C}H_3$).[103]

Ethyl (*Z*)-3-(methylamino)but-2-enoate 166



Silica gel (1.1 g) was added in a single portion to a stirred solution of methylamine (25% w/v in water, 15 mL, 120 mmol) and ethyl acetoacetate (13.0 g, 100 mmol) at room temperature. The resulting mixture was stirred at room temperature for 24 h after the addition of water (1 mL) then at 40 ºC for 6 days and 60 ºC for 24 h. The mixture was extracted with dichloromethane (3 x 10 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the *title compound* as an orange liquid (13.0 g, 83%); (Found: M+H^+, 144.1020. $C_7H_{14}NO_2^+$ requires 144.1019); ν_{max} (thin film)/cm^{-1} 3299, 2978, 1648, 1599, 1508, 1435, 1383, 1265; δ_H (400 MHz; $CDCl_3$) 8.45 (1H, br s, -N$\underline{H}$), 4.44 (1H, s, H2), 4.05 (2H, q, *J* 7.2, -OC$\underline{H}_2$), 2.88 (3H, *J* 5.2, -NHC$\underline{H}_3$), 1.89 (3H, s, -C$\underline{H}_3$), 1.22 (3H, t, *J* 7.2, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 170.6 (C1), 162.8 (C3), 81.8 (C2), 58.2 (-O$\underline{C}H_2$), 29.5 (-NH$\underline{C}H_3$), 19.1 (-$\underline{C}H_3$), 14.6 (-$\underline{C}H_3$).[278]

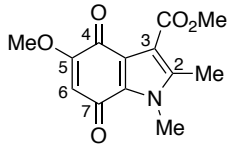
(*Z*)-4-(Methylamino)pent-3-en-2-one 167



Methylamine (15 mL, 11. 8 mmol) was added to a stirred suspension of pentan-2,4-dione (10.0 g, 10.0 mmol) and silica gel (1.0 g). The resulting mixture was stirred at rt for 18.7 h, filtered and washed with dichloromethane (60 mL). The mixture was separated and extracted with dichloromethane (3 x 10 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the *title compound* as a beige-yellow solid (11.3 g, 100%); mp 37-39 °C (lit.,[279] mp 37-38 °C); (Found: M+Na^+, 136.0731. $C_6H_{11}NONa^+$ requires 136.0733); ν_{max} (solid)/cm^{-1} 3163, 3076, 2996, 2939, 2891, 2821, 1557, 1519, 1460, 1422, 1377, 1349; δ_H (400 MHz; $CDCl_3$) 10.70 (1H, br s, -N$\underline{H}$), 4.98 (1H, s, H-2), 2.92 (3H, d, *J* 5.2, -NHC$\underline{H}_3$), 1.99 (3H, s, -COC$\underline{H}_3$), 1.91 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 195.2 (C1),164.5 (C3), 95.6 (C2), 29.9 (-NH$\underline{C}H_3$), 29.2 (-CO$\underline{C}H_3$), 19.1 (-$\underline{C}H_3$).[102]

5.3.3 SYNTHESIS OF INDOLEQUINONES ESTERS/KETONE

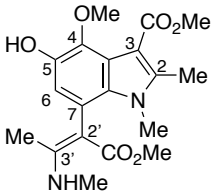
Methyl 5-methoxy-1,2-dimethyl-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxylate 168



A solution of sodium hydrogen carbonate (0.126 g, 1.5 mmol) in water (3.7 mL) and MeCN (1.1 mL) was added to a mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.162 g, 0.750 mmol), methyl (*Z*)-3-(methylamino)-

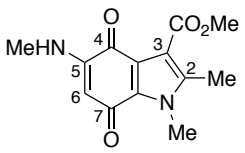
but-2-enoate **158** (0.108, 0.833 mmol) and copper(II) chloride (54.4 mg, 0.405 mmol). The resulting mixture was stirred at 80 °C for 10 min. The reaction mixture was cooled in an ice bath, filtered, washed with water (10 mL) and MeOH (2 mL) to give the *title compound* as a yellow solid (97.4 mg, 49%); mp 216-217 °C (lit.,[102] mp 212-214 °C); (Found: M+Na^+, 286.0695. $C_{13}H_{13}NO_5Na^+$ requires 286.0686); ν_{max} (solid)/cm^{-1} 2949, 1686, 1636, 1608, 1236; δ_H (400 MHz; $CDCl_3$) 5.67 (1H, s, H-6), 3.95 (3H, s, -NC$\underline{H}_3$), 3.92 (3H, s, -$CO_2C\underline{H}_3$), 3.84 (3H, s, -OC$\underline{H}_3$), 2.48 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.2 (C1 or C4), 175.5 (C1 or C4), 164.8 (-$\underline{C}O_2CH_3$), 160.2 (C5), 141.6 (C2), 129.5 (C7a), 121.7 (C3a), 112.5 (C3), 105.9 (C6), 56.6 (-O$\underline{C}H_3$), 51.9 (-$CO_2\underline{C}H_3$), 32.5 (-N$\underline{C}H_3$), 10.7 (-$\underline{C}H_3$).

Methyl (*Z*)-5-hydroxy-4-methoxy-7-(1-methoxy-3-(methylamino)-1-oxobut-2-en-2-yl)-1,2-dimethyl -1*H*-indole-3-carboxylate 168



A solution of water/TFE (83:17, 3 mL) was added to a mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.108 g, 0.499 mmol) and methyl (*Z*)-3-(methylamino)but-2-enoate **158** (97.5 mg, 0.755 mmol). The resulting mixture was stirred at 50 °C for 28 min**.** The reaction mixture was filtered, washed with water (5 mL) and MeOH (1 mL) to give the *title compound* as a colourless solid (5 mg, 18%); mp 256-257 ºC; (Found: M+H^+, 377.1725. $C_{19}H_{25}N_2O_6^+$ requires 377.1707); ν_{max} (solid)/cm^{-1} 3421, 1688, 1638, 1583, 1558, 1398, 1247, 1219; δ_H (400 MHz; $CDCl_3$) 9.82 (1H, s, -N$\underline{H}CH_3$), 6.39 (1H, s, H-6), 5.25 (1H, s, -O$\underline{H}$), 3.92 (3H, s, -OC$\underline{H}_3$), 3.91 (3H, s, -ArOC$\underline{H}_3$), 3.56 (3H, s, -OC$\underline{H}_3$), 3.51 (3H, s, -NC$\underline{H}_3$), 3.05 (3H, d, *J* 5.2, -NHC$\underline{H}_3$), 2.50 (3H, s, -C$\underline{H}_3$), 1.74 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 171.1 (-$\underline{C}O_2$Me, enamine), 167.4 (-$\underline{C}O_2$Me), 165.6 (C3'), 153.0 (C4), 151.4 (C5), 139.3 (C2), 136.9 (C3a), 110.4 (C7a), 105.2 (C3), 99.4 (C7), 92.0 (C6), 82.3 (C2'), 55.7 (-ArO$\underline{C}H_3$), 51.3 (-$CO_2\underline{C}H_3$,), 50.9 (-$CO_2\underline{C}H_3$, enamine), 31.3 (-N$\underline{C}H_3$), 30.5 (-NH$\underline{C}H_3$), 16.2 (-$\underline{C}H_3$), 11.8 (-$\underline{C}H_3$, enamine).

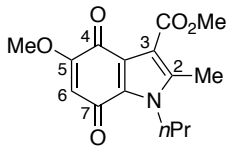
Methyl 1,2-dimethyl-5-(methylamino)-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxylate 175



Water (2.5 mL) was added to a mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.109 g, 0.500 mmol), methyl (*Z*)-3-(methylamino)-but-2-enoate **158** (0.258 g, 1.98 mmol) and potassium carbonate (69.2 mg, 0.501 mmol) in MeCN (0.5 mL). The resulting mixture was stirred at 50 ºC for 2.5 h. The reaction mixture was concentrated under reduced pressure to give the crude product as a red residue. Column chromatography eluting with 10-20% ethyl acetate/dichloromethane gave the *title compound* as a burgundy solid (23 mg, 17%); mp 203 ºC; (Found: M+Na^+, 285.0853.

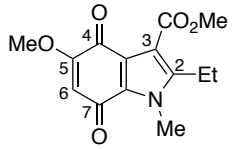
$C_{13}H_{14}N_2O_4Na^+$ requires: 285.0846); λ_{max} (MeOH)/nm 210 (log ε 3.65), 311 (3.33), 512 (2.42); ν_{max} (solid)/cm^{-1} 3373, 1697, 1673, 1624, 1604, 1503, 1430, 1311; δ_H (400 MHz; $CDCl_3$) 5.99 (1H, br s, -NH), 5.19 (1H, s, H-6), 3.98 (3H, s, -NCH_3), 3.92 (3H, s, -OCH_3), 2.89 (3H, d, *J* 5.6, -$NHCH_3$), 2.46 (3H, s, -CH_3); δ_C (100 MHz; $CDCl_3$) 178.7 (C1 or C4), 176.6 (C1 or C4), 165.8 (-CO_2CH_3), 149.6 (C5), 140.5 (C2), 132.1 (C7a), 119.5 (C3a), 96.0 (C6), 51.8 (-OCH_3), 32.5 (-NCH_3), 29.5 (-$NHCH_3$), 10.8 (-CH_3).

Methyl 5-methoxy-2-methyl-4,7-dioxo-1-propyl-4,7-dihydro-1*H*-indole-3-carboxylate 176



2-Bromo-5-methoxy-1,4-benzoquinone **156** (0.655 g, 3.02 mmol), methyl (*Z*)-3-(propylamino)but-2-enoate **160** (0.529 g, 3.36 mmol) and $CuCl_2$ (0.404 g, 3.00 mmol) were sequentially added to a solution of sodium hydrogen carbonate (0.504 g, in water/MeCN (75:25, 19.2 mL) stirred at 80 °C. The resulting mixture was stirred at 80 °C for 4 min. The reaction mixture was cooled in an ice bath, filtered, washed with water and MeOH to give a grey solid (0.612 g), identified as the quinone after the Michael addition. A second fraction was collected after filtration and washing with water (5 mL) to give the *title compound* as a yellow solid (0.170 g, 19%); mp 155-156 ºC; (Found: M+Na^+, 292.1179. $C_{15}H_{17}NO_5Na^+$ requires: 292.1187); ν_{max} (solid)/cm^{-1} 2947, 1699, 1680, 1635, 1602, 1506, 1423, 1340, 1280; δ_H (400 MHz; $CDCl_3$) 5.66 (1H, s, H-6), 4.32 (3H, tq, *J* 7.6, –NCH_2), 3.92 (3H, s, -CO_2CH_3), 3.84 (3H, s, -OCH_3), 2.48 (3H, s, -CH_3), 1.75 (2H, -CH_2), 1.00 (3H, t, *J* 7.6, CH_3); δ_C (100 MHz; $CDCl_3$) 178.8 (C1 or C4), 175.6 (C1 or C4), 164.9 (-CO_2CH_3), 160.0 (C5), 140.9 (C2), 129.1 (C7a), 121.9 (C3a), 112.7 (C3), 106.0 (C6), 56.5 (-CO_2CH_3), 51.9 (-OCH_3), 46.9 (-$NHCH_2$), 23.7 (-CH_2), 11.0 (-CH_3).

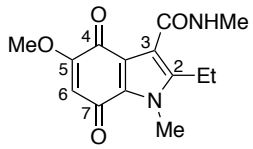
Methyl 2-ethyl-5-methoxy-1-methyl-4,7-dioxo-4,7-dihydro-*1H*-indole-3-carboxylate 177



A mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.966 g, 4.44 mmol) and $CuCl_2$ (0.274 g, 2.03 mmol) was added to a mixture of methyl (*Z*)-3-(methylamino)pent-2-enoate **164** (0.888 g, 6.20 mmol) in a solution of sodium hydrogen carbonate (0.678 g, 8.08 mmol) in water/MeCN (77:23, 26 mL) stirred at 80 °C. The resulting mixture was stirred at 80 °C for 5 min. The reaction mixture was concentrated under reduced pressure, extracted with dichloromethane (6 x 20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the crude product as a brown residue. Column chromatography eluting with 10% ethyl acetate/dichloromethane gave the *title compound* as a yellow solid (0.170 g, 14%); mp 163-165 ºC; (Found: M+Na^+, 300.0856. $C_{14}H_{15}NO_5Na^+$ requires 300.0842); ν_{max} (solid)/cm^{-1} 3691, 1709, 1686, 1641, 1608, 1506, 1458; δ_H (400 MHz; $CDCl_3$) 5.68 (1H, s, H6), 3.97 (3H, s, -NCH_3), 3.92 (3H, s, -OCH_3), 3.84 (3H, s, -

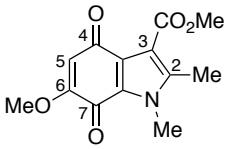
OC$\underline{H}_3$), 2.91 (2H, q, *J* 7.6, -C$\underline{H}_2$), 1.24 (3H, t, *J* 7.6, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.0 (C1 or C4), 178.8 (C1 or C4), 164.7 (-$\underline{C}O_2CH_3$), 160.2 (C5), 146.8 (C2), 129.5 (C7a), 121.8 (C3a), 112.0 (C3), 106.0 (C6), 56.6 (-O$\underline{C}H_3$), 51.9 (-$CO_2\underline{C}H_3$), 32.4 (-N$\underline{C}H_3$), 17.9 (-$\underline{C}H_2$), 14.0 (-$\underline{C}H_3$).

2-Ethyl-5-methoxy-*N*,1-dimethyl-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxamide 178



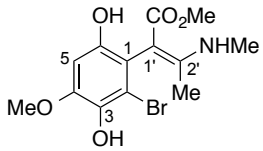
A solution of methyl (*Z*)-3-(methylamino)pent-2-enoate **164** (0.144 g, 1.00 mmol) in MeCN (3 mL) was added to a mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.110 g, 0.501 mmol), copper(II) acetate monohydrate (0.148 g, 0.739 mmol) and potassium carbonate (0.142 g, 1.03 mmol). The resulting mixture was stirred under reflux for 4.3 h. The reaction mixture was diluted with dichloromethane (5 mL), filtered through Celite and concentrated under reduced pressure. Column chromatography eluting with 10% ethyl acetate/dichloromethane gave the *title compound* as a red solid (8.4 mg, 6%); (Found: M+H$^+$, 277.1183. $C_{14}H_{17}N_2O_4^+$ requires: 277.1183); ν_{max} ($CHCl_3$)/ cm^{-1} 3690, 3297, 1708, 1634, 1613, 1580, 1496, 1466, 1413, 1337, 1239; δ_H (400 MHz; $CDCl_3$) 9.72 (1H, br s, -N$\underline{H}$), 5.71 (1H, s, H-6), 4.00 (3H, s, –NC$\underline{H}_3$), 3.87 (3H, s, -OC$\underline{H}_3$), 3.26 (2H, q, *J* 7.6, –C$\underline{H}_2$), 2.97 (3H, d, *J* 4.8, NHC$\underline{H}_3$), 1.26 (3H, t, *J* 7.6, C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.0 (C1 or C4), 178.8 (C1 or C4), 163.7 (-$\underline{C}$ONHC$\underline{H}_3$), 159.8 (C5), 149.5 (C2), 129.5 (C7a), 119.2 (C3a), 115.1 (C3), 106.2 (C6), 56.8 (-O$\underline{C}H_3$), 32.3 (-N$\underline{C}H_3$), 25.9 (-NH$\underline{C}H_3$), 18.2 (-$\underline{C}H_2$), 13.1 (-$\underline{C}H_3$).

Methyl 6-methoxy-1,2-dimethyl-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxylate 48



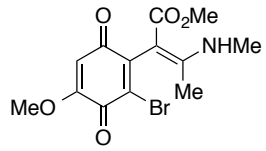
A solution of sodium hydrogen carbonate (42.1 mg, 0.501 mmol) in water (1.25 mL) and MeCN (0.25 mL) was added to a mixture of 2-bromo-6-methoxy-1,4-benzoquinone **113** (55.4 mg, 0.251 mmol), methyl (*Z*)-3-(methylamino)but-2-enoate **158** (48.7 mg, 0.376 mmol) and copper(II) chloride (33.6 mg, 0.250 mmol). The resulting mixture was stirred at 80 °C for 10 min. The reaction mixture was cooled in an ice bath, filtered, washed with water (5 mL) and MeOH (1 mL) to give the *title compound* as a yellow solid (54.6 mg, 81%); mp 204-206 °C (lit.,[102] mp 209-211 °C); (Found: M+H$^+$, 264.0881. $C_{13}H_{14}NO_5^+$ requires: 264.0866); ν_{max} (solid)/cm^{-1} 2955, 1696, 1665, 1652, 1606, 1538, 1502, 1429, 1345, 1300, 1261; δ_H (400 MHz; $CDCl_3$) 5.74 (1H, s, H-5), 3.96 (3H, s, -NC$\underline{H}_3$), 3.94 (3H, s, -$CO_2C\underline{H}_3$), 3.84 (3H, s, -OC$\underline{H}_3$), 2.47 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 181.4 (C4), 172.4 (C7), 164.8 (-$\underline{C}O_2CH_3$), 158.7 (C6), 142.6 (C2), 127.8 (C3a), 124.6 (C3a), 112.9 (C3), 107.5 (C5), 56.6 (O$\underline{C}H_3$), 52.0 (O$\underline{C}H_3$), 32.8 (-NH$\underline{C}H_3$), 11.0 ($\underline{C}H_3$).

Methyl (*Z*)-2-(2-bromo-3,6-dihydroxy-4-methoxyphenyl)-3-(methylamino)-but-2-enoate 179



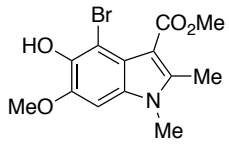
A mixture of water/MeCN (83:17, 12 mL) was added to a mixture of 2-bromo-6-methoxy-1,4-benzoquinone **113** (0.327 g, 1.50 mmol) and methyl (*Z*)-3-(methylamino)but-2-enoate **158** (0.293 g, 1.352 mmol). The resulting mixture was stirred for less than 1 min at rt, under an argon atmosphere. The reaction mixture was cooled in an ice bath, filtered, washed with water (8 mL) to give the *title compound* as a colourless solid (0.415 g, 80%); mp 184-186 °C; (Found: M+H$^+$, 346.0285. $C_{13}H_{17}{}^{79}BrNO_5^+$ requires: 346.0286); ν_{max} (solid)/cm^{-1} 3398, 2946, 1637, 1612, 1567, 1489,1436, 1300, 1254; δ_H (400 MHz; $CDCl_3$) 9.76 (1H, br s, -NHCH$_3$), 6.56 (1H, s, H-5), 5.51 (1H, s, -OH), 5.10 (1H, s, -OH), 3.91 (3H, s, -OCH$_3$), 3.59 (3H, s, -CO$_2$CH$_3$), 3.03 (3H, d, *J* 4.8, -NHCH$_3$), 1.74 (3H, s, -CH$_3$); δ_C (100 MHz; $CDCl_3$) 170.2 (-CO$_2$CH$_3$), 165.5 (C2'), 148.3 (C4), 147.1 (C6), 136.9 (C3), 116.8 (C1), 114.0 (C2), 97.5 (C5), 85.7 (C1'), 56.2 (-OCH$_3$), 50.9 (-CO$_2$CH$_3$), 30.3 (-NHCH$_3$), 15.6 (-CH$_3$).

Methyl (*Z*)-2-(2-bromo-4-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)-3-(methylamino) but-2-enoate 180



A solution of sodium hydrogen carbonate (21.9 mg, 0.258 mmol) in water (1.25 mL) and MeCN (0.25 mL) was added to a mixture of 2-bromo-6-methoxy-1,4-benzoquinone **113** (55.9 mg, 0.268 mmol), methyl (*Z*)-3-(methylamino)but-2-enoate **158** (49.0 mg, 0.379 mmol) and copper(II) bromide (56.1 mg, 0.251 mmol). The resulting mixture was stirred at 80 °C for 10 min. The reaction mixture was cooled in an ice bath, filtered, washed with water (8 mL) to give the *title compound* as a purple solid (82.6 mg, 93%); mp > 300 °C; (Found: M+H$^+$, 344.0140. $C_{13}H_{15}{}^{79}BrNO_5$ requires: 344.0128); ν_{max} (solid)/cm^{-1} 2945, 1637, 1568, 1433, 1345, 1301, 1260; δ_H (400 MHz; $CDCl_3$) 9.59 (1H, br s, -NHCH$_3$), 6.03 (1H, s, H-5), 3.93 (3H, s, -OCH$_3$), 3.58 (3H, s, -CO$_2$CH$_3$), 3.01 (3H, d, *J* 5.2, -NHCH$_3$), 1.83 (3H, s, -CH$_3$); δ_C (100 MHz; $CDCl_3$) 184.3 (C3), 175.6 (C6), 168.0 (-CO$_2$CH$_3$), 162.3 (C2'), 158.4 (C4), 147.5 (C1), 135.6 (C2), 107.7 (C5), 89.6 (C1'), 56.6 (-OCH$_3$,), 50.8 (-CO$_2$CH$_3$), 30.0 (-NHCH$_3$), 16.8 (CH$_3$).

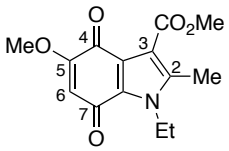
Methyl 4-bromo-5-hydroxy-6-methoxy-1,2-dimethyl-1*H*-indole-3-carboxylate 181



Water (2.5 mL) and MeCN (0.5 mL) were sequentially added to a mixture of 2-bromo-6-methoxy-1,4-benzoquinone **113** (0.108 g, 0.501 mmol), methyl (*Z*)-3-(methylamino)but-2-enoate **158** (99.5 mg g, 0.768 mmol) and copper(II) acetate monohydrate (0.100 g, 0.501 mmol). The resulting mixture was stirred for 5

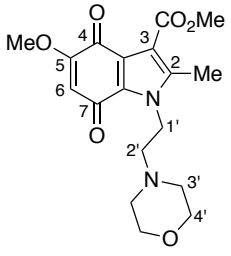
min at 80 °C. The reaction mixture was cooled in an ice bath, filtered, washed with water and MeOH to give the *title compound* as a yellow solid (69.2 mg, 42%); mp 173-175 °C; (Found: M+H$^+$ 328.0179. $C_{13}H_{15}{}^{79}BrNO_4$ requires: 328.0179); ν_{max} (solid)/cm^{-1} 3365, 2949, 1665, 1606, 1529, 1468, 1434, 1377, 1333, 1312, 1261; δ_H (400 MHz; $CDCl_3$) 6.74 (1H, s, H-7), 5.90 (1H, s, -O<u>H</u>), 3.99 (3H, s, -OC<u>H</u>$_3$), 3.94 (3H, s, -CO$_2$C<u>H</u>$_3$), 3.65 (3H, s, -NC<u>H</u>$_3$), 2.58 (3H, s, -C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 166.4 (-<u>C</u>O$_2$CH$_3$), 144.3 (C6), 141. (C2), 139.7 (C5), 130.9 (C7a), 107.5 (C3), 99.6 (C4), 91.8 (C5), 56.7 (-O<u>C</u>H$_3$), 51.1 (-CO$_2$<u>C</u>H$_3$), 30.0 (-N<u>C</u>H$_3$), 11.6 (-<u>C</u>H$_3$); 1C unobserved.

Methyl 1-ethyl-5-methoxy-2-methyl-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxylate 186



A mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.652 g, 3.00 mmol) and $CuCl_2$ (0.201 g, 1.49 mmol) was added to a mixture of methyl (*Z*)-3-(ethylamino)but-2-enoate **159** (0.434 g, 3.38 mmol) in water/MeCN (75:25, 19.2 mL) stirred at 80 °C. The resulting mixture was stirred at 80 °C for 13 min. The reaction mixture was cooled in an ice bath, MeCN (1 mL) was added and the suspension was filtered, washed with water (5 mL) and MeOH (1 mL) to give the *title compound* as a yellow solid (0.271 g, 34%); mp 172-174 ºC; (Found: M+H$^+$, 278.1023. $C_{14}H_{16}NO_5{}^+$ requires: 278.1024); ν_{max} (solid)/cm^{-1} 1680, 1636, 1602, 1508, 1423, 1339, 1278, 1237; δ_H (400 MHz; $CDCl_3$) 5.64 (1H, s, H-6), 4.41 (2H, q, *J* 7.2, -C<u>H</u>$_2$), 3.89 (3H, s, -CO$_2$C<u>H</u>$_3$), 3.81 (3H, s, -OC<u>H</u>$_3$), 2.46 (3H, s, -C<u>H</u>$_3$), 1.34 (3H, t, *J* 7.2, -C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 179.2 (C1 or C4), 176.0 (C1 or C4), 165.4 (-<u>C</u>O$_2$CH$_3$), 160.5 (C5), 141.1 (C2), 129.3 (C7a), 122.4 (C3a), 113.2 (C3), 106.4 (<u>C</u>H, C6), 57.0 (-O<u>C</u>H$_3$), 52.3 (-CO$_2$<u>C</u>H$_3$), 41.05 (-N<u>C</u>H$_2$), 15.76 (-<u>C</u>H$_3$), 10.75 (-<u>C</u>H$_3$).

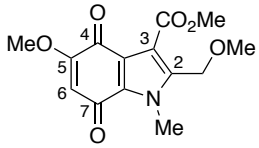
Methyl 5-methoxy-2-methyl-1-(2-morpholinoethyl)-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxylate 187



A mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.327 g, 1.50 mmol) and $CuCl_2$ (0.106 g, 0.786 mmol) and a solution of methyl (*Z*)-3-((2-morpholinoethyl)amino)but-2-enoate **161** (0.378, 1.65 mmol) in MeCN (1.4 mL) were sequentially added to a stirred solution of sodium hydrogen carbonate (0.251 g, 2.99 mmol) in water/MeCN (86:14, 8.2 mL) at 80 °C. The resulting mixture was stirred at 80 °C for 1.8 h. The reaction mixture was extracted with ethyl acetate (5 x 35 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The orange-red residue was purified by dry-flash chromatography, eluting with 50-98% ethyl acetate/dichloromethane and 2% triethylamine to give the *title compound* as a yellow solid (15.8 mg, 3%); mp 135-136 °C; (Found: M+H$^+$, 363.1553. $C_{18}H_{23}N_2O_6{}^+$ requires 363.1551);

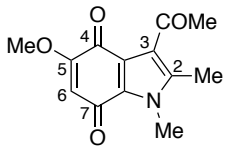
ν_{max} (solid)/cm^{-1} 2930, 2851, 2809, 1687, 1636, 1609, 1530, 1500, 1434, 1338; δ_H (400 MHz; $CDCl_3$) 5.63 (1H, s, H-6), 4.46 (2H, t, *J* 6.8, H-1'), 3.90 (3H, s, -$CO_2C\underline{H}_3$), 3.82 (3H, s, -$OC\underline{H}_3$), 3.71 (4H, t, *J* 4.8, H-4'), 2.63 (2H, t, *J* 6.8, H-2'), 2.56 (4H t, *J* 4.8, H-3'), 2.51 (3H, s, -$C\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.2 (C1 or C4), 176.0 (C1 or C4), 165.3 (-$\underline{C}O_2CH_3$), 160.6 (C5), 141.8 (C2), 129.6 (C7a), 122.5 (C3a), 113.2 (C3), 106.4 (C6), 67.4 (C4'), 58.7 (C2'), 57.0 (-$O\underline{C}H_3$), 54.5 (C3'), 52.4 (-$CO_2\underline{C}H_3$), 43.5 (C1'), 11.1 (-$\underline{C}H_3$).

Methyl 5-methoxy-2-(methoxymethyl)-1-methyl-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxylate 188



A mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.617 g, 2.84 mmol) and $CuCl_2$ (0.189 g, 1.40 mmol) and (*Z*)-5-methoxy-4-(methylamino)pent-3-en-2-one **165** (0.378, 1.65 mmol) were sequentially added to a stirred solution of sodium hydrogen carbonate (0.464 g, 5.53 mmol) in water/MeCN (73:27, 18.6 mL) at 80 °C. The resulting mixture was stirred at 80 °C for 10 min. The reaction mixture was extracted with dichloromethane (4 x 20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The dark residue was purified by column chromatography, eluting with 10-30% ethyl acetate/dichloromethane gave *title compound* as a yellow solid (54.4 mg, 6%); mp 147-148 °C (lit.,[103] 148-150 °C); (Found: M+H$^+$, 294.097607. $C_{14}H_{16}NO_6^+$ requires 294.097214); ν_{max} (solid)/cm^{-1} 3060, 2994, 2947, 2896, 2824, 2811, 1707, 1687, 1639, 1604, 1552, 1506, 1470, 1455, 1439, 1426, 1411, 1378, 1339; δ_H (400 MHz; $CDCl_3$) 5.70 (1H, s, H-6), 4.67 (2H, s, -$C\underline{H}_2$OMe), 4.02 (3H, s, -$NC\underline{H}_3$), 3.91 (3H, s, -$CO_2C\underline{H}_3$), 3.83 (3H, s, -$OC\underline{H}_3$), 3.36 (3H, s, -$CH_2OC\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.4 (C1 or C4), 175.5 (C1 or C4), 164.5 (-$\underline{C}O_2CH_3$), 160.6 (C5), 139.2 (C2), 130.7 (C7a), 115.0 (C3), 106.4 (C6), 62.5 (-$\underline{C}H_2$), 58.2 (-$CH_2O\underline{C}H_3$), 56.8 (-$O\underline{C}H_3$), 52.3 (-$CO_2\underline{C}H_3$), 33.4 (-$N\underline{C}H_3$).

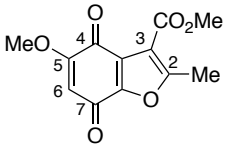
3-Acetyl-5-methoxy-1,2-dimethyl-*1H*-indole-4,7-dione 189



A mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.325 g, 1.50 mmol) and $CuCl_2$ (0.107 g, 0.786 mmol) and a solution of (*Z*)-4-(methylamino)pent-3-en-2-one **167** (0.233 g, 1.80 mmol) was added to a stirred solution of sodium hydrogen carbonate (0.261 g, 3.11 mmol) in water/MeCN (86:14, 8.2 mL) at 80 °C. The resulting mixture was stirred at 80 °C for 10 min. The reaction mixture was cooled in an ice bath, filtered and washed with water (6 mL) and MeOH (2 mL) to give the *title compound* as a yellow solid (99.2 mg, 27%); mp 229-230 °C (lit.,[280] 227-228 °C); (Found: M+Na$^+$, 270.0731. $C_{13}H_{13}NO_4Na^+$ requires 270.0737); ν_{max} (solid)/cm^{-1} 3051, 2993, 2950, 2849, 1666, 1635, 1596, 1524, 1498, 1447, 1402, 1374, 1356, 1336; δ_H (400 MHz; $CDCl_3$) 5.68 (1H,

s, H-6), 3.93 (3H, s, -NC<u>H</u>$_3$), 3.84 (3H, s, -OC<u>H</u>$_3$), 2.65 (3H, s, -COC<u>H</u>$_3$), 2.39 (3H, s, -C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 198.9 (-<u>C</u>OCH$_3$), 179.6 (C1 or C4), 177.4 (C1 or C4), 160.3 (C5), 141.4 (C2), 129.4 (C7a), 122.4 (C3a), 110.4 (C3), 106.6 (C6), 57.1 (-O<u>C</u>H$_3$), 32.9 (-N<u>C</u>H$_3$), 32.0 (-CO<u>C</u>H$_3$), 11.2 (-<u>C</u>H$_3$).

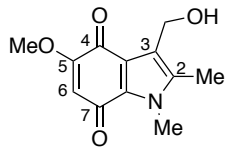
Methyl 5-methoxy-2-methyl-4,7-dioxo-4,7-dihydrobenzofuran-3-carboxylate 185



A mixture of 2-bromo-5-methoxy-1,4-benzoquinone **156** (0.327 g, 1.50 mmol) and $CuCl_2$ (0.106 g, 0.786 mmol) and a solution of methyl (*Z*)-3-(methylamino)but-2-enoate **158** (0.378 g, 1.65 mmol) in MeCN (1.4 mL) were sequentially added to a stirred solution of sodium hydrogen carbonate (0.251 g, 2.99 mmol) in water/acetonitrile (76:24, 8.2 mL) at 80 °C. The resulting mixture was stirred at 80 °C for 1.8 h. The reaction mixture was extracted with ethyl acetate (5 x 30 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The brown residue was purified by column chromatography, eluting with 10-100% ethyl acetate/dichloromethane to give the *title compound* as a yellow solid (18.0 mg, 5%); mp 209-210°C (lit.,[136] mp 203-205 °C); (Found: M+Na$^+$, 273.0371. $C_{12}H_{10}O_6Na^+$ requires 273.0370); ν_{max} (solid)/cm^{-1} 3154, 3054, 2925, 2849, 1731, 1698, 1656, 1603, 1572, 1545, 1458, 1392, 1373, 1342; δ_H (400 MHz; $CDCl_3$) 5.80 (1H, s, H-6), 3.92 (3H, s, -OC<u>H</u>$_3$), 3.86 (3H, s, -CO$_2$C<u>H</u>$_3$), 2.67 (3H, s, -C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 175.9 (C1 or C4), 175.1 (C1 or C4), 164.4 (C2), 162.8 (C5), 161.0 (-<u>C</u>O$_2$CH$_3$), 150.7 (C7a), 124.1 (C3a), 113.0 (C3), 105.0 (C6), 57.5 (-O<u>C</u>H$_3$), 52.7 (-CO$_2$<u>C</u>H$_3$), 14.4 (-<u>C</u>H$_3$).

5.3.4 SYNTHESIS OF ALCOHOLS

3-Hydroxymethyl-5-methoxy-1,2-dimethyl-*1H*-indole-4,7-dione 43

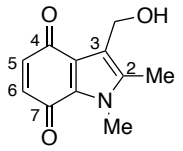


(a) A mixture of methyl 5-methoxy-1,2-dimethyl-4,7-dioxo-4,7-dihydro-*1H*-indole-3-carboxylate **168** (0.156 g, 0.592 mmol) and 10 wt% Pd/C (30.3 mg, 20 wt% of indolequinone) in TFE (4 mL) was stirred at rt under a hydrogen atmosphere for 2 h. The resulting mixture was diluted with dichloromethane (10 mL), filtered through Celite, washed with dichloromethane (10 mL) and concentrated under reduced pressure to give the crude hydroquinone as a white-beige solid.

(b) Lithium aluminium hydride (0.100 g, 2.65 mmol) was added to a suspension of the crude hydroquinone in THF (6 mL). The resulting mixture was stirred at rt for 1.7 h and sequentially quenched with ethyl acetate (5 mL), water (2.5 mL), NaOH (aq., 1 M, 2.5 mL) and SiO_2 (~1 g). The mixture was filtered, washed with ethyl acetate (6 x 10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Dry-flash chromatography of the residue eluting with

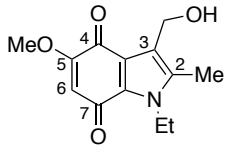
40-50% ethyl acetate/dichloromethane gave the *title compound* as a red solid (41.8 mg, 30%); mp 204-205 °C (lit.,[281] mp 199-200 °C); (Found: M+Na$^+$, 258.0731. $C_{12}H_{13}NO_4Na^+$ requires 258.0737); ν_{max} (solid)/cm^{-1} 3447, 2919, 2850, 1633, 1595, 1502, 1456, 1337; δ_H (400 MHz; $CDCl_3$) 5.61 (1H, s, H-6), 4.59 (2H, d, *J* 7.2, -C$\underline{H}_2$), 3.97 (1H, t, *J* 7.2, -O$\underline{H}$), 3.87 (3H, s, -NC$\underline{H}_3$), 3.83 (3H, s, -OC$\underline{H}_3$), 2.20 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.4 (C1 or C4), 178.7 (C1 or C4), 159.8 (C5), 134.7 (C2), 129.5 (C7a), 122.8 (C3), 122.1 (C3a), 107.2 (C6), 56.7 (-O$\underline{C}H_3$), 56.0 (-$\underline{C}H_2$), 32.5 (-N$\underline{C}H_3$), 9.7 (-$\underline{C}H_3$).

3-Hydroxymethyl-1,2-dimethyl-1*H*-indole-4,7-dione 139



Lithium aluminium hydride (83.9 mg, 881 mmol) was added portionwise to a stirred suspension of methyl 5-methoxy-1,2-dimethyl-4,7-dioxo-4,7-dihydro-1*H*-indole-3-carboxylate **168** (0.113 g, 0.429 mmol) in THF (6 mL) at 0 °C. The resulting mixture was stirred at rt for 3 h, under argon atmosphere. The mixture was quenched with ethyl acetate (5 mL), Rochelle's solution (1 M, 15 mL) and stirred under air for 30 min. The mixture was extracted with ethyl acetate (3 x 10 mL) and the combined organic phases were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Chromatography of the residue eluting with 17% ethyl acetate/dichloromethane gave *title compound* as a red solid (5.2 mg, 6%); (Found: M+Na$^+$, 228.0630. $C_{11}H_{11}NO_3Na^+$ requires 228.0631); δ_H (400 MHz; $CDCl_3$) 6.55 (1H, d, *J* 10.0, H-5 or H-6), 6.51 (1H, d, *J* 10.0, H-5 or H-6), 4.62 (2H, d, *J* 7.2, -C$\underline{H}_2$), 4.16 (1H, t, *J* 7.2, -O$\underline{H}$), 3.90 (3H, s, -NC$\underline{H}_3$), 2.25 (3H, s, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 185.9 (C1 or C4), 177.7 (C1 or C4), 138.2 (C5 or C6), 136.7 (C5 or C6), 135.8 (C2), 129.2 (C7a), 124.3 (C3), 123.4 (C3a), 56.4 (-C$\underline{H}_2$), 32.8 (-N$\underline{C}H_3$), 10.1 (-C$\underline{H}_3$).

1-Ethyl-3-hydroxymethyl-5-methoxy-2-methyl-*1H*-indole-4,7-dione 206

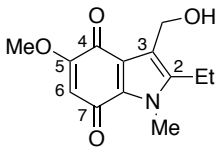


(a) A mixture of ethyl 1-ethyl-5-methoxy-2-methyl-4,7-dioxo-4,7-dihydro-*1H*-indole-3-carboxylate **186** (0.270 g, 0.973 mmol) and 10 wt% Pd/C (55.2 mg, 20 wt% of indolequinone) in TFE (4 mL) was stirred at rt under a hydrogen atmosphere for 3 h. The resulting mixture was diluted with chloroform (10 mL), filtered through Celite, washed with chloroform (20 mL) and concentrated under reduced pressure to give the crude hydroquinone as a pale yellow solid.

(b) Lithium aluminium hydride (0.149 g, 3.89 mmol) in THF (6 mL) was added to a stirred suspension of the crude material in THF (6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 10 min and then at rt for 20 h, under argon atmosphere. The mixture was quenched with ethyl acetate (10 mL) then Rochelle's solution (1 M, 20 mL) and stirred under air for 30 min. The mixture was extracted with ethyl acetate (4 x 10 mL) and the combined organic phases

were dried ($MgSO_4$), filtered and concentrated. Dry-flash chromatography of the residue eluting with 40-60% ethyl acetate/dichloromethane gave the *title compound* as a red solid (71.2 mg, 29%); mp 188-190 °C; (Found: M+Na^+, 272.0891. $C_{13}H_{15}NO_4Na^+$ requires 272.0893); ν_{max} (solid)/cm^{-1} 3420, 2920, 2851, 1668, 1625, 1593, 1501, 1432, 1382, 1335; δ_H (400 MHz; $CDCl_3$) 5.63 (1H, s, H-6), 4.61 (2H, d, *J* 7.2, -C$\underline{H}_2$), 4.34 (2H, q, *J* 7.2, -NC$\underline{H}_2$), 4.04 (1H, t, *J* 7.2, -O$\underline{H}$), 3.82 (3H, s, -OC$\underline{H}_3$), 2.25 (3H, s, -C$\underline{H}_3$), 1.37 (3H, t, *J* 7.2, -C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.6 (C1 or C4), 178.5 (C1 or C4), 159.8 (C5), 133.9 (C2), 129.0 (C7a), 123.2 (C3), 122.6 (C3a), 107.6 (C6), 56.8 (-O$\underline{C}H_3$), 56.2 (-$\underline{C}H_2$), 40.7 (-N$\underline{C}H_2$), 15.7 (-$\underline{C}H_3$), 9.51 (-$\underline{C}H_3$).

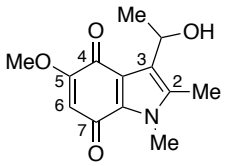
2-Ethyl-3-hydroxymethyl-5-methoxy-1-methyl-*1H*-indole-4,7-dione 207



A mixture of methyl 2-ethyl-5-methoxy-1-methyl-4,7-dioxo-4,7-dihydro-*1H*-indole-3-carboxylate **177** (0.165 g, 0.595 mmol) and 10 wt% Pd/C (21.0 mg, 15 wt% of indolequinone) in TFE (3 mL) was stirred at rt under a hydrogen atmosphere for 6.2 h. The resulting mixture was diluted with dichloromethane (10 mL), filtered through Celite, washed with dichloromethane (10 mL) and concentrated under reduced pressure to give the crude hydroquinone as a white-beige solid.

(b) Lithium aluminium hydride (97.6 mg, 2.79 mmol) was added to a suspension of the crude hydroquinone in THF (6 mL). The resulting mixture was stirred at rt for 17 h and sequentially quenched with ethyl acetate (10 mL) and Rochelle's solution (20 mL). The mixture was extracted with ethyl acetate (3 x 10 mL) and the combined organic phases were dried ($MgSO_4$), filtered and concentrated. Dry-flash chromatography of the residue eluting with 80-90% ethyl acetate/dichloromethane gave the *title compound* as a red solid (52.4 mg, 35%); mp 191-192 °C; (Found: M+Na^+, 272.0891. $C_{13}H_{15}NO_4Na^+$ requires 272.0893); ν_{max} (solid)/cm^{-1} 3395, 2929, 2874, 1667, 1633, 1594, 1498, 1454, 1408, 1376, 1351, 1334; δ_H (400 MHz; $CDCl_3$) 5.64 (1H, s, H-6), 4.62 (2H, d, *J* 6.8, -C$\underline{H}_2$OH), 4.02 (1H, t, *J* 6.8, -O$\underline{H}$), 3.93 (3H, s, -OC$\underline{H}_3$), 3.85 (3H, s, -NC$\underline{H}_3$), 2.66 (2H, q, *J* 7.6, -C$\underline{H}_2$CH$_3$), 1.16 (2H, t, *J* 7.6, -CH$_2$C$\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.3 (C1 or C4), 178.7 (C1 or C4), 159.7 (C5), 140.3 (C2), 129.4 (C7a), 122.4 (C3 or C3a), 122.2 (C3 or C3a), 107.2 (C6), 56.6 (-O$\underline{C}H_3$), 55.8 (-$\underline{C}H_2$OH), 32.3 (-N$\underline{C}H_3$), 17.0 (-$\underline{C}H_2$), 14.3 (-$\underline{C}H_3$).

3-(1-Hydroxyethyl)-5-methoxy-1,2-dimethyl-*1H*-indole-4,7-dione 208

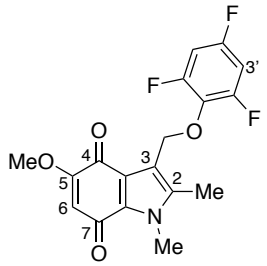


Sodium borohydride (0.396 g, 10.3 mmol) was added to a suspension of 3-acetyl-5-methoxy-1,2-dimethyl-*1H*-indole-4,7-dione **189** (0.255 g, 1.03 mmol) in anhydrous methanol (40 mL) and the resulting mixture was stirred at rt for 12 min. Water (3 mL) followed by ethyl acetate (4 mL) were added to the mixture and the mixture was stirred at rt under air. The solution was then extracted with

ethyl acetate (4 x 10 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography of the residue eluting with a gradient 17-33% ethyl acetate/dichloromethane gave the *title compound* as an orange-red solid (0.15 g, 59%); mp 138-139 °C (lit.,[280] 148-150 °C); (Found: M+Na^+, 272.0890. $C_{13}H_{15}NO_4Na^+$ requires 272.0893); δ_H (400 MHz; $CDCl_3$) 5.63 (1H, s, H-6), 4.91 - 4.80 (2H, m, -C<u>H</u> and -O<u>H</u>), 3.86 (3H, s, -NC<u>H</u>$_3$), 3.86 (3H, s, -OC<u>H</u>$_3$), 2.21 (3H, s, -C<u>H</u>$_3$), 1.44 (3H, d, *J* 6.2, -CHC<u>H</u>$_3$): δ_C (100 MHz; $CDCl_3$) 179.6 (C1 or C4), 179.1 (C1 or C4), 160.2 (C5), 134.1 (C2), 133.6 (C7), 130.2 (C7a), 128.7 (C3), 107.3 (C6), 64.0 (-<u>C</u>HOH), 57.1 (-O<u>C</u>H$_3$), 32.9 (-N<u>C</u>H$_3$), 25.0 (-CH<u>C</u>H$_3$), 10.1 (-<u>C</u>H$_3$).

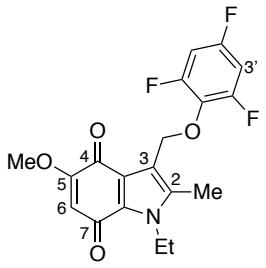
5.3.5 SYNTHESIS OF ARYL ETHERS

5-Methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 209



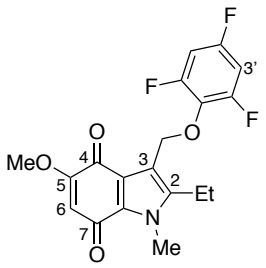
Thionyl chloride (0.515 g, 4.15 mmol) was added dropwise to a stirred solution of the 5-methoxy-3-hydroxymethyl-1,2-dimethylindole-4,7-dione **43** (0.103 g, 0.415 mmol) in dichloromethane (5 mL) at 0 °C. The reaction mixture was stirred at rt for 1.3 h. The solvent and excess thionyl chloride were removed under reduced pressure and the crude product was used directly in the next step without further purification. A suspension of the crude 3-chloromethylindolequinone in acetone (3 mL) was added to a stirred suspension of 2,4,6-trifluorophenol (92.5 mg, 0.621 mmol) and potassium carbonate (86.3 g, 0.622 mmol) under reflux. The resulting mixture was stirred under reflux for 1.8 h. The solvent was removed under reduced pressure and the residue was purified by chromatography eluting with 50-70% ethyl acetate/light petroleum to give the *title compound* as an orange solid (0.109 g, 72%); mp 209-210 °C (lit.,[271] mp 201-202 °C); (Found: M+Na^+, 388.0768. $C_{18}H_{14}F_3N\ O_4Na^+$ requires 388.0767); ν_{max} (solid)/cm^{-1} 2923, 2850, 1674, 1635, 1598, 1506, 1451, 1388, 1338; δ_H (400 MHz; $CDCl_3$) 6.61 (2H, t, J_{HF} 8.0, H-3'), 5.57 (1H, s, H-6), 5.28 (2H, s, -OC<u>H</u>$_2$), 3.88 (3H, s, -NC<u>H</u>$_3$), 3.76 (3H, s, -OC<u>H</u>$_3$), 2.31 (3H, s, -C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 179.3 (C1 or C4), 178.2 (C1 or C4), 160.1 (C5), 157.8 (d, J_{CF} 244, C4'), 157.1 (ddd, J_{CF} 248, 15, 8, C2'), 139.3 (C2), 132.3 (t, J_{CF} 15, C1'), 129.2 (C7a), 122.0 (C3), 116.7 (C3a), 107.1 (C6), 101.1 (t, J_{CF} 26, C3'), 66.0 (-O<u>C</u>H$_2$), 56.5 (-O<u>C</u>H$_3$), 32.5 (-N<u>C</u>H$_3$), 9.6 (-<u>C</u>H$_3$).

1-Ethyl-5-methoxy-2-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole -4,7-dione 210



Thionyl chloride (0.420 g, 3.42 mmol) was added dropwise to a stirred solution of the 1-ethyl-3-(hydroxymethyl)-5-methoxy-2-methyl-*1H*-indole-4,7-dione **206** (85.3 mg, 0.342 mmol) in dichloromethane (4 mL) at 0 °C. The reaction mixture was stirred at rt for 1.6 h. The solvent and excess thionyl chloride were removed under reduced pressure and the crude product was used directly in the next step without further purification. The crude 3-chloroethylindolequinone was added to a stirred suspension of 2,4,6-trifluorophenol (78.4 mg, 0.511 mmol) and potassium carbonate (82.3 g, 0.581 mmol) in acetone (6 mL) under reflux. The resulting mixture was stirred under reflux for 1.8 h. The solvent was removed under reduced pressure and the residue was purified by chromatography eluting with 50-80% ethyl acetate/light petroleum to give the *title compound* as an orange solid (89.9 mg, 69%); mp 159-160 °C; (Found: M+Na^+, 402.0923. $C_{19}H_{16}F_3NO_4Na^+$ requires 402.0924); ν_{max} (solid)/cm^{-1} 3058, 2979, 2922, 2850, 1674, 1628, 1599, 1504, 1445, 1381, 1337; δ_H (400 MHz; $CDCl_3$) 6.66 (2H, t, J_{HF} 8.4, H-3'), 5.59 (1H, s, H-6), 5.30 (2H, s, -OC$\underline{H}_2$), 4.40 (2H, q, *J* 7.2, -NC$\underline{H}_2$), 3.78 (3H, s, -OC$\underline{H}_3$), 2.35 (3H, s, -C$\underline{H}_3$), 1.36 (3H, t, *J* 7.2, -$CH_2C\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 178.9 (C1 or C4), 178.3 (C1 or C4), 160.0 (C5), 157.3 (dy, J_{CF} 244, 14, C4"), 156.6 (ddd, J_{CF} 248, 15, 8, C2"), 138.0 (C2), 131.7 (C1'), 128.0 (C7a), 121.8 (C3), 116.4 (C3a), 106.7 (C6), 100.6 (t, J_{CF} 26, C3'), 66.4 (-O$\underline{C}H_2$), 56.9 (-O$\underline{C}H_3$), 41.0 (-N$\underline{C}H_2$), 15.9 (-$\underline{C}H_3$), 9.7 (-$CH_2\underline{C}H_3$).

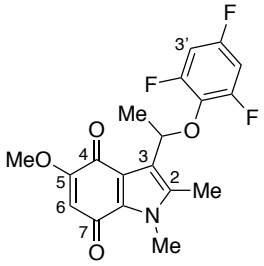
2-Ethyl-5-methoxy-1-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 211



Thionyl chloride (0.452 g, 3.74 mmol) was added dropwise to a stirred solution of the 5-methoxy-3-hydroxymethyl-1,2-dimethylindole-4,7-dione **207** (93.2 mg, 0.374 mmol) in dichloromethane (2 mL) at 0 °C. The reaction mixture was stirred at rt for 2 h. The solvent and excess thionyl chloride were removed under reduced pressure and the crude product was used directly in the next step without further purification. A suspension of the crude 3-chloromethylindolequinone, 2,4,6-trifluorophenol (84.8 mg, 0.573 mmol) and potassium carbonate (89.5 g, 0.648 mmol) in acetone (6 mL) was stirred under reflux for 4.5 h. The solvent was removed under reduced pressure and the residue was purified by chromatography eluting with 50% ethyl acetate/light petroleum to give the *title compound* as an orange solid (988 mg, 65%); mp 193-195 °C; (Found: M+Na^+, 402.0931. $C_{19}H_{16}F_3NO_4Na^+$ requires 402.0924); ν_{max} (solid)/cm^{-1} 3086, 2982, 2944, 1672, 1635, 1598, 1552, 1505, 1486, 1469, 1447, 1409, 1379, 1358, 1335; δ_H (500 MHz; $CDCl_3$) 6.65 (2H, t, J_{HF}

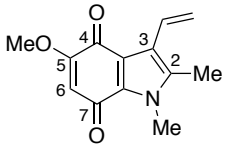
8.5, H-3'), 5.61 (1H, s, H-6), 5.28 (2H, s, -OC$\underline{H}_2$), 3.94 (3H, s, -NC$\underline{H}_3$), 3.79 (3H, s, -OC$\underline{H}_3$), 2.78 (2H, q, *J* 7.5, -C$\underline{H}_2$CH$_3$), 1.23 (3H, t, *J* 7.5, -CH$_2$C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 179.3 (C1 or C4), 178.1 (C1 or C4), 164.1 (C5), 157.7 (d, J_{CF} 244, C4'), 156.9 (ddd, J_{CF} 248, 15, 8, C2'), 144.6 (C2), 132.4 (td, J_{CF} 15, 5, C1'), 129.2 (C7a), 122.0 (C3), 116.2 (C3a), 107.1 (C6), 101.0 (td, J_{CF} 27, 7, C3'), 66.4 (-O$\underline{C}$H$_2$), 56.8 (-O$\underline{C}$H$_3$), 32.8 (-N$\underline{C}$H$_3$), 17.5 (-$\underline{C}$H$_2$CH$_3$), 14.2 (-CH$_2$$\underline{C}H_3$).

5-Methoxy-1,2-dimethyl-3-(1-(2,4,6-trifluorophenoxy)ethyl)-*1H*-indole-4,7-dione 212



Thionyl chloride (0.764 g, 6.41 mmol) was added dropwise to a stirred solution of the 3-(1-hydroxyethyl)-5-methoxy-1,2-dimethyl-*1H*-indole-4,7-dione **208** (0.152 g, 0.611 mmol) in dichloromethane (12 mL) at 0 °C. The reaction mixture was stirred at rt for 2.8 h. The solvent and excess thionyl chloride were removed under reduced pressure and the crude product was used directly in the next step without further purification. A suspension of the crude 3-chloromethylindolequinone in acetone (14 mL), 2,4,6-trifluorophenol (0.135 g, 0.910 mmol) and potassium carbonate (0.145 g, 1.05 mmol) was stirred under reflux for 10 h. The solvent was removed under reduced pressure and the residue was purified by chromatography eluting with 50% ethyl acetate/light petroleum to give the *title compound* as an orange solid (70.7 mg, 30%); mp 135-136 °C; (Found: M+Na$^+$, 402.0927. $C_{19}H_{16}F_3NO_4Na^+$ requires 402.0924); ν_{max} (solid)/cm^{-1} 3056, 2965, 2940, 1669, 1635, 1599, 1502, 1448, 1410, 1381, 1368, 1330; δ_H (500 MHz; $CDCl_3$) 6.61 (2H, t, J_{HF} 8.5, H-3'), 6.10 (1H, q, *J* 6.5, -OC$\underline{H}$), 5.57 (1H, s, H-6), 3.89 (3H, s, -OC$\underline{H}_3$), 3.78 (3H, s, -NC$\underline{H}_3$), 2.47 (3H, s, -C$\underline{H}_3$), 1.63 (3H, d, -OCHC$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 178.7 (C1 or C4), 177.9 (C1 or C4), 159.5 (C5), 157.1 (dt, J_{CF} 244, 14, C4'), 156.4 (ddd, J_{CF} 248, 15, 8, C2'), 136.5 (C2), 131.3 (td, J_{CF} 14, 5, C1'), 128.2 (C7a), 122.5 (C3), 120.4 (C3a), 106.6 (C6), 100.6 (td, J_{CF} 27, 8, C3'), 75.2 (-O$\underline{C}$H), 56.4 (-O$\underline{C}$H$_3$), 32.1 (-N$\underline{C}$H$_3$), 21.4 (-OCH$\underline{C}$H$_3$), 10.9 (-$\underline{C}$H$_3$).

5-Methoxy-1,2-dimethyl-3-vinyl-*1H*-indole-4,7-dione 213



Thionyl chloride (0.205 g, 1.72 mmol) was added dropwise to a stirred solution of the 3-(1-hydroxyethyl)-5-methoxy-1,2-dimethyl-*1H*-indole-4,7-dione **208** (40.7 mg, 0.160 mmol) in dichloromethane (3 mL) at 0 °C. The reaction mixture was stirred at rt for 1 h. The solvent and excess thionyl chloride were removed under reduced pressure and the crude product was used directly in the next step without further purification. A suspension of the crude 3-chloromethylindolequinone in acetone (3 mL), 2,4,6-trifluorophenol (35.3 mg, 0.238 mmol) and potassium carbonate (40.3 mg, 0.291 mmol) was stirred under reflux for 4.8 h. The solvent was removed under reduced pressure and the

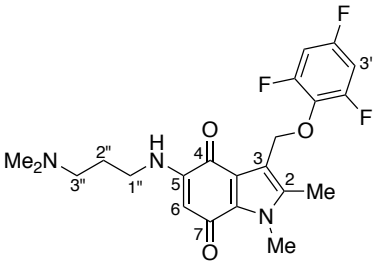
residue was purified by chromatography eluting with 50-60% ethyl acetate/light petroleum to give the *title compound* as a dark red solid (20.7 mg, 56%); mp 202-204 °C; (Found: M+H$^+$, 232.0963. $C_{13}H_{14}NO_3^+$ requires 232.0968); ν_{max} (solid)/cm^{-1} 2919, 2849, 1734, 1663, 1628, 1595, 1539, 1497, 1458, 1411, 1379, 1350, 1324; δ_H (500 MHz; $CDCl_3$) 7.04 (1H, dd, *J* 18.0, 11.5, -C<u>H</u>), 5.73 (1H, dd, *J* 18.0, 1.5, -C<u>H</u>$_2$), 5.62 (1H, s, H-6), 5.43 (1H, dd, *J* 11.5, 1.5, -C<u>H</u>$_2$), 3.93 (3H, s, -OC<u>H</u>$_3$), 3.81 (3H, s, -NC<u>H</u>$_3$), 2.34 (3H, s, -C<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 179.2 (C1 or C4), 178.1 (C1 or C4), 160.2 (C5), 136.1 (C2), 128.9 (C7a), 128.5 (-<u>C</u>H), 121.2 (C3), 121.0 (C3a), 118.1(-<u>C</u>H$_2$), 106.9 (C6), 56.8 (-OC<u>H</u>$_3$), 32.9 (-NC<u>H</u>$_3$), 11.1 (-C<u>H</u>$_3$).

5.3.6 DISPLACEMENT OF METHOXY GROUP BY AN AMINO GROUP

General Procedure 1

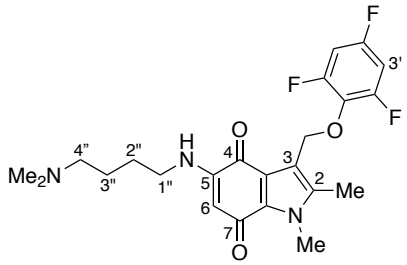
To a solution of the 5-methoxyindolequinone (39.3 – 84.8 μmol) in acetonitrile (0.5 – 1.0 mL) was added the corresponding amine (245 - 1072 μmol). The mixture was stirred at rt. The solvent and excess amine were removed under reduced pressure. When mentioned, the residue was extracted with dichloromethane (3 x 20 mL), washed with water (3 x 20 mL), dried (Na_2SO_4), filtered, and the filtrate concentrated under reduced pressure.

5-((3-(Dimethylamino)propyl)amino)-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 37



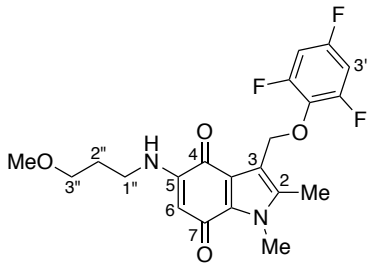
According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione **209** (30.0 mg, 84.8 μmol) in acetonitrile (0.5 mL) was treated with 3-dimethylamino-1-propylamine (50 μL, 393 μmol). The mixture was stirred for 3.5 h. Following workup, the residue was purified by dry-flash chromatography, eluting with 20-90% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (28.2 mg, 74%); mp 93-94 °C (lit.,[52] mp 101-102 °C); (Found: M+H$^+$, 436.1851. $C_{22}H_{25}F_3N_3O_3^+$ requires 436.1843); ν_{max} (solid)/cm^{-1} 3315, 2921, 1664, 1597, 1502, 1461, 1378; δ_H (500 MHz; $CDCl_3$) 6.66 (2H, t, J_{HF} 8.5, H-3'), 6.64 (1H, br s, -N<u>H</u>), 5.27 (2H, s, -OC<u>H</u>$_2$), 5.11 (1H, s, H-6), 3.92 (3H, s, -NC<u>H</u>$_3$), 3.17 (2H, q, *J* 6.5, H-1"), 2.41 (2H, t, *J* 6.5, H-3"), 2.23 (3H, s, -C<u>H</u>$_3$), 2.22 (6H, s, N(C<u>H</u>$_3$)$_2$), 1.81 (2H, quint, *J* 6.5, H-2"); δ_C (125 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.0 (C1 or C4), 157.8 (dt, J_{CF} 243, 15, C4'), 157.1 (ddd, J_{CF} 248, 15, 8, C2'), 148.9 (C5), 137.1 (C2), 131.9 (td, J_{CF} 15, 5, C1'), 131.6 (C7a), 120.0 (C3), 115.5 (C3a), 100.8 (td, J_{CF} 26, 7, C3'), 96.7 (C6), 66.7 (-O<u>C</u>H$_2$), 57.9 (C3"), 45.7 (-N(<u>C</u>H$_3$)$_2$), 42.1 (C1"), 32.7 (-N<u>C</u>H$_3$), 25.9 (C2"), 9.7 (-<u>C</u>H$_3$).

5-((4-(Dimethylamino)butyl)amino)-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 64



According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4,7-dione **209** (21.4 mg, 58.6 μmol) in acetonitrile (1 mL) was treated with N^1,N^1-dimethylbutane-1,4-diamine (75 μL, 358 μmol). The mixture was stirred at rt for 8 days. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 50-98% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a pink solid (20.4 mg, 78%); mp 107-109 °C; (Found: M+H$^+$, 450.2008. $C_{23}H_{27}F_3N_3O_3^+$ requires 450.1999); λ_{max} (MeOH)/nm 205 (log ε 4.20), 246 (log ε 4.16), 316 (log ε 4.05), 526 (log ε 3.35); ν_{max} (solid)/cm^{-1} 3307, 2940, 2856, 2814, 2762, 1666, 1612, 1593, 1501, 1474, 1461, 1413, 1378, 1315; δ_H (500 MHz; $CDCl_3$) 6.64 (2H, t, J_{HF} 8.5, H-3'), 6.30 (1H, t, *J* 5.5, -NH), 5.26 (2H, s, -OC$\underline{H}_2$), 5.11 (1H, s, H-6), 3.92 (3H, s, -NC$\underline{H}_3$), 3.09 (2H, td, *J* 7.0, 1.5, H-1"), 2.28 (2H, t, *J* 7.0, H-4"), 2.26 (9H, s, -N(C$\underline{H}_3$)$_2$ + C$\underline{H}_3$), 1.69 (2H, quint, *J* 7.0, H-2"), 1.57 (2H, quint, *J* 7.0, H-3"); δ_C (125 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.0 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4'), 157.0 (ddd, J_{CF} 249, 14, 8, C2'), 148.8 (C5), 137.2 (C2), 132.3 (td, J_{CF} 14, 5, C1'), 131.6 (C7a), 120.0 (C3), 115.6 (C3a), 101.1 (td, J_{CF} 27, 7, C4'), 96.9 (C6), 66.6 (-O$\underline{C}H_2$), 59.4 (C4"), 45.7 (-N($\underline{C}H_3$)$_2$), 43.1 (C2"), 32.7 (-N$\underline{C}H_3$), 26.4 (C2"), 25.6 (C3"), 9.7 (-$\underline{C}H_3$).

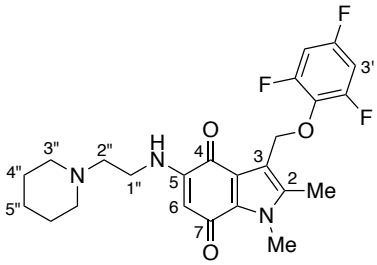
5-((3-Methoxypropyl)amino)-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 53



According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4,7-dione **209** (22.1 mg, 62.3 μmol) in acetonitrile (0.5 mL) was treated with 3-methoxypropan-1-amine (50 μL, 485 μmol). The mixture was stirred at rt for 91 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 50-80% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a pink solid (23.5 mg, 89%); mp 155-156 °C; (Found: M+H$^+$, 423.1532. $C_{21}H_{22}F_3N_2O_4^+$ requires 423.1526); λ_{max} (MeOH)/nm 201 (log ε 4.36), 245 (log ε 4.23), 316 (log ε 4.05), 525 (log ε 3.34); ν_{max} (solid)/cm^{-1} 3296, 2933, 2886, 2838, 1671, 1595, 1503, 1461, 1449, 1411, 1383, 1354, 1316; δ_H (500 MHz; $CDCl_3$) 6.64 (2H, t, J_{HF} 8.5, H-3'), 6.14 (1H, t, *J* 5.5, -NH), 5.27 (2H, s, -OC$\underline{H}_2$), 5.13 (1H, s, H-6), 3.92 (3H, t, *J* 4.0, -OC$\underline{H}_3$), 3.47

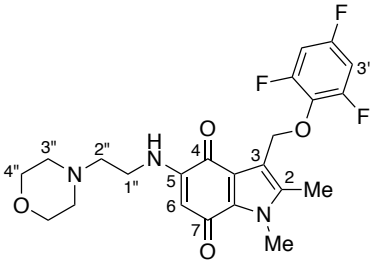
(3H, s, -NC$\underline{H}_3$), 3.36 (2H, t, *J* 6.0, H-3"), 3.21 (2H, q, *J* 6.0, H-1"), 2.26 (3H, s, -C$\underline{H}_3$), 1.93 (2H, quint, *J* 6.0, H-2"); δ_C (125 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.0 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4'), 157.0 (ddd, J_{CF} 248, 15, 8, C2'), 148.7 (C5), 137.2 (C2), 132.2 (td, J_{CF} 14, 5, C1'), 131.6 (C7a), 120.0 (C3), 115.6 (C3a), 101.0 (td, J_{CF} 27, 7, C3'), 96.8 (C6), 70.8 (-O$\underline{C}H_3$), 66.6 (-O$\underline{C}H_2$), 59.2 (C3"), 41.0 (C1"), 32.7 (-N$\underline{C}H_3$), 28.6 (C2"), 9.73 (-$\underline{C}H_3$).

1,2-Dimethyl-5-((2-(piperidin-1-yl)ethyl)amino)-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 65



According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione **209** (30.3 mg, 82.9 μmol) in acetonitrile (0.5 mL) was treated with 1-(2-aminoethyl)piperidine (100 μL, 245 μmol). The mixture was stirred at rt for 23.5 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by dry-flash chromatography, eluting with 20-90% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (28.5 mg, 78%); mp 110-111 °C; (Found: M+H$^+$, 462.0008. $C_{24}H_{27}F_3N_3O_3^+$ requires 462.1999); λ_{max} (MeCN)/nm 244 (log ε 4.13), 312 (log ε 3.95), 516 (log ε 3.11); ν_{max} (solid)/cm^{-1} 3360, 2817, 2849, 2809, 2793, 2752, 1659, 1610, 1503, 1461, 1409, 1380; δ_H (500 MHz; $CDCl_3$) 6.64 (2H, t, J_{HF} 7.6, H-3'), 6.39 (1H, br s, -N$\underline{H}$), 5.27 (2H, s, -OC$\underline{H}_2$), 5.10 (1H, s, H-6), 3.92 (3H, s, -NC$\underline{H}_3$), 3.13 (2H, td, *J* 6.0, 5.6, H-1"), 2.58 (2H, t, *J* 6.0, H-2"), 2.39 (4H, m, H-3"), 2.25 (3H, s, -C$\underline{H}_3$), 1.59 (4H, m, H-4"), 1.44 (2H, quint, *J* 6.0, H-5"); δ_C (125 MHz; $CDCl_3$) 179.3 (C1 or C4), 179.0 (C1 or C4), 157.2 (dt, J_{CF} 244, 14, C4'), 156.5 (ddd, J_{CF} 248, 15, 8, C2'), 148.7 (C5), 137.1 (C2), 131.8 (td, J_{CF} 15, 5, C1'), 131.5 (C7a), 120.0 (C3), 115.2 (C3a), 100.9 (t, J_{CF} 26, C3'), 97.0 (C6), 66.6 (-O$\underline{C}H_2$), 56.3 (C2"), 54.6 (C3'), 39.6 (C1"), 32.7 (-N$\underline{C}H_3$), 26.2 (C4"), 24.7 (C5"), 9.7 (-$\underline{C}H_3$).

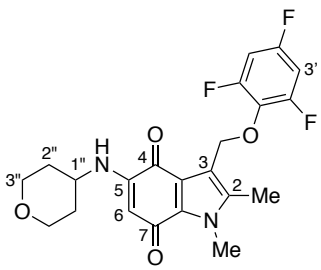
1,2-Dimethyl-5-((2-morpholinoethyl)amino)-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 214



According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4,7-dione **209** (24.7 mg, 67.6 μmol) in acetonitrile (0.5 mL) was treated with 2-morpholinoethan-1-amine (80 μL, 603 μmol). The mixture was stirred at rt for 68 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with ethyl acetate/light petroleum and 2% triethylamine to give the

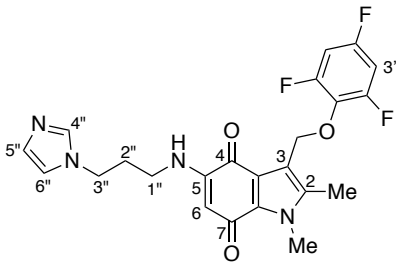
title compound as a purple solid (17.1 mg, 55%); mp 130-132 °C; (Found: M+H$^+$, 464.1791. $C_{23}H_{25}F_3N_3O_4^+$ requires 464.1792); λ_{max} (MeOH)/nm 244 (log ε 4.25), 316 (log ε 4.06), 522 (log ε 3.34); ν_{max} (solid)/cm^{-1} 3355, 2956, 2930, 2839, 2814, 1661, 1619, 1600, 1503, 1462, 1410, 1381, 1353, 1333, 1316; δ_H (500 MHz; $CDCl_3$) 6.65 (2H, t, J_{HF} 8.5, H-3'), 6.34 (1H, br s, -NH), 5.27 (2H, s, -OC$\underline{H}_2$), 5.11 (1H, s, H-6), 3.92 (3H, s, -NC$\underline{H}_3$), 3.73 (4H, t, *J* 4.5, H-4"), 3.15 (2H, q, *J* 6.0, H-1"), 2.64 (2H, t, *J* 6.0, H-2"), 2.47 (4H, t, *J* 4.5, H-3"), 2.26 (3H, s, -C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.0 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4'), 157.0 (ddd, J_{CF} 248, 15, 7, C2'), 148.6 (C5), 137.3 (C2), 132.2 (td, J_{CF} 15, 5, C1'), 131.5 (C7a), 120.1 (C3), 115.6 (C3a), 101.0 (td, J_{CF} 26, 7, C3'), 97.3 (C6), 67.3 (-O$\underline{C}H_2$), 66.7 (C4"), 56.1 (C2"), 53.6 (C3"), 39.1 (C1"), 32.8 (-N$\underline{C}H_3$), 9.7 (-$\underline{C}H_3$).

1,2-Dimethyl-5-((tetrahydro-2H-pyran-4-yl)amino)-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 215



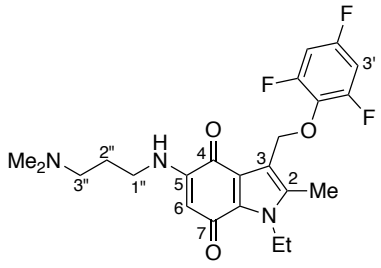
According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione **209** (22.1 mg, 62.3 μmol) in acetonitrile (0.5 mL) was treated with tetrahydro-2*H*-pyran-4-amine (100 μL, 936 μmol). The mixture was stirred at rt for 13 days. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 20-50% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a pink-red solid (11.2 mg, 41%); mp 168-170 °C; (Found: M+H$^+$, 435.1526. $C_{22}H_{22}F_3N_2O_4^+$ requires 435.1526); λ_{max} (MeOH)/nm 244 (log ε 4.30), 316 (log ε 4.09), 523 (log ε 3.40); ν_{max} (solid)/cm^{-1} 3355, 3060, 2967, 2939, 2848, 2547, 1739, 1666, 1613, 1594, 1545, 1500, 1461, 1448, 1433, 1381, 1311; δ_H (500 MHz; $CDCl_3$) 6.66 (2H, t, J_{HF} 8.5, H-3'), 5.76 (1H, d, *J* 8.0, -NH), 5.26 (2H, s, -OC$\underline{H}_2$), 5.16 (1H, s, H-6), 3.99 (2H, dt, *J* 11.5, 4.0, H-3"), 3.92 (3H, s, -NC$\underline{H}_3$), 3.49 (2H, dt, *J* 11.5, 4.0, H-3"), 3.42 (1H, tt, *J* 11.0, 8.0, H-1"), 2.27 (3H, s, -C$\underline{H}_3$), 1.99 (2H, d, *J* 11.5, H-2"), 1.59 (2H, dd, *J* 11.0, 4.0, H-2"); δ_C (100 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.0 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4'), 156.9 (ddd, J_{CF} 248, 15, 8, C2'), 146.9 (C5), 137.4 (C2), 132.3 (td, J_{CF} 14, 5, C1'), 131.3 (C7a), 120.0 (C3), 115.7 (C3a), 101.0 (td, J_{CF} 26, 7, C3'), 97.5 (C6), 66.8 (C3"), 66.6 (-O$\underline{C}H_2$), 49.0 (C1"), 32.8 (-N$\underline{C}H_3$), 32.3 (C2"), 9.7 (-$\underline{C}H_3$).

5-((3-(*1H*-Imidazol-1-yl)propyl)amino)-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 66



According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4, 7-dione **209** (10.0 mg, 27.4 μmol) in acetonitrile (0.5 mL) was treated with 3-(*1H*-imidazol-1-yl)propan-1-amine (40 μL, 325 μmol). The mixture was stirred at rt for 5 days. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 50-98% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a pink solid (12.0 mg, 96%); mp 191-192 °C; (Found: M+H$^+$, 459.1645. $C_{23}H_{22}F_3N_4O_3^+$ requires 459.1639); λ_{max} (MeOH)/nm 244 (log ε 4.24), 314 (log ε 4.02), 519 (log ε 3.33); ν_{max} (solid)/cm^{-1} 3368, 3144, 3111, 1662, 1610, 1588, 1506, 1494, 1462, 1413, 1378, 1332, 1304; δ_H (500 MHz; $CDCl_3$) 7.48 (1H, s, H-4"), 7.10 (1H, d, *J* 1.5, H-5"), 6.91 (1H, d, *J* 1.5, H-6"), 6.66 (2H, t, J_{HF} 8.0, H-3'), 5.74 (1H, t, *J* 6.0, -NH), 5.26 (2H, s, -OC$\underline{H}_2$), 5.09 (1H, s, H-6), 4.04 (2H, t, *J* 7.0, H-1"), 3.91 (3H, s, -NC$\underline{H}_3$), 3.11 (2H, t, *J* 7.0, H-3"), 2.27 (3H, s, -C$\underline{H}_3$), 2.14 (2H, quint, *J* 7.0, H-2"); δ_C (125 MHz; $CDCl_3$) 179.2 (C1 or C4), 178.8 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4'), 156.9 (ddd, J_{CF} 246, 15, 8, C2'), 148.1 (C5), 137.6 (C4"), 137.4 (C2), 132.2 (td, J_{CF} 13, 5, C1'), 131.3 (C7a), 130.5 (C5"), 120.1 (C3), 119.0 (C6"), 115.8 (C3a), 101.0 (td, J_{CF} 26, 7, C4') 97.8 (C6), 66.6 (-O$\underline{C}H_2$), 44.6 (C1"), 39.9 (C3"), 32.8 (-N$\underline{C}H_3$), 29.9 (C2"), 9.7 (-$\underline{C}H_3$).

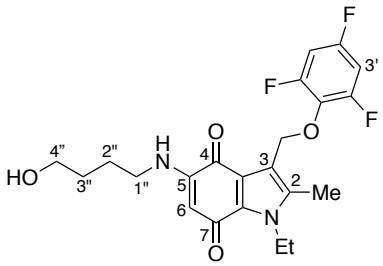
5-((3-(Dimethylamino)propyl)amino)-1-ethyl-2-methyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4,7-dione 67



According to the general procedure 1, a solution of 1-ethyl-5-methoxy-2-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione **210** (26.3 mg, 69.3 μmol) in acetonitrile (0.5 mL) was treated with 3-dimethylamino-1-propylamine (40 μL, 314 μmol). The mixture was stirred at rt for 23 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by dry-flash chromatography, eluting with 20-90% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (27.6 mg, 89%); mp 94-95 °C; (Found: M+H$^+$, 450.2010. $C_{23}H_{27}F_3N_3O_3^+$ requires 450.1999); λ_{max} (MeCN)/nm 245 (log ε 4.08), 314 (log ε 3.91), 516 (log ε 1.09); ν_{max} (solid)/cm^{-1} 3373, 2921, 2853, 2761, 1663, 1618, 1498, 1462, 1375; δ_H (500 MHz; $CDCl_3$) 6.64 (2H, t, J_{HF} 8.8, H-3'), 6.58 (1H, t, *J* 5.0, -NH), 5.27 (2H, s, -OC$\underline{H}_2$), 5.11 (1H, s, H-6), 4.40 (2H, q, *J* 7.5, -NC$\underline{H}_2$), 3.16 (2H, q, *J* 6.5, -C$\underline{H}_2$, H-3"), 2.38 (2H,

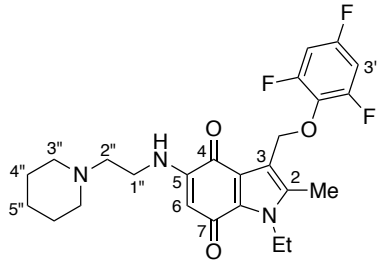
d, *J* 6.5, -C<u>H</u>$_2$, H-1"), 2.27 (3H, s, -C<u>H</u>$_3$), 2.25 (6H, s, N(CH$_3$)$_2$), 1.79 (2H, quint, *J* 6.5, H-2"), 1.32 (3H, t, *J* 7.5, - CH$_2$C<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 179.1 (C1 or C4), 179.0 (C1 or C4), 157.8 (dt, J_{CF} 244, 14, C4'), 157.0 (ddd, J_{CF} 248, 15, 8, C2'), 148.8 (C5), 136.2 (C2), 132.3 (td, J_{CF} 15, 5, C1'), 130.9 (C7a), 120.3 (C3), 115.7 (C3a), 101.0 (ddd J_{CF} 27, 26, 8, C3'), 96.8 (C6), 66.7 (-O<u>C</u>H$_2$), 58.0 (C3"), 45.8 (-N(<u>C</u>H$_3$)$_2$), 42.1 (C1"), 40.7 (-N<u>C</u>H$_2$), 26.0 (C2"), 15.9 (-<u>C</u>H$_3$), 9.4 (-<u>C</u>H$_3$).

1-Ethyl-5-((4-hydroxybutyl)amino)-2-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 68



According to the general procedure 1, a solution of 1-ethyl-5-methoxy-2-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione **210** (29.7 mg, 78.3 μmol) in acetonitrile (0.5 mL) was treated with 4-aminobutan-1-ol (50 μL, 531 μmol). The mixture was stirred at rt for 2 days. The solvent and excess amine were removed under reduced pressure and the residue was purified by dry-flash chromatography, eluting with 50-70% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (33.8 mg, 99%); mp 137-139 °C; (Found: M+H$^+$, 437.1692. $C_{22}H_{24}F_3N_2O_4^+$ requires 437.1683); λ_{max} (MeOH)/nm 246 (log ε 4.19), 316 (log ε 4.04), 527 (log ε 3.32); ν_{max} (solid)/cm^{-1} 3378, 3068, 2934, 2867, 1664, 1620, 1598, 1502, 1463, 1446, 1381, 1322 ; δ_H (500 MHz; $CDCl_3$) 6.64 (2H, t, J_{HF} 8.5, H-3'), 5.89 (1H, br s, -N<u>H</u>), 5.26 (2H, s, -OC<u>H</u>$_2$), 5.12 (1H, s, H-6), 4.40 (2H, q, *J* 7.0, -NC<u>H</u>$_2$), 3.69 (2H, t, *J* 6.0, H-4"), 3.11 (2H, q, *J* 6.0, H-1"), 2.28 (3H, s, -C<u>H</u>$_3$), 1.75 (2H, quint, *J* 6.0, H-2" or H-3"), 1.65 (2H, quint, *J* 6.0, H-2" or H-3"), 1.32 (3H, t, *J* 7.0, -NCH$_2$C<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 179.1 (C1 or C4), 179.0 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4'), 157.0 (ddd, J_{CF} 248, 15, 8, C2'), 148.4 (C5), 136.4 (C2), 132.2 (td, J_{CF} 15, 5, C1'), 130.8 (C7a), 120.2 (C3), 115.8 (C3a), 100.9 (td, J_{CF} 27, 6, C3'), 97.1 (C6), 66.6 (-O<u>C</u>H$_2$), 62.6 (C4"), 42.9 (C1"), 40.7 (-N<u>C</u>H$_2$), 30.3, 25.1, 15.9 (-<u>C</u>H$_3$), 9.4 (-<u>C</u>H$_3$).

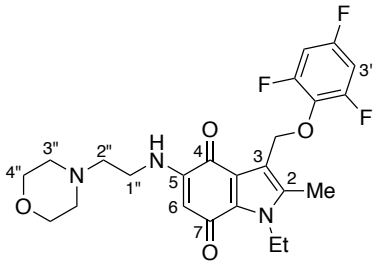
1-Ethyl-2-methyl-5-((2-(piperidin-1-yl)ethyl)amino)-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4,7-dione 69



According to the general procedure 1, a solution of 1-ethyl-5-methoxy-2-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione **210** (29.0 mg, 76.4 μmol) in acetonitrile (1.2 mL) was treated with 1-(2-aminoethyl)piperidine (100 μL, 1072 μmol). The mixture was stirred at rt for 49 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by

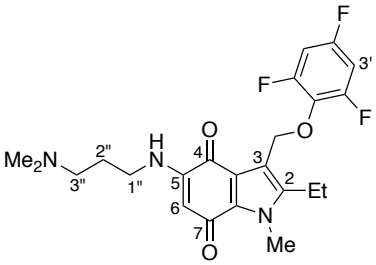
chromatography, eluting with 20-70% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (29.3 mg, 81%); mp 103-105 °C; (Found: M+H$^+$, 476.2162. $C_{25}H_{29}F_3N_3O_3^+$ requires 476.2156); λ_{max} (MeOH)/nm 203 (log ε 4.32), 245 (log ε 4.24), 315 (log ε 4.09), 524 (log ε 3.39); ν_{max} (solid)/cm^{-1} 3360, 3070, 2926, 1660, 1619, 1610, 1597, 1502, 1491, 1464, 1444, 1433, 1381, 1362, 1349, 1317, 1304; δ_H (400 MHz; $CDCl_3$) 6.64 (2H, t, J_{HF} 8.0 H-3'), 6.36 (1H, br s, -NH), 5.27 (2H, s, -OC$\underline{H}_2$), 5.09 (1H, s, H-6), 4.40 (2H, q, *J* 7.0, -NC$\underline{H}_2$), 3.12 (2H, q, *J* 6.0, H-1"), 2.58 (2H, t, *J* 6.0, H-2"), 2.39 (4H, m, H-3"), 2.27 (3H, s, -C$\underline{H}_3$), 1.58 (4H, m, H-4"), 1.44 (2H, quint, *J* 6.0, H-5"), 1.32 (3H, t, *J* 7.0, -$CH_2C\underline{H}_3$); δ_C (100 MHz; $CDCl_3$) 179.1 (C1 or C4), 179.0 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4'), 157.1 (ddd, J_{CF} 248, 15, 8, C2'), 148.6 (C5), 136.2 (C2), 132.3 (C7a), 130.9 (td, J_{CF} 15, 5, C1'), 120.3 (C3), 115.8 (C3a), 101.2 (t, J_{CF} 27, C3'), 97.2 (C6), 66.7 (-O$\underline{C}H_2$), 56.4 (C2"), 54.7 (C3"), 40.7 (-N$\underline{C}H_2$), 39.7 (C1"), 26.3 (C5"), 24.8 (C4"), 15.9 (-$CH_2\underline{C}H_3$), 9.4 (-$\underline{C}H_3$).

1-Ethyl-2-methyl-5-((2-morpholinoethyl)amino)-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 70



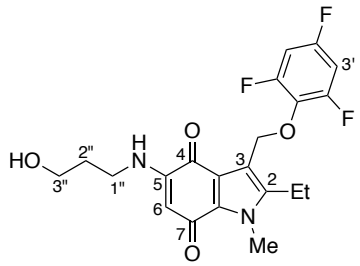
According to the general procedure 1, a solution of 1-ethyl-5-methoxy-2-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4, 7-dione **210** (31.4 mg, 82.8 μmol) in acetonitrile (0.5 mL) was treated with 4-(2-aminoethyl)morpholine (50 μL, 378 μmol). The mixture was stirred at rt for 27.5 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by dry-flash chromatography, eluting with 20-90% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (37.8 mg, 96%); mp 119-121 °C; (Found: M+H$^+$, 478.1952. $C_{24}H_{27}F_3N_3O_4^+$ requires 478.1948); λ_{max} (MeCN)/nm 244 (log ε 4.25), 314 (log ε 4.09), 516 (log ε 3.23); ν_{max} (solid)/cm^{-1} 3354, 2955, 2920, 2848, 2817, 1662, 1600, 1490, 1381; δ_H (500 MHz; $CDCl_3$) 6.66 (2H, t, J_{HF} 8.5, H-3'), 6.31 (1H, t, *J* 8.4, -NH), 5.27 (2H, s, OC$\underline{H}_2$), 5.10 (1H, s, H-6), 4.40 (2H, t, *J* 7.0, -NC$\underline{H}_2$), 3.73 (4H, t, *J* 4.5, H-4"), 3.15 (2H, q, *J* 6.0, H-1"), 2.64 (2H, t, *J* 6.0, H-2"), 2.49 (4H, t, *J* 4.5, H-3"), 2.27 (3H, s, -C$\underline{H}_3$), 1.32 (3H, t, *J* 7.0, -$CH_2C\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 179.1 (C1 or C4), 179.0 (C1 or C4), 158.0 (dt, J_{CF} 244, 15, C4'), 157.0 (ddd, J_{CF} 241, 15, 8, C2'), 148.4 (C5), 136.4 (C2), 131.8 (td, J_{CF} 15, 5, C1'), 130.8 (C7a), 120.3 (C3), 115.8 (C3a), 101.0 (ddd J_{CF} 27, 15, 8, C3'), 97.4 (C6), 67.3 (C4"), 66.7 (-O$\underline{C}H_2$), 56.1 (C2"), 53.6 (C3"), 40.7 (-N$\underline{C}H_2$), 39.1 (C1"), 15.9 (-$\underline{C}H_3$), 9.4 (-$\underline{C}H_3$).

5-((3-(Dimethylamino)propyl)amino)-2-ethyl-1-methyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4,7-dione 71



According to the general procedure 1, a solution of 2-ethyl-5-methoxy-1-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione **211** (28.0 mg, 73.8 μmol) in acetonitrile (0.7 mL) was treated with 3-dimethylamino-1-propylamine (70 μL, 551 μmol). The mixture was stirred at rt for 2 days. The solvent and excess amine were removed under reduced pressure and the residue was purified by chromatography, eluting with 50-90% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (25.4 mg, 77%); mp 112-114 °C; (Found: M+H$^+$, 450.2007. $C_{23}H_{27}F_3N_3O_3^+$ requires 450.1999); λ_{max} (MeOH)/nm 244 (log ε 4.09), 316 (log ε 4.01), 524 (log ε 3.35); ν_{max} (solid)/cm^{-1} 3310, 2937, 2876, 2815, 2763, 1665, 1596, 1501, 1450, 1407, 1377, 1317; δ_H (500 MHz; $CDCl_3$) 6.65 (2H, t, J_{HF} 8.5, H-3'), 6.61 (1H, br s, -NH), 5.25 (2H, s, -OC$\underline{H}_2$), 5.13 (1H, s, H-6), 3.95 (3H, s, -NC$\underline{H}_3$), 3.17 (2H, q, *J* 6.5, H-3"), 2.72 (2H, t, *J* 7.5, -C$\underline{H}_2$), 2.38 (2H, t, *J* 6.5, H-1"), 2.22 (6H, s, -N(C$\underline{H}_3$)$_2$), 1.79 (2H, quint, *J* 6.5, H-2"), 1.19 (3H, t, *J* 7.5, -CH$_2$C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.1 (C1 or C4), 157.7 (dt, J_{CF} 244, 15, C4'), 157.0 (ddd, J_{CF} 248, 15, 7, C2'), 149.0 (C5), 142.6 (C2), 132.5 (td, J_{CF} 15, 5, C1'), 131.7 (C7a), 120.1 (C3), 115.1 (C3a), 101.0 (td, J_{CF} 27, 7 C3'), 96.8 (C6), 66.7 (-O$\underline{C}$H$_2$), 58.0 (C3"), 45.8 (-N($\underline{C}$H$_3$)$_2$), 42.2 (C1"), 32.7 (-N$\underline{C}$H$_3$), 26.0 (C2"), 17.3 (-$\underline{C}$H$_2$CH$_3$), 14.4 (-CH$_2\underline{C}$H$_3$).

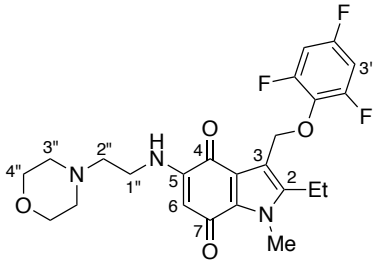
2-Ethyl-5-((3-hydroxypropyl)amino)-1-methyl-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 72



According to the general procedure 1, a solution of 2-ethyl-5-methoxy-1-methyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4,7-dione **211** (29.4 mg, 77.5 μmol) in acetonitrile (0.5 mL) was treated with 3-aminopropan-1-ol (50 μL, 647 μmol). The mixture was stirred at rt for 21.5 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by chromatography, eluting with 20-98% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (14.9 mg, 46%); mp 214-215 °C; (Found: M+H$^+$, 423.1530. $C_{21}H_{22}F_3N_2O_4^+$ requires 423.1526); λ_{max} (MeOH)/nm 205 (log ε 4.22), 246 (log ε 4.17), 316 (log ε 4.02), 523 (log ε 3.31); ν_{max} (solid)/cm^{-1} 3383, 3342, 3093, 2943, 2879, 1665, 16111, 1596, 1497, 1457, 1409, 1380, 1353, 1316; δ_H (500 MHz; DMSO-d_6) 7.25 (1H, t, *J* 6.0, -NH), 7.20 (2H, t, J_{HF} 9.0, H-3'), 5.21 (2H, s, -OC$\underline{H}_2$), 5.07 (1H, s, H-6), 4.61 (1H, t, *J* 5.0, -OH), 3.90 (3H, s, -NC$\underline{H}_3$), 3.46

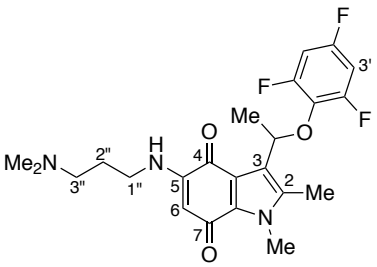
(2H, q, *J* 6.5, H-3”, 3.14 (2H, q, *J* 6.5, H-1”), 2.68 (2H, q, *J* 7.5, -C<u>H</u>$_2$CH$_3$), 1.69 (2H, quint, *J* 6.5, H-2”), 1.08 (3H, t, *J* 7.5, -CH$_2$C<u>H</u>$_3$); δ_C (125 MHz; DMSO-d$_6$) 178.6 (C1 or C4), 178.0 (C1 or C4), 157.1 (dt, J_{CF} 241, 15, C4’), 156.4 (ddd, J_{CF} 247, 16, 8, C2’), 148.8 (C5), 142.8 (C2), 131.8 (C7a), 132.0 (t, J_{CF} 15, C1’), 119.4 (C3), 114.3 (C3a), 101.61 (td, J_{CF} 27, 7, C3’), 95.5 (C6), 66.2 (-O<u>C</u>H$_2$), 59.0 (C3”), 40.6 (C1”), 32.3 (-N<u>C</u>H$_3$), 30.8 (C2”), 16.4 (-<u>C</u>H$_2$CH$_3$), 14.0 (-CH$_2$<u>C</u>H$_3$).

2-Ethyl-1-methyl-5-((2-morpholinoethyl)amino)-3-((2,4,6-trifluorophenoxy)methyl)-*1H*-indole-4,7-dione 73



According to the general procedure 1, a solution of 2-ethyl-5-methoxy-1-methyl-3-((2,4,6-trifluorophenoxy)-methyl)-*1H*-indole-4, 7-dione **211** (28.1 mg, 74.1 μmol) in acetonitrile (0.7 mL) was treated with 2-morpholinoethan-1-amine (100 μL, 759 μmol). The mixture was stirred at rt for 2 days. The solvent and excess amine were removed under reduced pressure and the residue was purified by chromatography, eluting with 50-60% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (32.8 mg, 93%); mp 125-127 °C; (Found: M+H$^+$, 478.1957. $C_{24}H_{27}F_3N_3O_4^+$ requires 478.1948); λ_{max} (MeOH)/nm 204 (log ε 4.36), 246 (log ε 4.28), 314 (log ε 4.08), 522 (log ε 3.38); ν_{max} (solid)/cm^{-1} 3307, 3064, 2930, 2853, 1666, 1594, 1555, 1502, 1450, 1408, 1378, 1357, 1309; δ_H (500 MHz; $CDCl_3$) 6.66 (2H, t, J_{HF} 8.0, H-3’), 6.33 (1H, br s, -N<u>H</u>), 5.25 (2H, s, -OC<u>H</u>$_2$), 5.12 (1H, s, H-6), 3.95 (3H, s, -NC<u>H</u>$_3$), 3.73 (4H, t, *J* 4.5, H-4”), 3.15 (2H, dd, *J* 6.0, 5.5, H-1”), 2.70 (2H, q, *J* 7.5, -C<u>H</u>$_2$CH$_3$), 2.64 (2H, t, *J* 6.0, H-2”), 2.46 (4H, t, *J* 4.5, H-3”), 1.18 (3H, t, *J* 7.5, -CH$_2$C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 179.4 (C1 or C4), 179.0 (C1 or C4), 157.7 (dt, J_{CF} 244, 14, C4’), 157.0 (ddd, J_{CF} 240, 15, 8, C2’), 148.6 (C5), 142.7 (C2), 132.4 (td, J_{CF} 15, 8, C1’), 131.6 (C7a), 120.1 (C3), 115.2 (C3a), 101.0 (td, J_{CF} 27, 7, C3’), 97.3 (C6), 67.3 (C4”), 66.7 (-O<u>C</u>H$_2$), 56.1(C2”), 53.6 (C3”), 39.2 (C1”), 32.8 (-N<u>C</u>H$_3$), 17.3 (-<u>C</u>H$_2$CH$_3$), 14.4 (-CH$_2$<u>C</u>H$_3$).

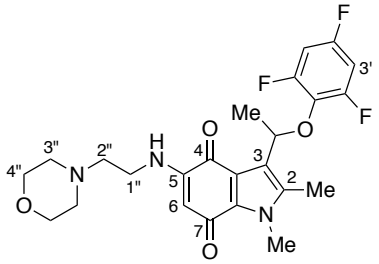
5-((3-(Dimethylamino)propyl)amino)-1,2-dimethyl-3-(1-(2,4,6-trifluorophenoxy)ethyl)-*1H*-indole-4,7-dione 74



According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-(1-(2,4,6-trifluorophenoxy)-ethyl)-*1H*-indole-4,7-dione **212** (15.3 mg, 40.3 μmol) in acetonitrile (0.5 mL) was treated with *N*^{1},*N*^{1}-dimethylpropane-1,3-diamine (25 μL, 196 μmol). The mixture was stirred at rt for 43 h. The solvent and

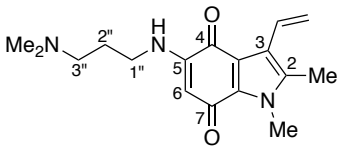
excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 50-98% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a pink solid (18.0 mg, 99%); mp 87-89 °C; (Found: M+H$^+$, 450.2008. $C_{23}H_{27}F_3N_3O_3^+$ requires 450.1999); λ_{max} (MeOH)/nm 204 (log ε 4.31), 245 (log ε 4.24), 316 (log ε 4.09), 528 (log ε 3.37); ν_{max} (solid)/cm^{-1} 3149, 2980, 2947, 2861, 2820, 2773, 1660, 1607, 1595, 1496, 1448, 1408, 1377, 1311; δ_H (500 MHz; $CDCl_3$) 6.62 (2H, t, J_{HF} 8.5, H-3'), 6.55 (1H, t, *J* 5.0, -NH), 6.00 (1H, q, *J* 6.5, -OCH), 5.10 (1H, s, H-6), 3.90 (3H, s, -NCH_3), 3.15 (2H, q, *J* 6.5, H-1"), 2.39 (3H, s, -CH_3), 2.37 (2H, t, *J* 6.5, H-3"), 2.24 (6H, s, -N(CH_3)$_2$), 1.78 (2H, quint, *J* 6.5, H-2"), 1.65 (3H, d, *J* 6.5, -CHCH_3); δ_C (125 MHz; $CDCl_3$) 179.3 (C1 or C4), 179.0 (C1 or C4), 157.4 (dt, J_{CF} 244, 14, C4'), 156.9 (ddd, J_{CF} 248, 15, 8, C2'), 148.9 (C5), 134.6 (C2), 131.7 (td, J_{CF} 14, 5, C1'), 131.1 (C7a), 121.7 (C3), 118.9 (C3a), 100.9 (td, J_{CF} 14, 5, C3'), 96.7 (C6), 75.7 (-OCH), 58.0 (C3"), 45.8 (-N(CH_3)$_2$), 42.2 (C1"), 32.4 (-NCH_3), 26.1(C2"), 21.8 (-CHCH_3), 11.0 (-CH_3).

1,2-Dimethyl-5-((2-morpholinoethyl)amino)-3-(1-(2,4,6-trifluorophenoxy)ethyl)-1H-indole-4,7-dione 216



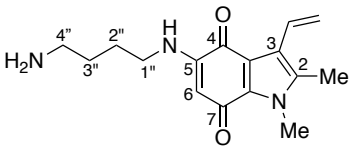
According to the general procedure 1, a solution of 5-methoxy-1,2-dimethyl-3-(1-(2,4,6-trifluorophenoxy)-ethyl)-*1H*-indole-4,7-dione **212** (12.4 mg, 32.7 μmol) in acetonitrile (0.5 mL) was treated with 2-morpholinoethan-1-amine (40 μL, 302 μmol). The mixture was stirred at rt for 32 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 50% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a burgundy solid (14.9 mg, 95%); mp 133-136 °C; (Found: M+H$^+$, 478.1960. $C_{24}H_{27}F_3N_3O_4$ requires 478.1948); λ_{max} (MeOH)/nm 246 (log ε 4.24), 315 (log ε 4.13), 523 (log ε 3.52); ν_{max} (solid)/cm^{-1} 3309, 2966, 2917, 2852, 2798, 1732, 1695, 1656, 1635, 1599, 1580, 1506, 1493, 1475, 1447, 1402, 1378, 1356, 1336; δ_H (500 MHz; $CDCl_3$) 6.62 (2H, t, J_{HF} 8.0, H-3'), 6.33 (1H, br s, -NH), 5.98 (1H, q, *J* 6.5, -OCH), 5.09 (1H, s, H-6), 3.90 (3H, s, -NCH_3), 3.73 (4H, t, *J* 4.5, H-4"), 3.13 (2H, q, *J* 4.5, H-1"), 2.64 (2H, q, *J* 4.5, H-2"), 2.45 (4H, t, *J* 4.5, H-3"), 2.39 (3H, s, -CH_3), 1.66 (3H, d, *J* 6.5, -CHCH_3); δ_C (125 MHz; $CDCl_3$) 179.3 (C1 or C4), 178.9 (C1 or C4), 157.5 (dt, J_{CF} 244, 14, C4'), 156.9 (ddd, J_{CF} 248, 15, 8, C2'), 148.6 (C5), 134.8 (C2), 1388 (td, J_{CF} 14, 5, C1'), 131.0 (C7a), 121.8 (C3), 119.0 (C3a), 101.0 (td, J_{CF} 26, 5, C3'), 97.2 (C6), 75.8 (-OCH), 67.3 (C4"), 56.2 (C2"), 53.6 (C3"), 39.1 (C1"), 32.5 (-NCH_3), 21.8 (-CHCH_3), 11.0 (-CH_3).

5-((3-(Dimethylamino)propyl)amino)-1,2-dimethyl-3-vinyl-*1H*-indole-4,7-dione 217



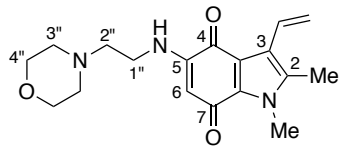
A solution of 5-methoxy-1,2-dimethyl-3-vinyl-*1H*-indole-4,7-dione **213** (18.5 mg, 80.0 μmol) in acetonitrile (0.5 mL) was treated with N^1,N^1-dimethylpropane-1,3-diamine (50 μL, 393 μmol). The mixture was stirred at rt for 91 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 50-98% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (21.3 mg, 88%); mp 88-89 °C; (Found: M+H$^+$, 302.1862. $C_{17}H_{24}N_3O_2^+$ requires 302.1863); λ_{max} (MeOH)/nm 201 (log ε 4.30), 236 (log ε 4.25), 319 (log ε 4.11), 384 (log ε 3.58), 534 (log ε 3.45); ν_{max} (solid)/cm^{-1} 3320, 2969, 2943, 2915, 2852, 2809, 2758, 1660, 1583, 1523, 1495, 1458, 1446, 1403, 1382, 1349, 1328; δ_H (500 MHz; $CDCl_3$) 7.01 (1H, dd, *J* 18.0, 11.5, -C<u>H</u>), 6.81 (1H, br s, -N<u>H</u>), 5.69 (1H, dd, *J* 18.0, 2.0, -C<u>H</u>$_2$), 5.39 (1H, dd, *J* 11.5, 2.0 -C<u>H</u>$_2$), 5.13 (1H, s, H-6), 3.95 (3H, s, -NC<u>H</u>$_3$), 3.18 (2H, q, *J* 6.5, H-3"), 2.38 (2H, t, *J* 6.5, H-1"), 2.30 (3H, s, -C<u>H</u>$_3$), 2.25 (6H, s, -N(C<u>H</u>$_3$)$_2$), 1.79 (2H, t, *J* 6.5, H-2"); δ_C (125 MHz; $CDCl_3$) 179.3 (C1 or C4), 179.0 (C1 or C4), 149.3 (C5), 133.9 (C2), 131.4 (C7a), 128.9 (<u>C</u>H), 120.3 (C3), 119.1(C3a), 117.4 (<u>C</u>H$_2$), 96.6 (C6), 58.2 (C3"), 45.8 (-N(<u>C</u>H$_3$)$_2$), 42.4 (C1"), 32.8 (-N<u>C</u>H$_3$), 25.9 (C2"), 11.0 (-<u>C</u>H$_3$).

5-((4-Aminobutyl)amino)-1,2-dimethyl-3-vinyl-*1H*-indole-4,7-dione 218



A solution of 5-methoxy-1,2-dimethyl-3-vinyl-*1H*-indole-4,7-dione **213** (16.3 mg, 70.5 μmol) in acetonitrile (0.5 mL) was treated with butane-1,4-diamine (25 μL, 246 μmol). The mixture was stirred at rt for 67.5 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 10-15% methanol/ dichloromethane and 2% triethylamine to give the *title compound* as a purple solid (20.0 mg, 99%); mp 108-110 °C; (Found: M+H$^+$, 288.1701. $C_{16}H_{22}N_3O_2^+$ requires 288.1707); λ_{max} (MeOH)/nm 203 (log ε 4.22), 237 (log ε 4.16), 319 (log ε 4.00), 382 (log ε 3.46), 535 (log ε 3.33); ν_{max} (solid)/cm^{-1} 3358, 3285, 2970, 2935, 2863, 1739, 1652, 1598, 1579, 1491, 1440, 1406, 1375, 1316; δ_H (500 MHz; $CDCl_3$) 6.99 (1H, dd, *J* 17.5, 11.5, -C<u>H</u>), 6.08 (1H, br s, -N<u>H</u>), 5.72 (1H, dd, *J* 17.5, 2.0, -C<u>H</u>$_2$), 5.39 (1H, d, *J* 11.5, 2.0, -C<u>H</u>$_2$), 5.14 (1H, s, H-6), 3.95 (3H, s, -NC<u>H</u>$_3$), 3.13 (2H, q, *J* 7.0, H-1"), 2.74 (2H, t, *J* 7.0, H-4"), 2.31 (3H, s, -C<u>H</u>$_3$), 1.71 (2H, quint, *J* 7.0, H-2"), 1.54 (2H, quint, *J* 7.0, H-3"), 1.47 (2H, br s, -N<u>H</u>$_2$); δ_C (125 MHz; $CDCl_3$) 179.3 (C1 or C4), 178.9 (C1 or C4), 148.9 (C5), 134.1 (C2), 131.3 (C7a), 128.8 (-<u>C</u>H), 120.4 (C3), 119.0 (C3a), 117.5 (-<u>C</u>H$_2$), 96.9 (C6), 43.0 (C1"), 42.1 (C4"), 32.8 (-NC<u>H</u>$_3$), 31.4 (C3"), 26.0 (C2"), 10.9 (-<u>C</u>H$_3$).

1,2-Dimethyl-5-((2-morpholinoethyl)amino)-3-vinyl-*1H*-indole-4,7-dione 219



A solution of 5-methoxy-1,2-dimethyl-3-vinyl-*1H*-indole-4,7-dione **213** (12.6 mg, 54.5 μmol) in acetonitrile (0.5 mL) was treated with 2-morpholinoethan-1-amine (50 μL, 376 μmol). The mixture was stirred at rt for 91 h. The solvent and excess amine were removed under reduced pressure and the residue was purified by column chromatography, eluting with 50-60% ethyl acetate/light petroleum and 2% triethylamine to give the *title compound* as a purple solid (17.0 mg, 95%); mp 115-116 °C; (Found: $M+H^+$, 330.1821. $C_{18}H_{24}N_3O_3^+$ requires 330.18120); λ_{max} (MeOH)/nm 201 (log ε 4.41), 236 (log ε 4.21), 321 (log ε 4.01), 382 (log ε 3.48), 532 (log ε 3.36); ν_{max} (solid)/cm^{-1} 3372, 2849, 2817, 1647, 1609, 1588, 1483, 1454, 1404, 1354, 1335; δ_H (500 MHz; $CDCl_3$) 7.00 (1H, dd, *J* 18.0, 11.5, -C<u>H</u>), 6.43 (1H, br s, -N<u>H</u>), 5.71 (1H, dd, *J* 18.0, 2.0, -C<u>H</u>$_2$), 5.40 (1H, dd, *J* 11.5, 2.0, -C<u>H</u>$_2$), 3.95 (3H, s, -NC<u>H</u>$_3$) 3.73 (4H, t, *J* 4.5, H-4"), 3.15 (2H, q, *J* 6.0, H-1"), 2.65 (2H, t, *J* 6.0, H-2"), 2.47 (4H, *J* 4.5, H-3"), 2.31 (3H, s, -C<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 179.3 (C1 or C4), 178.8 (C1 or C4), 148.8 (C5), 134.1 (C2), 131.2 (C7a), 128.8 (-<u>C</u>H), 120.4 (C3), 119.1 (C3a), 117.5 (-<u>C</u>H$_2$), 97.2 (C6), 67.3 (C4"), 56.1 (C2"), 53.6 (C3"), 39.1 (C1"), 32.8 (-NC<u>H</u>$_3$), 10.9 (-<u>C</u>H$_3$).

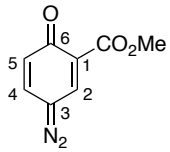
5.4 Syntheses Chapter 4

5.4.1 Preparation of quinone diazides

General Procedure 2

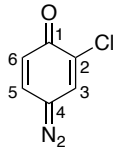
Conc. hydrochloric acid (37%, 4.2 - 5.6 mL) was added to a solution of aminophenol (5.04 - 7.23 mmol) in EtOH (25 - 35 mL), cooled at 0 °C. The resulting mixture was stirred at 0 °C for approximately 15 min then an ice cold solution of $NaNO_2$ (15.2 - 20.4 mmol) in water (1.7 - 2.0 mL) was added dropwise over 4 - 8 min. The resulting mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with cold dichloromethane (150 - 200 mL), ice (~ 10 g) was added and it was vigorously stirred while a cold solution of potassium carbonate (5.5 - 6.2 g) in water (5 - 5.6 mL) was added. The organic layers were separated and the aqueous layer was extracted with dichloromethane (2 - 3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered, and the filtrate concentrated under reduced pressure and used in the next step without purification.

Methyl 3-diazo-6-oxocyclohexa-1,4-diene-1-carboxylate 264



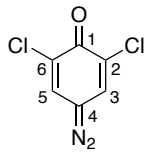
According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of methyl 5-aminosalicylate (1.02 g, 5.90 mmol) in EtOH (30 mL). After 10 min, a solution of $NaNO_2$ (1.24 g, 18.0 mmol) in water (2 mL) was added over 8 min. Upon completion and dilution with dichloromethane (150 mL), the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a yellow-brown solid (0.420 g, 40%); mp 143-146 °C (dec.); (Found: $M+H^+$, 179.0460. $C_8H_7N_2O_3^+$ requires 179.0451); ν_{max} (solid)/cm^{-1} 3094, 3044, 2999, 2836, 2148, 1682, 1590, 1576, 1508, 1441, 1426, 1379, 1333; δ_H (500 MHz; $CDCl_3$) 8.30 (1H, d, *J* 3.0, H-2), 7.35 (1H, dd, *J* 10.0, 3.0, H-4), 6.54 (1H, d, *J* 10.0, H-5), 3.89 (3H, s, -OC$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 178.4 (C6), 165.5 (-$\underline{C}O_2CH_3$), 137.4 (C2), 136.0 (C3), 130.6 (C5), 128.4 (C4), 126.7 (C1), 52.9 (-O$\underline{C}H_3$).

2-Chloro-4-diazocyclohexa-2,5-dien-1-one 265



According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 2-chloro-4-aminophenol (1.09 g, 6.05 mmol) in EtOH (30 mL). After 20 min a solution of $NaNO_2$ (1.24 g, 18.0 mmol) in water (2 mL) was added over 8 min. Upon completion and dilution with dichloromethane (150 mL) and ice, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a yellow solid (0.924 g, 99%); mp 80-82 °C (dec.); (Found: $M+H^+$, 155.0012. $C_6H_4{}^{35}ClN_2O^+$ requires 155.0007); ν_{max} (solid)/cm^{-1} 2110, 1578, 1450, 1374, 1306; δ_H (500 MHz; $CDCl_3$) 7.63(1H, d, *J* 3.0, H-3), 7.43 (1H, dd, *J* 10.0, 3.0, H-5), 6.52 (1H, d, *J* 10.0, H-6); δ_C (125 MHz; $CDCl_3$) 176.4 (C1), 129.9 (C2), 129.7 (C5), 127.3 (C3), 126.4 (C6), one C unobserved. The compound is reported in the literature but not fully characterised.[231]

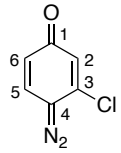
2,6-Dichloro-4-diazocyclohexa-2,5-dien-1-one 266



According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 2,6-dichloro-4-aminophenol (1.10 g, 6.04 mmol) in EtOH (30 mL). After 10 min, a solution of $NaNO_2$ (1.25 g, 18.0 mmol) in water (2 mL) was added over 5 min. Upon completion and dilution with dichloromethane (200 mL) and ice, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a yellow-brown solid (1.10 g, 96%); mp 155-156 °C (exp.); (Found: $M+Na^+$, 210.9441; $C_6H_2Cl_2N_2ONa^+$ requires 210.9436); ν_{max} (solid)/cm^{-1} 3056, 3083, 3025, 2121, 1578, 1555, 1523, 1443, 1350, 1304; δ_H (400 MHz; $CDCl_3$) 7.64 (2H, s, H-3); δ_C

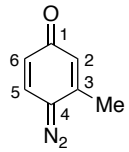
(100 MHz; $CDCl_3$) 171.3 (C1), 134.3 (C4), 129.4 (C3), 126.7 (C2), one C unobserved. The compound is reported in the literature but not fully characterised.[231]

3-Chloro-4-diazocyclohexa-2,5-dien-1-one 267



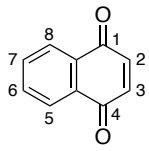
According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 3-chloro-4-aminophenol (1.08 g, 6.03 mmol) in EtOH (30 mL). After 20 min, a solution of $NaNO_2$ (1.25 g, 18.1 mmol) in water (2 mL) was added over 5 min. Upon completion and dilution with dichloromethane (100 mL) and ice, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a yellow solid (0.847 g, 91%); mp 115-116 °C (dec.); (Found: $M+H^+$, 155.0018. $C_6H_4ClN_2O^+$ requires 155.0007); ν_{max} (solid)/cm^{-1} 2120, 1584, 1444, 1378; δ_H (500 MHz; $CDCl_3$) 7.36 (1H, *J* 10.0, H-5), 6.55 (1H, *J* 2.0, H-2), 6.37 (1H, *J* 10.0, H-6); δ_C (125 MHz; $CDCl_3$) 181.6 (C1), 135.0 (C4), 129.4 (C5), 127.4 (C2), 126.7 (C6), one C unobserved. The compound is reported in the literature but not fully characterised.[231]

4-Diazo-3-methylcyclohexa-2,5-dien-1-one 268



According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 4-amino-3-methylphenol (0.76 g, 6.00 mmol) in EtOH (30 mL). After 20 min, a solution of $NaNO_2$ (1.24 g, 18.0 mmol) in water (2 mL) was added over 8 min. Upon completion and dilution, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a brown solid (0.649 g, 81%); mp 81-82 °C (dec.); (Found: $M+H^+$, 135.0560. $C_7H_7N_2O^+$ requires 135.0553); ν_{max} (solid)/cm^{-1} 2099, 1575, 1550, 1476, 1437, 1372; δ_H (500 MHz; $CDCl_3$) 7.38 (1H, d, *J* 7.6, H-5), 6.35 (1H, dd, *J* 7.6, 1.6, H-6) 6.31 (1H, dd, *J* 1.6, 1.2, H-2), 2.25 (3H, d, *J* 1.2, -C<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 182.7 (C1), 140.5 (C3), 130.4 (C5), 127.1 (C2), 126.4 (C6), 18.6 (-<u>C</u>H$_3$), one C unobserved.

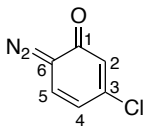
Naphthalene-1,4-dione 269



According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 4-amino-1-naphthol hydrochloride (1.30 g, 6.00 mmol) in EtOH (40 mL). After 17 min, a solution of $NaNO_2$ (1.25 g, 18.1 mmol) in water (2 mL) was added over 7 min. Upon completion and dilution, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a brown solid (0.260 g, 27%); (Found: $M+H^+$, 171.0554. $C_{10}H_7N_2O^+$ requires 171.0553); mp 94-95 °C (dec.); ν_{max} (solid)/cm^{-1} 3058, 3029, 2922, 1657, 1586, 1538, 1482, 1455, 1377, 1328;

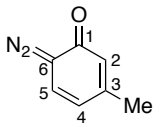
δ_H (500 MHz; $CDCl_3$) 8.10 (2H, dd, *J* 6.0, 3.5), 7.77 (2H, dd, *J* 6.0, 3.5), 6.99 (2H, s, H-2/H3); δ_C (125 MHz; $CDCl_3$) 185.4 (C1/C4), 139.1 (C2/C3), 134.3 (C6/C7), 132.3 (C4a/C8a), 126.8 (C5/C8).

3-Chloro-6-diazocyclohexa-2,4-dien-1-one 270



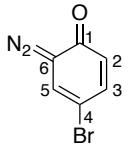
According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 2-amino-3-chlorophenol (0.89 g, 5.98 mmol) in EtOH (30 mL). After 20 min, a solution of $NaNO_2$ (1.24 g, 18.0 mmol) in water (2 mL) was added dropwise over 7 min. Upon completion and dilution, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a dark yellow solid (0.908 g, 98%); mp 120-121 °C (dec.) (lit.,[282] mp 122 °C (dec.)); (Found: $M+H^+$, 155.0018. $C_6H_4ClN_2O^+$ requires 155.0007); ν_{max} (solid)/cm^{-1} 3083, 2183, 2127, 1603, 1539, 1486, 1428; δ_H (500 MHz; $CDCl_3$) 7.13 (1H, d, *J* 7.6, H-3), 6.72 (1H, d, *J* 1.6, H-6), 6.23 (1H, dd, *J* 7.6, 1.6, H-4); δ_C (125 MHz; $CDCl_3$) 177.1 (C1), 147.5 (C5), 124.8 (C3), 124.5 (C6), 117.4 (C4), 84.8 (C2).

6-Diazo-3-methylcyclohexa-2,4-dien-1-one 271



According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 2-amino-5-methylphenol (0.740 g, 6.01 mmol) in EtOH (30 mL). After 10 min, a solution $NaNO_2$ (1.24 g, 18.0 mmol) in water (2 mL) was added over 4 min. Upon completion and dilution with dichloromethane (150 mL) and ice, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a black gooey solid (0.779 g, 97%); (Found: $M+H^+$, 135.0555. $C_7H_7N_2O^+$ requires 135.0553); ν_{max} (solid)/cm^{-1} 3058, 3020, 2914, 2856, 2192, 2171, 2108, 1691, 1630, 1552, 1512, 1491, 1356, 1306; δ_H (500 MHz; $CDCl_3$) 7.16 (1H, dd, *J* 9.5, 2.5, H-4), 6.89 (1H, d, *J* 2.5, H-2), 6.65 (1H, d, *J* 9.5, H-5), 2.16 (3H, s, -C$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 177.3 (C1), 141.8 (C4), 134.2 (C6), 125.4 (C3), 123.9 (C5), 119.7 (C2), 20.7 (-$\underline{C}H_3$). The compound is reported in the literature but not fully characterised.[231]

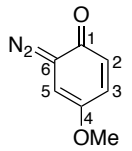
4-Bromo-6-diazocyclohexa-2,4-dien-1-one 272



According to the general procedure 2, conc. hydrochloric acid (4.2 mL) was added to a solution of 2-amino-4-bromophenol (1.01 g, 5.04 mmol) in EtOH (25 mL). After 17 min, a solution of $NaNO_2$ (1.04 g, 15.2 mmol) in water (1.7 mL) was added over 7 min. Upon completion and dilution, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as a yellow

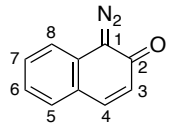
solid (1.00 g, 99%); mp 97-98 °C (dec.); (Found: M+H$^+$, 198.9496. $C_6H_4{}^{79}BrN_2O^+$ requires 198.9502); ν_{max} (solid)/cm^{-1} 3061, 2191, 2169, 2128, 1607, 1540, 1485, 1409, 1379, 1349; δ_H (500 MHz; $CDCl_3$) 7.34 (1H, d, *J* 2.0, H-3), 7.28 (1H, dd, *J* 8.0, 2.0, H-5), 6.59 (1H, d, *J* 8.0, H-6); δ_C (125 MHz; $CDCl_3$) 175.6 (C1), 142.5 (C5), 125.8 (C6), 124.1 (C3), 105.2 (C4), 90.4 (C2). The compound is reported in the literature but not fully characterised.[231]

6-Diazo-4-methoxycyclohexa-2,4-dien-1-one 273



According to the general procedure 2, conc. hydrochloric acid (5.6 mL) was added to a solution of 2-amino-4-methoxyphenol (1.06 g, 7.23 mmol) in EtOH (35 mL). After 16 min, a solution of $NaNO_2$ (1.42 g, 20.4 mmol) in water (2 mL) was added over 7 min. Upon completion and dilution, the reaction mixture was quenched with a solution of K_2CO_3 (6.2 g) in water (5.6 mL). Following workup, the *title compound* was isolated as a yellow-orange solid (1.06 g, 98%); mp 63-66 °C (dec.); (Found: M+H$^+$, 151.0510. $C_7H_7N_2O_2^+$ requires 151.0502); ν_{max} (solid)/cm^{-1} 3088, 3065, 2998, 2930, 2113, 1632, 1552, 1511, 1442, 1426, 1357, 1306; δ_H (500 MHz; $CDCl_3$) 7.12 (1H, dd, *J* 7.6, 2.6, H-5), 6.79 (1H, d, *J* 7.6, H-6), 6.38 (1H, d, *J* 2.6, H-3), 3.76 (3H, s, -OC$\underline{H}_3$); δ_C (125 MHz; $CDCl_3$) 172.9 (C1), 149.2 (C4), 132.8 (C5), 123.0 (C6), 96.0 (C3), 55.6 (-OC$\underline{H}_3$), one C unobserved.

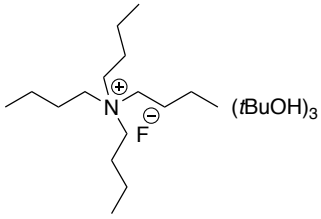
1-Diazonaphthalen-2(1*H*)-one 274



According to the general procedure 2, conc. hydrochloric acid (5 mL) was added to a solution of 1-amino-2-naphthol hydrochloride (1.10 g, 6.04 mmol) in EtOH (30 mL). After 10 min, a solution of $NaNO_2$ (1.25 g, 18.0 mmol) in water (2 mL). was added over 5 min. Upon completion and dilution with dichloromethane (150 mL) and ice, the reaction mixture was quenched with a solution of K_2CO_3 (5.5 g) in water (5 mL). Following workup, the *title compound* was isolated as an orange-red solid (0.704 g, 62%); mp 66-68 °C (lit.,[234] mp 82-84 °C); (Found: M+H$^+$, 171.0561. $C_{10}H_7N_2O^+$ requires 171.0553); ν_{max} (solid)/cm^{-1} 3058, 2915, 2171, 2107, 1631, 1553, 1512, 1492, 1356, 1306; δ_H (500 MHz; $CDCl_3$) 7.64 (1H, d, *J* 10.0), 7.58 (1H, dd, *J* 7.0, 1.0), 7.52 (1H, td, *J* 1.0), 7.28 (2H, m), 6.67 (1H, d, *J* 10.0); δ_C (125 MHz; $CDCl_3$) 180.6 (C1), 140.7, 136.2 (C2), 130.4, 130.2, 127.6, 126.4, 126.0, 125.1, 120.1.

5.4.2 PREPARATION OF A FLUORIDE SOURCE

Tetrabutylammonium tri *t*-butanol coordinated fluoride 282

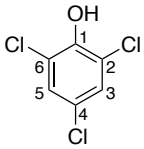


A solution of *t*-BuOH (88 mL) and *n*-hexane (22 mL) was added to TBAF hydrate 284 (1.09 g, 3.83 mmol) and the resulting mixture was stirred at 90 °C for 30 min. The reaction mixture was cooled to rt then in an ice bath. The precipitated colourless crystalline solid was rapidly filtered and washed with 70% tBuOH/hexane (40 mL). The filtrate was kept under reduced pressure for 30 min to give the *title compound* as a colourless crystalline solid (1.96 g, 92%); δ_H (400 MHz; $CDCl_3$)[a] 3.34 (8H, t, *J* 7.6), 1.62-1.70 (8H, m), 1.45 (8H,[a] sext, *J* 7.6), 1.25 (25H, s), 1.00 (12H, t, *J* 7.6); δ_C (100 MHz; $CDCl_3$) 69.4, 59.3, 31.6, 24.5, 20.2, 14.1; δ_F (376 MHz; $CDCl_3$) 129.04.[251]

a) unobserved H - the integrations do not account for 66 hydrogens.

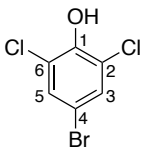
5.4.3 PREPARATION OF PHENOLS

2,4,6-Trichlorophenol 280



LiCl (0.105 g, 2.49 mmol) was added to a mixture of 2,6-dichloro-4-diazocyclohexa-2,5-dien-1-one **266** (94.5 mg, 0.500 mmol) and $Pd(OAc)_2$ (11.5 mg, 51.2 μmol) in AcOH (1 mL). The resulting mixture was stirred at 70 °C for 2.5 h. The reaction was quenched with sodium hydrogen carbonate (aq sat., 4 mL) and extracted with dichloromethane (3 x 4 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the *title compound* as a beige-grey solid (84.9 mg. 86%); mp 63-66 °C (lit.,[283] mp 64-66 °C); (Found: M-H, 194.9170. $C_6H_2Cl_3O$ requires 194.9177); ν_{max} (solid)/cm^{-1} 3501, 3088, 3076, 1572, 1386, 1317; δ_H (400 MHz; $CDCl_3$) 7.28 (2H, s, H-3); δ_C (100 MHz, $CDCl_3$) 147.4 (C1), 128.6 (C3), 125.8 (C4), 122.0 (C2).

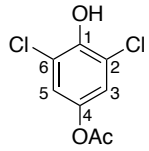
4-Bromo-2,6-dichlorophenol 278



AcOH (1 mL) was added to a mixture of LiBr (0.218 g, 2.51 mmol), 2,6-dichloro-4-diazocyclohexa-2,5-dien-1-one **266** (95.3 mg, 0.504 mmol) and $Pd(OAc)_2$ (11.8 mg, 52.6 μmol). The resulting mixture was stirred at 70 °C for 55 min. The reaction was quenched with sodium hydrogen carbonate (aq sat., 15 mL) and extracted with dichloromethane (3 x 10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the *title compound* as a beige crystalline solid (0.111 g, 91%); mp 64-67 °C; (lit.,[284] mp 66 °C); (Found: M-H, 238.8665. $C_6H_2BrCl_2O$ requires 238.8672); ν_{max} (solid)/cm^{-1}

3441, 3205, 3076, 1565, 1506, 1468, 1458, 1415, 1388, 1360, 1312; δ_H (400 MHz; $CDCl_3$) 7.41 (2H, s, H-3); δ_C (100 MHz; $CDCl_3$) 147.8 (C1), 131.3 (C3), 122.4 (C2), 118.8 (C4).

3,5-Dichloro-4-hydroxyphenyl acetate 279

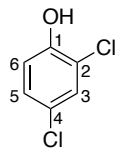


AcOH (1 mL) was added to a mixture of NaF (0.105 g, 2.51 mmol), 2,6-dichloro-4-diazocyclohexa-2,5-dien-1-one **266** (94.1 mg, 0.498 mmol) and $Pd(OAc)_2$ (12.1 mg, 53.9 μmol). The resulting mixture was stirred at 70 °C for 2.5 h. The reaction was quenched with sodium hydrogen carbonate (aq sat., 10 mL) and extracted with dichloromethane (3 x 10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the *title compound* as a beige solid (0.109 g, 99%); mp 98-99 °C; (Found: M-H, 218.9621. $C_8H_5Cl_2O_3$ requires 218.9621); ν_{max} (solid)/cm^{-1} 3296, 1650, 1581, 1481, 1409, 1369, 1318; δ_H (400 MHz; $CDCl_3$) 7.08 (2H, s, H-3), 5.78 (1H, s, -O<u>H</u>), 2.28 (3H, s, -OC<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 169.5 (-O<u>C</u>OCH$_3$), 146.5 (C6), 143.6 (C1), 122.3 (C2), 121.4 (C3), 21.4 (-<u>C</u>H$_3$).

General Procedure 3

AcOH (0.5 mL) was added to a mixture of quinone diazide (0.500 mmol), Li-X, where X = Cl, Br, I (2.50 mmol, 5 eq.), and $Rh_2(OAc)_4$ (2.50 μmol, 0.5 mol%). The resulting mixture was stirred at 70 °C for 0.2 - 3.8 h. The reaction was diluted with dichloromethane (15 - 30 mL) and quenched with a saturated solution of sodium hydrogen carbonate (20 - 40 mL). The organic phase was separated and the aqueous layer was extracted with dichloromethane (1-3 x 10 mL). The combined organic phases were washed with brine (10 - 20 mL), dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure. When mentioned, following workup, the residue was purified by column chromatography, eluting with hexane/ethyl acetate.

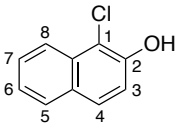
2,4-Dichlorophenol 297



According to the general procedure 3, a mixture of LiCl (0.106 g, 2.51 mmol), 2-chloro-4-diazocyclohexa-2,5-dien-1-one **265** (78.0 mg, 0.505 mmol) and $Rh_2(OAc)_4$ (1.1 mg, 2.49 μmol) in AcOH (0.5 mL) was stirred for 70 min. The reaction mixture was diluted with dichloromethane (25 mL) and quenched with sodium hydrogen carbonate (20 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (10 mL). After workup, the *title compound* was isolated as a colourless solid (40.8 mg, 50%); mp 40-42 °C (lit.,[285] mp 41-44 °C); (Found: M-H, 160.9560. $C_6H_3{}^{35}Cl_2O$ requires 160.9566); ν_{max} (solid)/cm^{-1} 3410, 1583, 1474, 1404, 1324; δ_H (400 MHz; $CDCl_3$) 7.33 (1H, d, *J* 2.4, H-3), 7.16

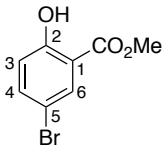
(1H, dd, *J* 8.8, 2.4, H-5*)*, 6.95 (1H, d, *J* 8.8, H-6), 5.48 (1H, s, -OH); δ_C (100 MHz; $CDCl_3$) 150.7 (C1), 129.0 (C5), 128.9 (C3), 126.1 (C4), 120.8 (C2), 117.6 (C6).

1-Chloronaphthalen-2-ol 300



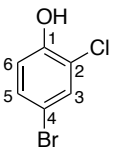
According to the general procedure 3, a mixture of LiCl (0.109 g, 2.56 mmol), 1-diazonaphthalen-2(1*H*)-one **274** (85.3 mg, 0.501 mmol) and $Rh_2(OAc)_4$ (1.1 mg, 2.49 μmol) in AcOH (0.5 mL) was stirred for 48 min. The reaction mixture was diluted with dichloromethane (30 mL) and quenched with sodium hydrogen carbonate (20 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (10 mL). After workup, a burgundy residue was isolated and purification eluting with hexane/ethyl acetate (3:2) gave the *title compound* as a burgundy solid (31.8 mg, 36%); mp 65-67 °C (lit.,[192] mp 64-65 °C); (Found: M-H, 177.0106. $C_{10}H_6{}^{35}ClO$ requires 177.0113); ν_{max} (solid)/cm^{-1} 3301, 3061, 1630, 1605, 1573, 1504, 1467, 1433, 1409, 1387, 1367, 1346; δ_H (400 MHz; $CDCl_3$) 8.06 (1H, dd, *J* 8.8, 1.0, H-8), 7.79 (1H, dd, *J* 8.8, 1.0, H-5), 7.72 (1H, d, *J* 8.8, H-4), 7.57 (1H, td, *J* 8.4, 1.2, H-7), 7.41 (1H, td, *J* 8.4, 1.2, H-6), 7.26 (1H, d, *J* 8.8, H-3), 5.88 (1H, s, -OH); δ_C (100 MHz, $CDCl_3$) 149.8 (C2), 131.5 (C4a), 129.9 (C8a), 128.9 (C4), 128.7 (C5), 128.0 (C7), 124.6 (C6), 123.2 (C8), 117.7 (C3), 113.8 (C1).

Methyl 5-bromo-2-hydroxybenzoate 301



According to the general procedure 3, a mixture of LiBr (0.218 g, 2.51 mmol), methyl 3-diazo-6-oxocyclohexa-1,4-diene-1-carboxylate **264** (99.2 mg, 0.557 mmol) and $Rh_2(OAc)_4$ (1.0 mg, 886 μmol) in AcOH (0.5 mL) was stirred for 1.8 h. The reaction mixture was diluted with dichloromethane (20 mL) and quenched with sodium hydrogen carbonate (30 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (10 mL). After workup, the *title compound* was isolated as a beige solid (0.105 mg, 83%); mp 57-59 °C; ν_{max} (solid)/cm^{-1} 3186, 2957, 1676, 1606, 1578, 1535, 1492, 1469, 1434, 1378, 1333; δ_H (400 MHz; $CDCl_3$) 10.69 (1H, s, -OH), 7.95 (1H, d, *J* 2.4, H-6), 7.53 (1H, dd, *J* 8.8, 2.4, H-4*)*, 6.90 (1H, d, *J* 8.8, H-3), 3.96. (3H, s, -OCH_3); δ_C (100 MHz; $CDCl_3$) 169.9 (-CO_2CH_3), 161.0 (C2), 138.9 (C4), 132.7 (C6), 120.0 (C3), 114.3 (C1), 111.3 (C4), 53.0 (-OCH_3). The compound is reported in the literature but not fully characterised.[286]

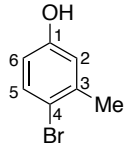
4-Bromo-2-chlorophenol 302



According to the general procedure 3, a mixture of LiBr (0.218 g, 2.51 mmol), 2-chloro-4-diazocyclohexa-2,5-dien-1-one **265** (77.3 mg, 0.500 mmol) and $Rh_2(OAc)_4$ (11.0 mg, 25.0 μmol) in AcOH (1 mL) was stirred for 14 min. The reaction mixture

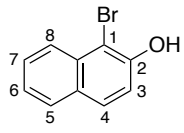
was diluted with dichloromethane (15 mL), and quenched with sodium hydrogen carbonate (40 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (10 mL). After workup, the *title compound* was isolated as a colourless needles solid (59.8 mg, 58%); mp 48-50 °C (lit.,[287] mp 48-50 °C); (Found: M-H, 204.9059. $C_6H_3{}^{79}Br^{35}ClO$ requires 204.9061); ν_{max} (solid)/cm^{-1} 3402, 1577, 1466, 1397, 1363, 1320; δ_H (400 MHz; $CDCl_3$) δ 7.49 (1H, d, *J* 2.4, H-3), 7.30 (1H, dd, *J* 8.4, 2.4, H-5*)*, 6.93 (1H, d, *J* 8.4, H-6), 5.52 (1H, s, -O<u>H</u>); δ_C (100 MHz; $CDCl_3$) 150.5 (C1), 131.3 (C5), 131.1 (C3), 120.6 (C2), 117.4 (C6), 112.1 (C4).

4-Bromo-3-methylphenol 305



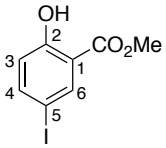
According to the general procedure 3, a mixture of LiBr (0.217 g, 2.50 mmol), 4-diazo-3-methylcyclohexa-2,5-dien-1-one **268** (67.4 mg, 0.502 mmol) and $Rh_2(OAc)_4$ (1.0 mg, 886 μmol) in AcOH (0.5 mL) was stirred for 3.8 h. The reaction was diluted with dichloromethane (15 mL) and quenched with sodium hydrogen carbonate (20 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (15 mL). After workup, the residue was purified eluting with hexane/ethyl acetate (2:3) to give the *title compound* as a beige crystalline solid (5.7 mg, 6%); mp 60-61°C (lit.,[287] mp 59-60 °C); (Found: M-H, 184.9608. $C_7H_6{}^{79}BrO$ requires 184.9608); ν_{max} (solid)/cm^{-1} 3186, 1601, 1585, 1478, 1450, 1420, 1378; δ_H (400 MHz; $CDCl_3$) 7.35 (1H, d, *J* 8.4*,* H-5), 6.73 (1H, d, *J* 2.8, H-2), 6.55 (1H, dd, *J* 8.4, 2.8, H-6*)*, 4.69 (1H, s, -O<u>H</u>), 2.34 (3H, s, -C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) δ 155.2 (C1), 139.6 (C3), 133.5 (C5), 118.2 (C2), 115.9 (C4), 114.9 (C6), 23.44 (-<u>C</u>H$_3$).

1-Bromonaphthalen-2-ol 307



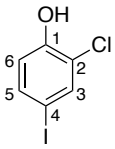
According to the general procedure 3, a mixture of LiBr (0.218 g, 2.50 mmol), 1-diazonaphthalen-2(1*H*)-one **274** (85.3 mg, 0.501 mmol) and $Rh_2(OAc)_4$ (11.1 mg, 25.1 μmol) in AcOH (1 mL) was stirred for 41 min. The reaction mixture was diluted with dichloromethane (20 mL), and quenched with sodium hydrogen carbonate (40 mL), followed by extraction with dichloromethane (2 x 10 mL) and washing with brine (10 mL). After workup, a dark red residue was isolated and purification eluting with hexane/ethyl acetate (50:50) gave the title compound as a red-pink solid (43.4 mg, 39%); mp 77-79°C (lit.,[288] mp 78 °C); (Found: M-H, 220.9609. $C_{10}H_6{}^{79}BrO$ requires 220.9608); ν_{max} (solid)/cm^{-1} 3275, 1631, 1602, 1569, 1498, 1464, 1432, 1410, 1386, 1365, 1346; δ_H (500 MHz; $CDCl_3$) 8.03 (1H, dd, *J* 8.0, 1.0, H-8), 7.78 (1H, dd, *J* 8.0, 1.0, H-5), 7.75 (1H, d, *J* 9.0, H-4), 7.57 (1H, td, *J* 8.0, 1.0, H-5), 7.40 (1H, td, *J* 8.0, 1.0, H-6), 7.27 (1H, d, *J* 9.0, H-3), 5.91 (1H, s, -O<u>H</u>); δ_C (125 MHz; $CDCl_3$) 151.0 (C2), 132.7 (C4a), 130.1 (C8a), 129.7 (C4), 128.6 (C7), 128.2 (C5), 125.7 (C8), 124.6 (C6), 117.6 (C3), 106.5 (C1).

Methyl 2-hydroxy-5-iodobenzoate 308



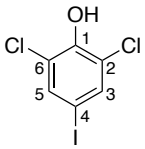
According to the general procedure 3, a mixture of LiI (0.336 g, 2.51 mmol), methyl 3-diazo-6-oxocyclohexa-1,4-diene-1-carboxylate **264** (99.4 mg, 0.558 mmol) and $Rh_2(OAc)_4$ (1.0 mg, 886 μmol) in AcOH (0.5 mL) was stirred for 3.2 h. The reaction mixture was diluted with dichloromethane (30 mL) and quenched with sodium hydrogen carbonate (25 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (10 mL). After workup, the *title compound* was isolated as an orange solid (0.146 g, 94%); mp 71-74°C (dec.) (lit.,[289] mp 78 °C); ν_{max} (solid)/cm^{-1} 3161, 2950, 1666, 1600, 1469, 1434, 1375, 1328; δ_H (400 MHz; $CDCl_3$) 10.71 (1H, s, -O<u>H</u>), 8.14 (1H, d, *J* 2.4, H-6*)*, 7.69 (1H, dd, *J* 8.8, 2.4, H-4), 6.77 (1H, d, *J* 2.4, H-3), 3.95 (3H, s, -OC<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 169.8 (-<u>C</u>O_2CH_3), 161.7 (C2), 144.5 (C4), 138.8 (C6), 120.5 (C3), 115.0 (C1), 80.5 (C5), 53.1 (-O<u>C</u>H_3).

2-Chloro-4-iodophenol 309



According to the general procedure 3, a mixture of LiI (0.337 g, 2.52 mmol), 2-chloro-4-diazocyclohexa-2,5-dien-1-one **265** (79.1 mg, 0.512 mmol) and $Rh_2(OAc)_4$ (1.2 mg, 2.71 μmol) in AcOH (0.5 mL) was stirred for 14 min. The reaction mixture was diluted with dichloromethane (20 mL) and quenched with sodium hydrogen carbonate (20 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (10 mL). After workup, a brown residue was isolated and purification eluting with hexane/ethyl acetate (50:50) gave the *title compound* as a colourless crystalline solid (54.7 mg, 42%); mp 53-54°C; (lit.,[290] mp 54 °C); (Found: M-H, 252.8932. $C_6H_3{}^{35}ClIO$ requires 252.8923); ν_{max} (solid)/cm^{-1} 3340, 3056, 1584, 1471, 1416, 1396, 1334; δ_H (400 MHz; $CDCl_3$) 7.62 (1H, d, *J* 2.0, H-3), 7.46 (1H, dd, *J* 8.4, 2.0, H-5*)*, 6.78 (1H, d, *J* 8.4, H-6), 5.52 (1H, s, -O<u>H</u>); δ_C (100 MHz; $CDCl_3$) 151.8 (C1), 137.8 (C5), 137.4 (C3), 121.6 (C2), 118.7 (C6), 82.0 (C4).

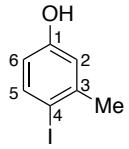
2,6-Dichloro-4-iodophenol 310



According to the general procedure 3, a mixture of LiI (0.336 g, 2.51 mmol), 2,6-dichloro-4-diazocyclohexa-2,5-dien-1-one **266** (94.5 mg, 0.500 mmol) and $Rh_2(OAc)_4$ (1.1 mg, 2.49 μmol) in AcOH (0.5 mL) was stirred for 35 min. The reaction mixture was diluted with dichloromethane (25 mL), quenched with sodium hydrogen carbonate (30 mL), followed by extraction with dichloromethane (3 x 10 mL) and washing with brine (20 mL). After workup, a brown solid was isolated and purification eluting with hexane/ethyl acetate (50:50) gave the *title compound* as an orange crystalline solid (0.108 g, 75%); mp 87-89 °C (lit.,[290] mp 91-92 °C); (Found: M-H, 286.8539. $C_6H_2{}^{35}Cl_2IO$ requires

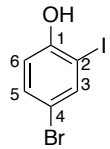
286.8533); ν_{max} (solid)/cm^{-1} 3364, 3065, 1562, 1456, 1382, 1356, 1328; δ_H (400 MHz; $CDCl_3$) 7.57 (2H, s, H-3), 5.84 (1H, s, -O<u>H</u>); δ_C (100 MHz; $CDCl_3$) 148.02 (C1), 136.4 (C3), 122.1 (C2), 80.4 (C4).

4-Iodo-3-methylphenol 311



According to the general procedure 3, a mixture of LiI (0.217 g, 2.50 mmol), 4-diazo-3-methylcyclohexa-2,5-dien-1-one **268** (67.1 mg, 0.500 mmol) and $Rh_2(OAc)_4$ (1.0 mg, 886 μmol) in AcOH (0.5 mL) was stirred for 1.3 h. The reaction was diluted with dichloromethane (20 mL) and quenched with sodium hydrogen carbonate (25 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (15 mL). After workup, the residue was purified eluting with hexane/ethyl acetate (2:3) to give the *title compound* as a pale pink solid (20.0 mg, 17%); mp 78-81 °C; (Found: M-H, 232.9478. C_7H_6IO requires 232.9469); ν_{max} (solid)/cm^{-1} 3193, 1568, 1464, 1428, 1309; δ_H (400 MHz; $CDCl_3$) 7.61 (1H, d, *J* 8.4, H-5), 6.76 (1H, d, *J* 2.8, H-2), 6.42 (1H, dd, *J* 8.4, 2.8, H-6), 4.74 (1H, s, -O<u>H</u>), 2.34 (3H, s, -C<u>H</u>$_3$); δ_C (100 MHz; $CDCl_3$) 155.8 (C1), 142.7 (C3), 139.6 (C5), 117.1 (C2), 114.9 (C6), 89.6 (C4), 28.1 (-<u>C</u>H$_3$). The compound is reported in the literature but not fully characterised.[291]

4-Bromo-2-iodophenol 313



According to the general procedure 3, a mixture of LiI (0.339 g, 2.53 mmol), 4-bromo-6-diazocyclohexa-2,4-dien-1-one **272** (0.100 g, 0.505 mmol) and $Rh_2(OAc)_4$ (1.1 mg, 2.49 μmol) in AcOH (0.5 mL) was stirred for 26 min. The reaction was diluted with dichloromethane (20 mL) and quenched with sodium hydrogen carbonate (25 mL), followed by extraction with dichloromethane (10 mL) and washing with brine (15 mL). After workup, the residue was purified eluting with hexane/ethyl acetate (50:50) to give the *title compound* as a colourless crystalline solid (78.2 mg, 52%); mp 70-72 °C (lit.,[292] mp 71 °C); (Found: M-H, 296.8429. $C_6H_3{}^{79}BrIO$ requires 296.8417); ν_{max} (solid)/cm^{-1} 3280, 3081, 1568, 1481, 1397, 1338; δ_H (400 MHz; $CDCl_3$) 7.76 (1H, d, *J* 2.4, H-3), 7.35 (1H, dd, *J* 8.8, 2.4, H-5), 6.87 (1H, d, *J* 8.8, H-6), 5.27 (1H, s, -O<u>H</u>); δ_C (100 MHz; $CDCl_3$) 154.7 (C1), 140.2 (C3), 133.5 (C5), 116.8 (C6), 113.5 (C4), 86.6 (C2).

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