

1-Hydroxy-xanthine Inhibitors of Cxcl12 Receptor as a Potential Treatment for Osteoporosis

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Abstract

Caf1 is a subunit of the CCR4-NOT complex. The role of Caf1 is that of a magnesium dependent deadenylase enzyme, which removes the poly(A) tail of mRNA (deadenylation). The degradation of the poly(A) tail has a downstream effect on mRNA translation and subsequent protein synthesis. Caf1 is implicated in several biological processes, an important one of which being the inhibition of osteoblasts. Osteoblasts are cells responsible for the formation of bone tissue and their inhibition leads to negative regulation of bone mass. Inhibition of Caf1 has been proposed as a potential pharmacological method of increasing osteoblast activity. This may serve as a novel approach to increasing bone density as a treatment for brittle bone diseases such as osteoporosis.

1-hydroxy-xanthines have previously been identified to act as inhibitors of Caf1 via the chelation of the magnesium ions present in the active site. This project aims to continue to develop novel 1-hydroxy xanthine compounds that act as Caf1 inhibitors by exploring the chemical space surrounding the compound's substituents and the nature of their interaction with the Caf1 enzyme.

This thesis reports the design, synthesis, chemical characterisation and pharmacological data of a library of novel compounds that act as Caf1 inhibitors using 3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione, **8j** as a lead compound. **8j** was selected as the lead because it was to the best of our knowledge, the most potent small molecule Caf1 inhibitor reported in the literature ($IC_{50} = 3.30 \mu M$). There exists a proposed binding mode for the interaction of **8j** with Caf1 which was used to aid the design of new compounds as well as being assessed and re-examined in the context of new SAR findings.

Derivatisation was explored around 3 substituents on the xanthine scaffold. A range of substituents and functional groups were tested at the 3- position. However, none showed improvement over the 3-substituent already present in **8j**. A new synthetic pathway was developed to more easily derivatise the xanthine 7-position. Modification of substituents at the 7-position afforded several compounds (**23k**), (**23o**), (**23p**), and (**23s**) that were several times more potency than **8j**. Specific substituents at the 3-position of benzene ring on the phenethyl fragment proved beneficial to potency. There was also found to be no strong relationship between the benzene ring electronics and the

compounds binding, in disagreement with the proposed binding mode. These findings in combination with molecular modelling were used to reevaluate the hypothesised binding mode and propose a new alternative. An additional series of compounds were designed and synthesised with an additional functional group incorporated at the xanthine 8-position. This series gave rise to the compound with the highest potency towards Caf1 so far (**35b**) with a IC_{50} of 0.65 μ M.

Differential scanning fluorimetry was used to measure the binding of a diverse set of compounds to wild type Caf1 as well as variants containing substitutions of amino acids Phe⁴³ and Tyr¹⁶⁰. This was used to demonstrate the role of these residues in inhibitor binding. The residues were shown to be contributing towards the binding of certain compounds, in agreement with the newly proposed binding mode.

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Abbreviations

ΔT_m	change in half maximal melting temperature
ADP	Adenosine di-phosphate
AMP	Adenosine mono-phosphate
atm	Atmosphere
ATP	Adenosine tri-phosphate
BEI	Binding efficiency index
Boc	<i>tert</i> -Butyloxycarbonyl
CBz	Carboxybenzyl
CDI	Carbonyldiimidazole
d	Doublet
DCM	Dichloromethane
dd	Doublet of doublets
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEDD	aspartic acid - glutamic acid - aspartic acid - aspartic acid
DIAD	Diisopropylazodicarboxylate
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
DNA	Deoxy ribose nucleic acid
dq	Doublet of quartets
DSF	Differential scanning fluorimetry
dt	Doublet of triplets
EEP	Endonuclease /Exonuclease phosphate
eq	Equivalent
ESI	Electrospray ionisation
FEN1	Flap structure-specific endonuclease 1

Flc	Fluorescein
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
FRET	Fluorescence resonance energy transfer
HRMS	High resolution mass spectrometry
IC ₅₀	Half maximal inhibitory concentration
LiPE	Lipophilic efficiency
mRNA	mitochondrial ribose nucleic acid
NCS	<i>N</i> -chloro succinimide
NFSI	<i>N</i> -Fluorobenzenesulfonimide
NMR	Nuclear magnetic resonance
PARN	Poly(A)-specific ribonuclease
Pd/C	Palladium on carbon
Phe	Phenylalanine
PMB	Paramethoxybenzyl
Poly (A)	Poly adenosine
ppm	Parts per million
PSA	Polar surface area
PTH	Para thyroid hormone
q	Quartet
quin	Quintet
s	Singlet
SAR	Structure activity relationship
SEM	Standard error mean
sex	Sextet
t	Triplet

TAMRA	Tertramethylrhodamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
T_m	Half maximal melting temperature
TOF	Time of flight
Tyr	Tyrosine

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1. Introduction

1.1 Nuclease Enzymes

Many enzymes exist in nature for the purpose of cleaving the phosphodiester bonds between nucleic acids, these enzymes are known as nucleases. A large variety of nucleases exist for a range of specialised functions. Nucleases can be separated into two categories. Exonucleases cleave single nucleic acids from the terminus of the polymer, whilst endonucleases cleave in the middle of the polymer. These categories can also be subdivided into ribonucleases, which act on RNA, and deoxyribonucleases, which act on DNA. Nucleases are further differentiated by their selectivity towards double or single stranded DNA/RNA and also have differing selectivity towards specific nucleic acid sequences. ^{[1][2]}

Some of the most well-known nucleases are prokaryotic restriction endonucleases. Restriction endonucleases cleave double stranded DNA at sequence specific sites ^[3] and are utilised by bacteria as a defence against invading viruses. ^{[4][5]} Restriction endonucleases are widely utilised for molecular cloning to create recombinant DNA, with over 600 such enzymes commercially available. ^[6] Some nuclease enzymes are dependent on metal cofactors for substrate binding, examples of such enzymes discussed in this thesis include Flap structure-specific endonuclease 1 (FEN1), influenza RNA endonuclease PA, HIV-1 Ribonuclease H, and Cfl. FEN1 is a structure specific endodeoxyribonuclease in humans that is responsible for the removal of 5' "flaps" which are single strands of DNA protruding from the end of a strand. This process is used in DNA replication and repair. ^[7] Influenza RNA endonuclease PA and HIV-1 Ribonuclease H are both endoribonucleases utilised during viral replication by the influenza virus and human immunodeficiency virus (HIV) respectively. ^{[8][9]} Cfl is an exoribonuclease found in many organisms that specifically targets the poly(A) tail of mRNA, Cfl is a type of deadenylase enzyme. ^[10]

1.2 Deadenylase Enzymes

Deadenylase enzymes are a type of exoribonuclease responsible for catalysing the shortening of the poly(A) tail of mRNA, a process known as

deadenylation. The poly(A) tail is a terminal chain of adenine nucleotides present at the 3' end of nearly all eukaryotic mRNA (except animal replication-dependent histone mRNAs).^[11] The mRNA poly(A) tail binds to poly(A)-binding protein (PABP) which allows transportation of the mRNA out of the nucleus, leading to its translation. The poly(A) tail also prevents enzymatic degradation of the mRNA.^[12] The presence, and length of the poly(A) tail has a direct influence on both the translation and stability of mRNA therefore influencing downstream protein synthesis.^[13]

Deadenylase enzymes shorten the poly(A) tail by the scission of adenine nucleotides from the terminus of the mRNA. Absence of the poly(A) tail allows for the enzymatic removal of the 5' cap leaving the uncapped mRNA unstable, leading to its degradation.^[14]

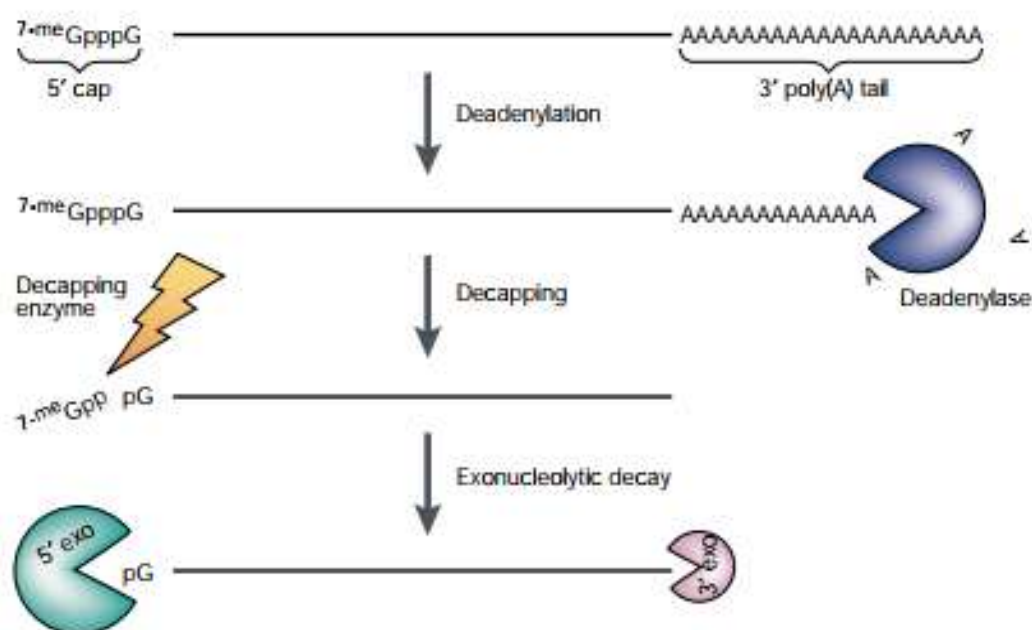


Figure. 1 deadenylase dependent mRNA degradation^[14]

Deadenylation is a process occurring under normal physiological conditions and is often the rate determining step during mRNA degradation and subsequent translational silencing.^[15] Deadenylation is therefore an important method for the body to modulate gene expression. Many different deadenylase enzymes exist in nature, mostly as complexes consisting of multiple subunits. There are two families of deadenylase enzyme, all of which contain two Mg^{2+} ions in their active site. These magnesiums are coordinated by the scissile

phosphate group of the poly(A) tail during substrate binding. The EEP family contain their magnesium within a conserved endonuclease-exonuclease-phosphate domain, whilst the DEDD family chelate magnesium using a conserved Asp-Glu-Asp-Asp sequence. Much of the knowledge of deadenylases was primarily inferred from studies in yeast. While most deadenylases are conserved across eukaryotes, studies in animals are often hindered as many are essential and often work synergistically in multicellular organisms. ^[16] Ten deadenylases are known to be encoded in the human genome and several biological processes have been linked to specific deadenylase enzymes or subunits (briefly outlined in table 1). ^[15] This range of deadenylase mediated biological processes makes the concept of a selective deadenylase inhibitor attractive as a pharmacological intervention.

Table. 1 Examples of biological processes known to be mediated by specific deadenylase enzymes or subunits. ^[15]

Deadenylase/subunit	Family	Biological Functions	References
CCR4	EEP	DNA damage, cell cycle and replication, oogenesis, nonsense mediated decay	[17] [22] [24] [27] [37]
NOCTURIN	EEP	Circadian rhythm	[33][39]
ANGEL	EEP	RNA processing	[19][20][21]
PARN	DEDD	Oocyte maturation, nonsense mediated decay	[17][22][25][32] [34]
PAN2	DEDD	Replication stress, Nuclear poly(A) shortening	[17][22][38]
CAF1Z	DEDD	Nuclear decay	[20]
Not/Caf1	DEDD	DNA damage, Cell cycle, Fertility/spermatogenesis, Bone mass	[17][18][22][23] [26][28] [29][30][31][35] [36][37]

1.3 CCR4-NOT complex

Caf1, encoded by the CNOT7 or CNOT8 genes, and Ccr4 encoded by the CNOT6 and CNOT6L genes are the catalytic subunits of the CCR4-NOT complex. This complex is made up of a number of subunits that varies slightly

by species. Some subunits (Ccr4, Caf1, Not1, Not2, Not3 and Not5) and their interactions remain conserved across all species examined. ^[40]

The names of these subunits can be confusing as they can vary between species and some publications use different names. For clarity, a table is included of common names of the CCR4-NOT complex subunits (table 2).

Table. 2 Common names for CCR4-NOT complex subunits in yeast (*Saccharomyces cerevisiae*) and humans.

Yeast Subunit	Other names	Human Subunit	Other names	Domain/motif
Not1	Cdc39	CNOT1	Human Not1	LxxLL
Not2	Cdc36	CNOT2	Human Not2	
Not3		CNOT3	Human Not3	
Not4	Mot2	CNOT4	Human Not4	RING
Not5		Unknown		homologous to Not3
Ccr4		CNOT6	Human Ccr4	LRR, EEP
		CNOT6L	Human Ccr4	LRR, EEP
Pop2	Caf1	CNOT7	Human Caf1	DEDD
		CNOT8	Human Caf1, Human Pop2, Calif	DEDD
Caf40		RQCD1	Rcd1, CNOT9	
Caf130		CNOT10		
		CNOT11	C2ORF29	

Caf1 and Ccr4 are both Mg^{2+} dependent exonucleases. Sequence comparison ^[41] and X-ray crystal ^[42] structure reveal that Ccr4 belongs to the EEP family, is selective towards poly(A) tails and interacts with Caf1 *via* a leucine rich repeat surface. ^[43] Caf1 however belongs to the DEDD family and degrades RNA with a strong selectivity towards poly(A) tails. ^[44] Not1 is a large, central protein that holds together many of the other subunits. ^[45] This is achieved using structures that are typically involved in protein-protein binding. ^{[46][47]} Less is known about the functionality of Not2 and Not3/5, although they have been shown to only exist within the complex. ^[48] The complex is conserved in humans and yeast, in yeast an additional three subunits (Not4, Caf40 and Caf130) are present. The structure has been shown by electron microscopy to be two roughly perpendicular arms of similar length making up the Not1 structural subunit that holds the others in place. There is a

central cavity containing the catalytic subunits (Ccr4 and Caf1), that is thought to act as the deadenylation surface. ^[49]

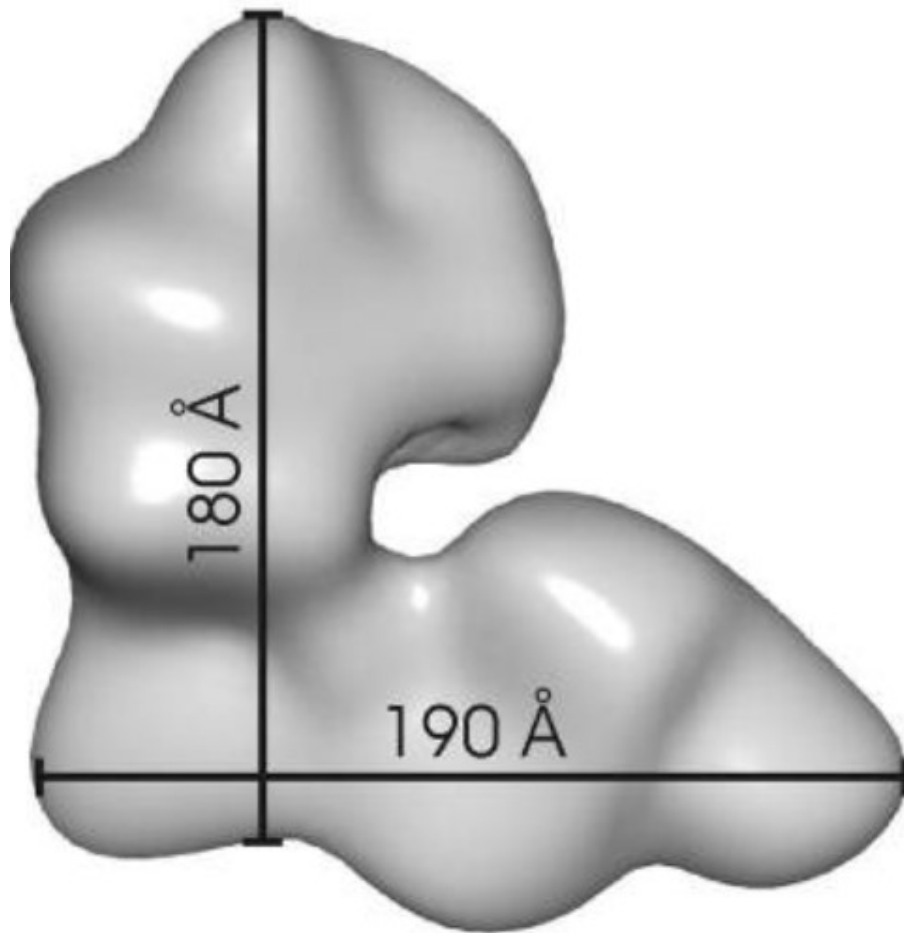


Figure. 2 Surface representations of yeast Ccr4-Not complex, side and front view, distance labelled in angstroms. ^[49]

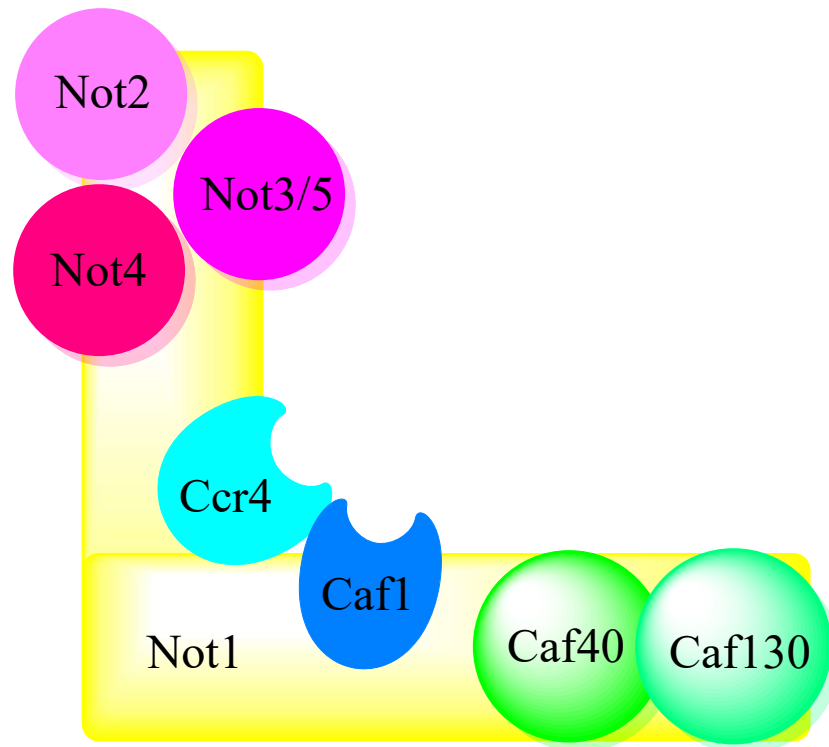


Figure. 3 Diagram of yeast Ccr4-Not complex indicating rough positions and interactions of the subunits. ^{[40][49]}

Surprisingly, the roles of these various subunits are often non-overlapping making it unclear why association into a single complex is necessary. The complexity of the complex is also far greater than would be expected for its current known functions. ^[50] Studies on mutant mice have however implicated Ccr4-Not complex in several roles including spermatogenesis, ^[51] energy metabolism ^[52] and normal cardiovascular function. ^[53]

1.4 Caf1

1.4.1 The Structure of Caf1

The function of Caf1 is known to be that of a poly(A) tail selective exonuclease enzyme, e.g. a deadenylase. The structure of human Caf1 has been elucidated by X-ray crystallography of Caf1 co-crystalised with the NOT1 MIF4G domain (figure 4). ^[54] The active site of Caf1 is a hydrophobic pocket except for the typical DEDD domain responsible for the coordination of the magnesium. The magnesium in the active site binds to the scissile phosphate group of the mRNA whilst the aromatic regions in the hydrophobic pocket are likely to be involved in binding to the adenosine.

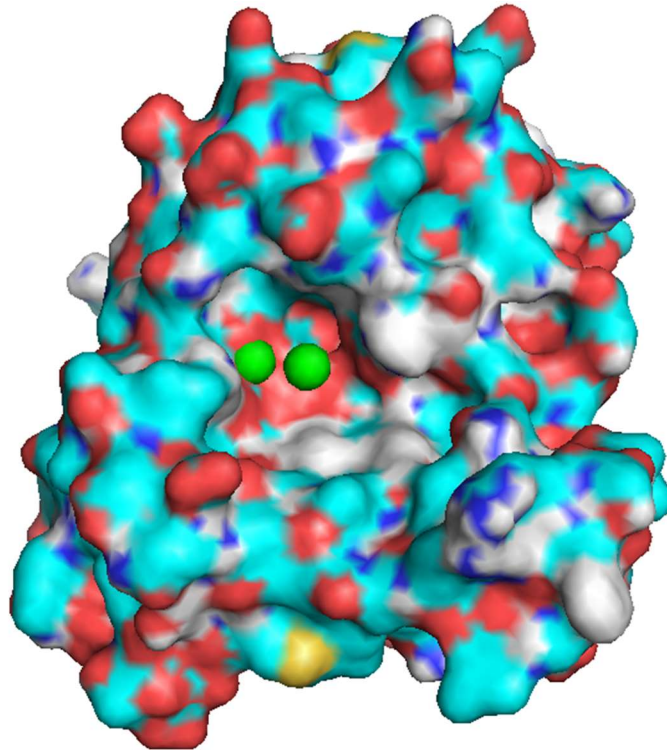


Figure. 4 Surface representation of the X-ray crystal structure of Caf1 (PDB 4GMJ chain B). ^{[54][55]} Oxygen = red, Nitrogen = blue, Sulphur = yellow, Aromatic carbon = grey, and aliphatic carbon = turquoise. Magnesium is represented by green spheres.

1.4.2 The Role of Caf1

Caf1 is believed to interact with the BTG/TOB proteins to modulate their antiproliferative activity, which are essential to some of their functions. ^[56] Certain BTG proteins can also be required for the deadenylation of several mRNAs. ^[57] Caf1 seems to repress the expression of genes involved in the cell replication cycle and encourages expression of genes required for division. ^[58]

The physiological roles of Caf1 in mice have been investigated by the use of gene knockout strategies. Caf1 has been shown to be essential for spermatogenesis and is highly expressed in the testes. Mice lacking Cnot7 display no obvious abnormalities except for male infertility. Female Cnot7^{-/-} mice reproduce normally to produce healthy Cnot7^{+/-} offspring. When Cnot7^{+/-} mice reproduce together, Cnot7 deficient offspring are produced in the Mendelian ratio. ^{[59][60]} Cnot7 has also been identified as an endogenous suppressor of bone mass by suppressing osteoblast activity. Cnot7 knockout increases mineralisation in bone marrow cultures and increases bone density in mice. In addition to the increased bone density, the Cnot7^{-/-} phenotype in mice suffered no ill effects aside from male infertility. ^[60]

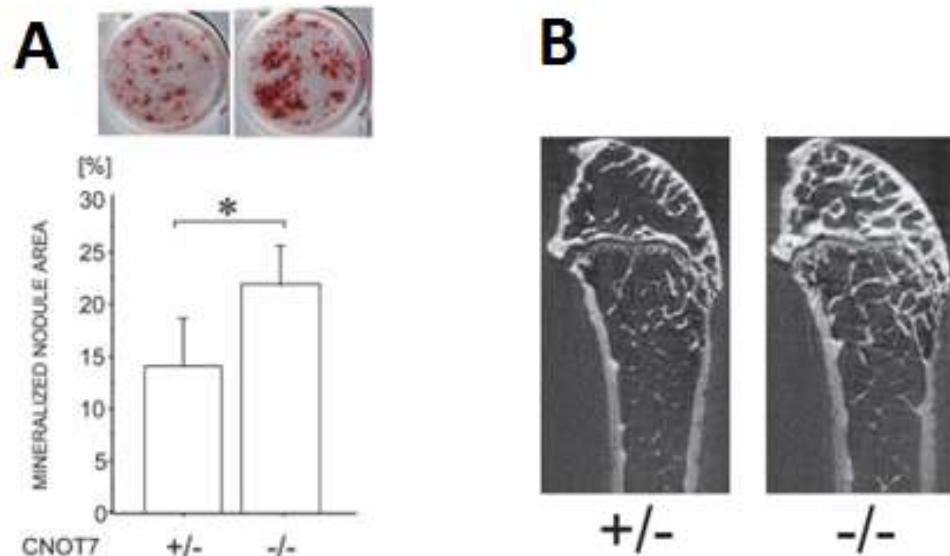


Figure. 5 (A) Comparison of CNOT7 ^{+/−} and CNOT7 ^{−/−} bone marrow cell culture showing increased mineralisation in the absence of Caf1. (B) Comparison of CNOT7 ^{+/−} and CNOT7 ^{−/−} mouse femurs showing increased bone density in the absence of Caf1. ^[60]

1.4.3 The relationship between Caf1 and Other Deadenylases

Ccr4 and Caf1 are both subunits of the CNOT7 complex and appear to overlap in many areas of functionality. ^[58] Ccr4 is a member of the EEP family whilst Caf1 is a member of the DEDD family meaning their magnesium cofactors are contained in different domains in their active sites. ^[61] It is unknown whether they have specialised or co-operative roles and the exact relationship between Caf1 and Ccr4 remains unclear.

In yeast (*Saccharomyces cerevisiae*) Ccr4 is the main deadenylase ^{[62][63]} and Caf1 is not essential for deadenylase activity. ^[64] However, in some other species of yeast (*Schizosaccharomyces pombe* and *Aspergillus nidulans*) both Ccr4 and Caf1 are required for deadenylation. ^{[65][66]} In human cells, both Ccr4 and Caf1 are required for the functioning of the Ccr4-Not complex. ^[67] However the knockdown of Caf1 and Ccr4 each have a different impact on gene expression. ^{[68][69]} It has also been shown that selective Caf1 inhibitors with no activity towards Ccr4 are effective at inhibiting the activity of a Caf1-Ccr4 complex. ^[67]

Poly(A)-specific ribonuclease (PARN) is the most well understood deadenylase enzyme as it is the predominant deadenylase in mammalian cells. PARN can uniquely interact with both the poly(A) tail and the mRNA 5' cap. ^[70] The exact role of PARN is uncertain but it has been linked to the

deadenylation of many different mRNAs decreasing the rate of transcription associated with cell adhesion and cell movement. ^[71]

Whilst PARN is not known to share any functionality with Caf1 it bears a close structural similarity. PARN is also dependent on a magnesium cofactor and is also a member of the DEDD family, sharing the same DEDD active site domain as Caf1. ^[72] The close structural similarity means that PARN has the potential to also be affected by compounds with high affinity towards Caf1.

1.5 Osteoporosis

Osteoporosis is a brittle bone disease that progressively reduces bone density. The term osteoporosis is derived from the Greek for “porous bones”. The lower density of osteoporotic bone tissue leads to an increased risk of fractures. Fractures become common under mild stress or even spontaneously, particularly in the spine and hip. Osteoporosis becomes more prevalent with age and is more common in women, especially post menopause. It is estimated that osteoporosis effects 200 million people worldwide ^[73] and is responsible for 8.9 million fractures per year (one every three seconds) ^[74].

The cause of osteoporosis is an imbalance between the processes of bone formation, which is carried out by osteoblasts, and bone resorption, which is carried out by osteoclasts. Both these processes are present in healthy bone as part of the constant remodelling that occurs in all bone tissues. ^[75]

Pathogenesis arises when the activity of the osteoclasts exceeds that of the osteoblasts leading to an overall reduction in bone tissue. ^[76] The balance between osteoblast and osteoclast activity is regulated by parathyroid hormone (PTH), calcitonin and 1.25 dihydroxycholecalciferol (a form of vitamin D), which respond to dietary and physiological variations to maintain calcium/phosphate homeostasis. Oestrogen deficiency also causes increased osteoclast formation and bone loss, because of this, the highest incidence of osteoporosis is in postmenopausal women.

Current therapies for the management of osteoporosis involve exercise regimes to strengthen bones and reduce the risk of fractures, ^[77] along with several pharmacological options. Various hormones and hormone modulators have been shown to reduce the occurrence of fractures in post-menopausal osteoporosis, ^[78] but these treatments are not without risks. Hormone replacement therapy (oestrogen based treatment) is no longer commonly used because of an increased incidence of breast cancer, strokes and embolic

cardiovascular disease.^[79] PTH based treatments are also given as a daily injection which may not be acceptable to many sufferers of the disease.

Bisphosphonates are a class of drug that reduce loss in bone density by stimulating apoptosis in osteoclasts. Bisphosphonates are structurally related to pyrophosphates (Figure 6).^[80]

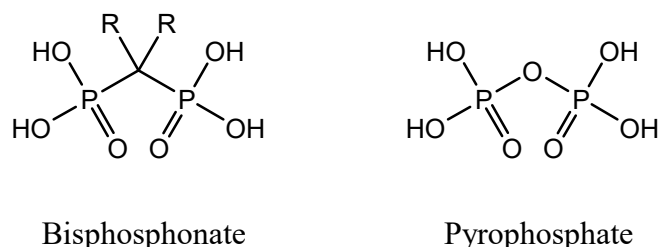


Figure. 6 Generic chemical structure of bisphosphonates compared to pyrophosphates.

However, bisphosphonates are also responsible for causing a range of side effects including oesophageal and gastric erosions, which renders bisphosphonates difficult for some people to tolerate as oral medication. If given intravenously they can cause the severe side effect of osteonecrosis of the jaw.^[81] These side effects can often be immediate whilst therapeutic effects are felt in the long term which causes issues in patient compliance. Strontium ranelate is a drug promoted as a “dual action bone agent”. It is a chelator of strontium which deposits strontium into bone tissue in the place of calcium.

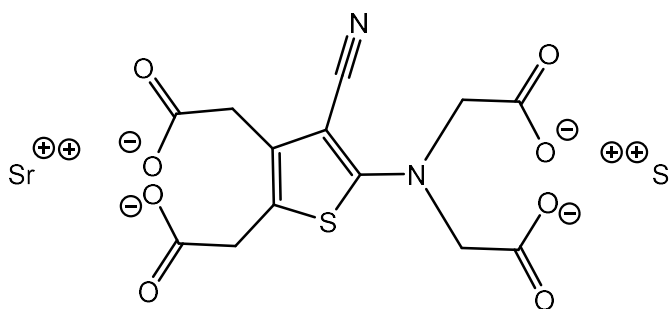


Figure. 7 Chemical structure of strontium ranelate.

Strontium has a similar sized nucleus and can stimulate calcium sensing receptors. This helps aid bone growth by both inhibiting the formation of osteoclasts by the secretion of osteoprotegerin, as well as increasing the formation of osteoblasts.^[82] Unfortunately, the use of strontium ranelate has recently been restricted to only severe cases of osteoporosis that are refractory to other therapies due to an increased occurrence of myocardial infarction and

deep vein thrombosis.^[83] Most current therapeutic interventions show limited evidence of increasing bone density but rather simply preventing or slowing further reduction in bone density.

Osteoporosis and brittle bone diseases represent a clear and significant unmet clinical need, with novel and innovative treatment options being required for the side effect free management and prevention of decreasing bone density. A small molecule, oral drug that could promote bone formation by osteoblasts or reduce bone resorption by osteoclasts would be particularly attractive.

Pharmacological options promoting osteoblasts bone forming activity would reduce the large burden to society and individuals, of the occurrence of fractures, and prevent premature morbidity in those suffering from osteoporosis. The promotion of osteoblast activity may also have more general implications in bone repair and regeneration.

1.6 Inhibition of Caf1

1.6.1 The *N*-hydroxy-imide Moiety

Various nucleoside analogues are reported to act as inhibitors of poly (A) specific ribonuclease (PARN), as similar magnesium dependent deadenylase. However, few non-nucleoside small molecule inhibitors are known for other magnesium dependent ribonucleases.^{[84][85]} The first small molecule compounds identified to be inhibitors of Caf1 were discovered using structure based design using a Caf1 x-ray crystal structure^[54] and the University of Nottingham Managed Chemical Compound Collection, (MCCC) totalling 83086 potential leads. The modelling was carried out using autodock tools. The 1440 compounds with the highest binding scores and best docking modes were then selected to be screened in a fluorescence resonance energy transfer (FRET) deadenylase assay.^[86] To further narrow down the series of compounds, the *N*-hydroxyimide group was selected as a desired pharmacophore due to its ability to chelate metal ions. Many nuclease enzymes rely on two divalent metal cations within their active site that are responsible for the binding of the enzyme to its substrate DNA/RNA. Chelation of these essential cations by a small molecule has been utilised as a strategy to inhibit such enzymes. Notable examples include HIV RNase H,^[87] human flap endonuclease FEN1^{[88][89]} and influenza RNA endonuclease PA.^{[90][91]} Several different functional groups, often incorporated into a ring structure are used as chelating moieties. The *N*-hydroxy imide group was

predicted to be the most suitable for Caf1 based on the distance between the two magnesium ions in the Caf1 active site (3.9 – 4.0 angstroms).^{[93][54]}

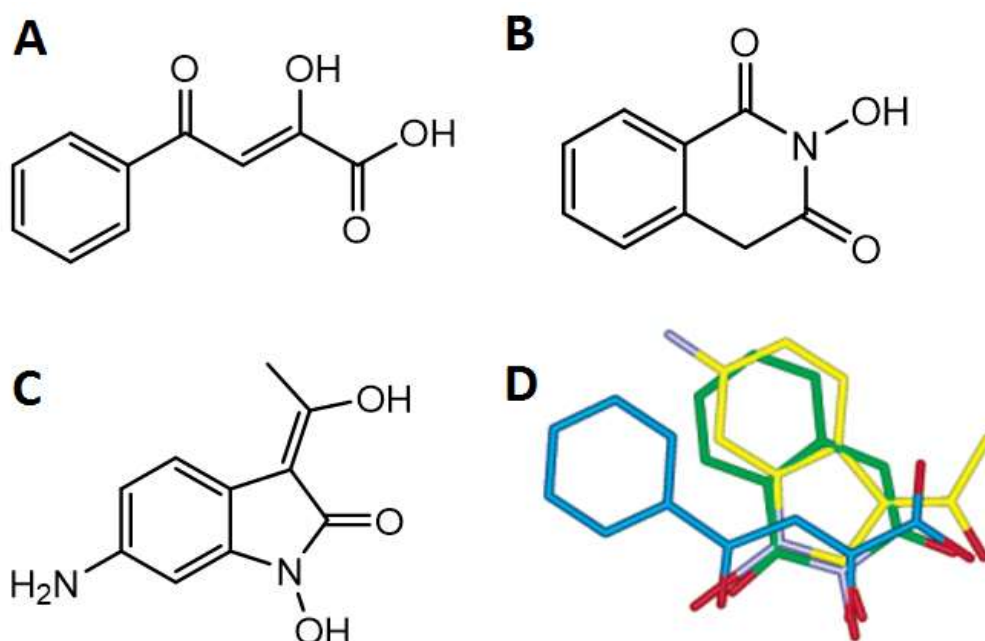


Figure. 8 Three influenza RNA endonuclease PA inhibitors each with different functional group designed to chelate active site metal cations. (A) an inhibitor of the diketobutanoic acid class.^[94] (B) bicyclic ring compound containing *N*-hydroxy imide moiety. (C) *N*-hydroxytetramic acid-based compound. (D) overlay of all three, displaying similarity of the chelating groups.^[91]

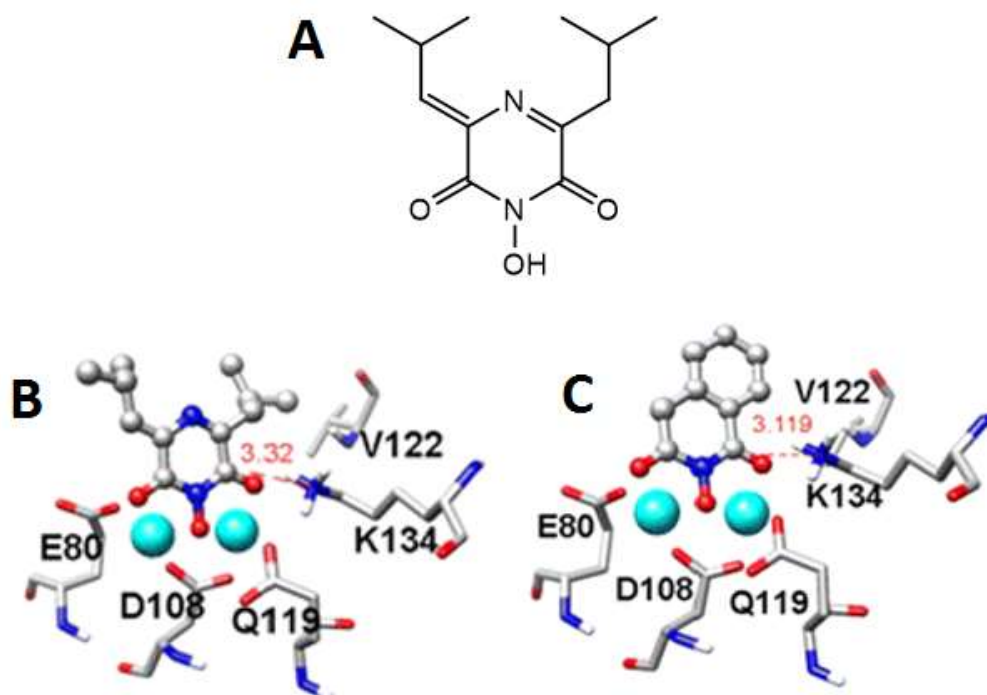


Figure. 9 (A) Flutimide, a known influenza RNA endonuclease PA inhibitor. (B) model of flutimides mode of binding, demonstrating its chelation of two Mn^{2+} ions in the active site. (C) *N*-hydroxyimide compound showing similar binding mode. ^[90]

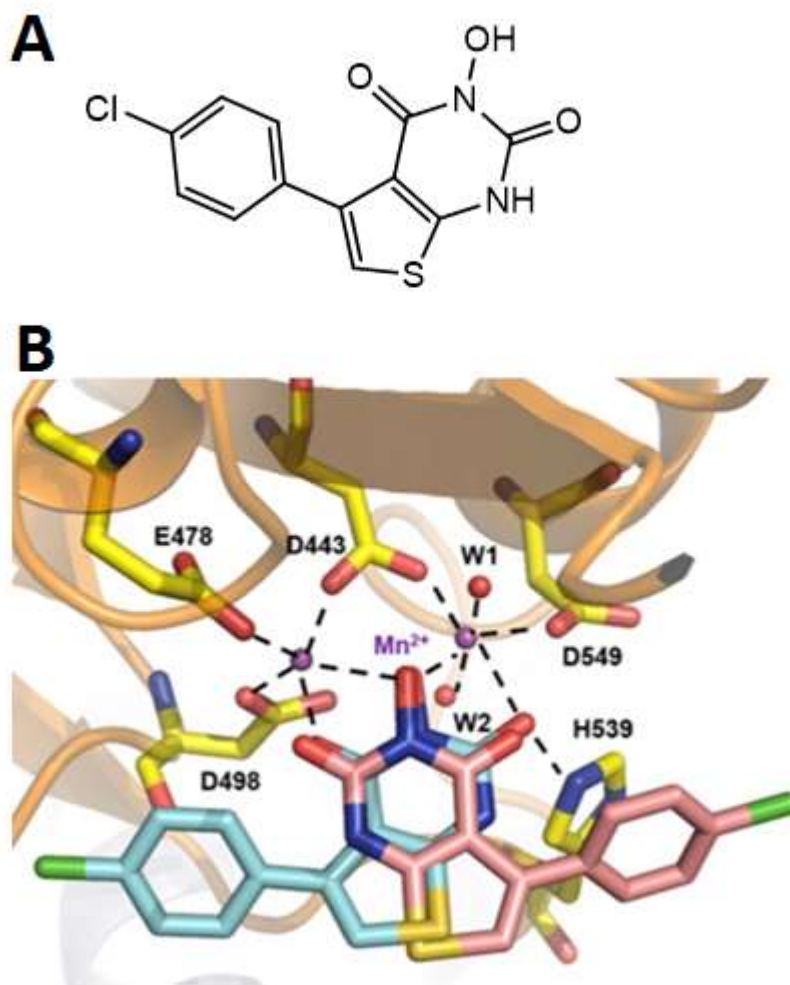


Figure. 10 (A) *N*-hydroxy imide-based HIV RNase H inhibitor. (B) Model of inhibitor chelating two Mn^{2+} ions in HIV RNase H (PDB 5J1E) active site by two different binding modes. ^[93]

The *N*-hydroxyimide moiety has also been used to inhibit human FEN1, a magnesium dependent nuclease enzyme with an X-ray crystal structure used to demonstrate the inhibitor chelation of the catalytic magnesiums. ^[89] This series *N*-hydroxyimide containing FEN1 inhibitors have demonstrated cellular activity showing that even though the *N*-hydroxyimide is ionisable (with a calculated pKa of 5.20 using marvinsketch) it does not prevent membrane permeability.

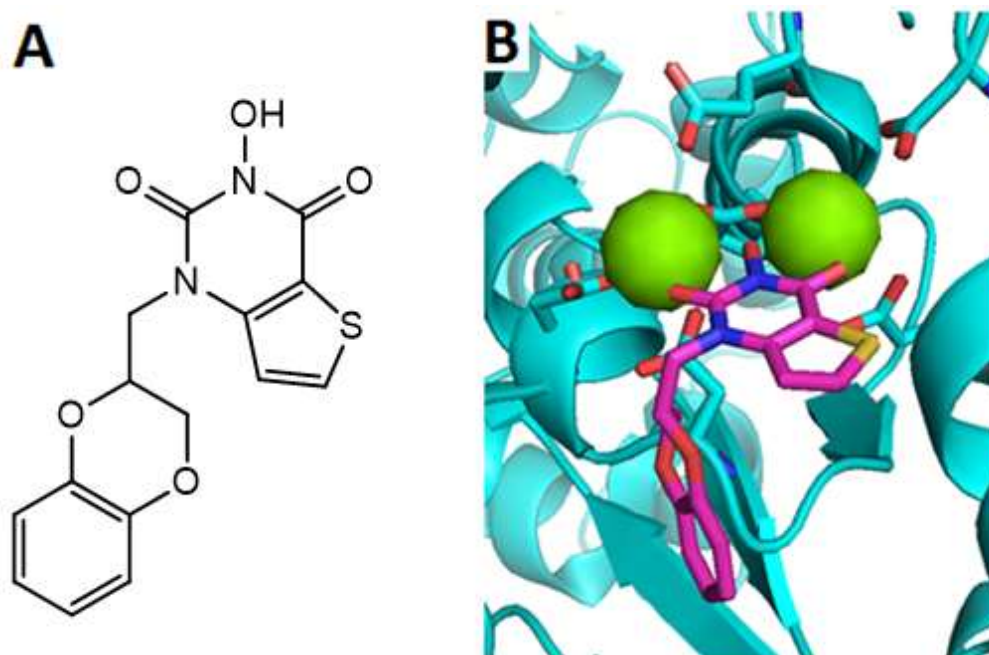
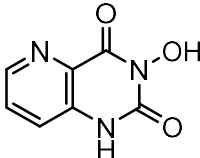
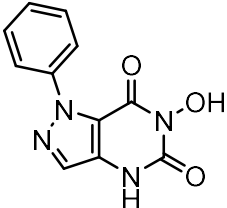
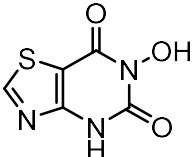
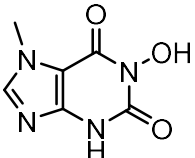
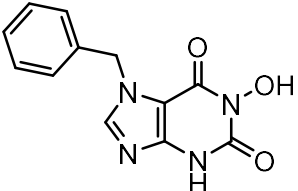


Figure. 11 (A) *N*-hydroxy imide-based HIV RNase H inhibitor. (B) X-ray crystal structure of inhibitor binding to human FEN1 (PDB 5FV7). Magnesiums are represented by the green spheres. ^[89]

A group of suitable compounds containing the *N*-hydroxy imide functional group were identified from the open chemical repository collection. These compounds all contained a fused ring system incorporating a 1-hydroxy pyrimidine. Binding towards Caf1 was determined using FRET assays (table 3). A 7-benzyl substituted 1-hydroxy-xanthine (**5**) was the most potent of the compounds selected but its 7-methyl analogue had ten-fold lower potency. Because of this, 7-substituted 1-hydroxy-xanthines were chosen for further investigation.

Table. 3 IC₅₀ values and cLogP (calculated using chemdraw) for selected open chemical repository collection *N*-hydroxy-pyrimidine compounds. Data collected by FRET assay. ^[97]

compound	Structure	CafI IC ₅₀ (μM)	IC ₅₀ standard error (μM) (n=3)	cLogP
1		525	13.9	-2.39
2		38.6	2.79	-0.84
3		28.7	3.80	-2.55
4		251	18.4	-3.05
5		22.8	0.13	-1.53

1.6.2 Fluorescence assay

A FRET assay was developed specifically to test the inhibitory activity of 1-hydroxy-xanthines towards deadenylases. The assay measures inhibition by looking at the change in the enzyme's ability to degrade mRNA. This is quantified by measuring the fluorescence *vs.* the concentration of the inhibitor at a gradient of different concentrations. The assay uses a 5' fluorescein (Flc) labelled RNA oligonucleotide and a complimentary 3' tetramethylrhodamine (TAMRA) labelled DNA oligonucleotide probe. The complementarity of the RNA and DNA strands keeps the fluorophores in close proximity forming a FRET pair and quenching the fluorescence of the fluorescein. When the activity of the CafI enzyme cleaves the RNA poly(A) tail, the two strands are

separated and so are the fluorescent probes, fluorescence can then be observed. Fluorescence is not observed in the presence of catalytically inactive Caf1 enzyme. ^[95] Caf1 inhibitors will prevent the activity of the enzyme and therefore reduce the fluorescence observed.

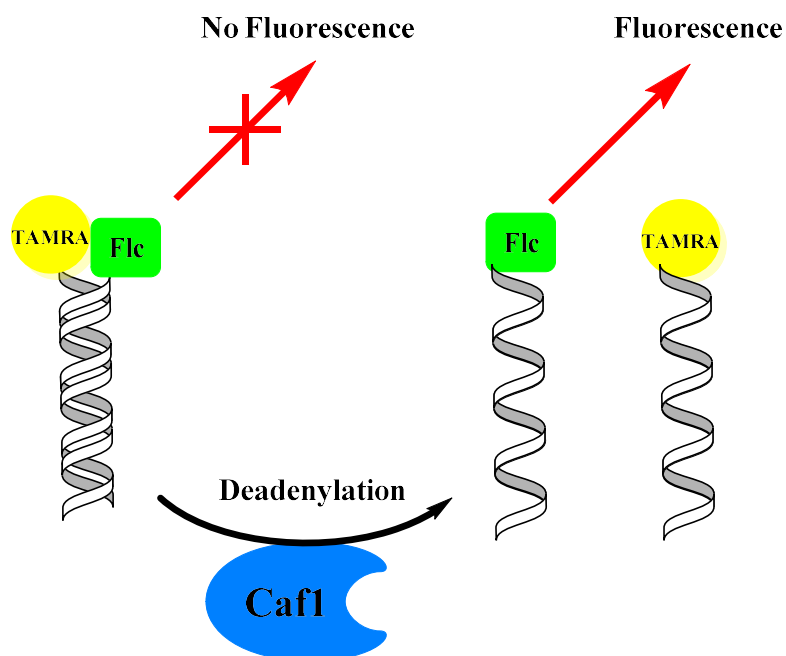


Figure. 12 Diagram of Caf1 mediated deadenylation leading to Flc fluorescence

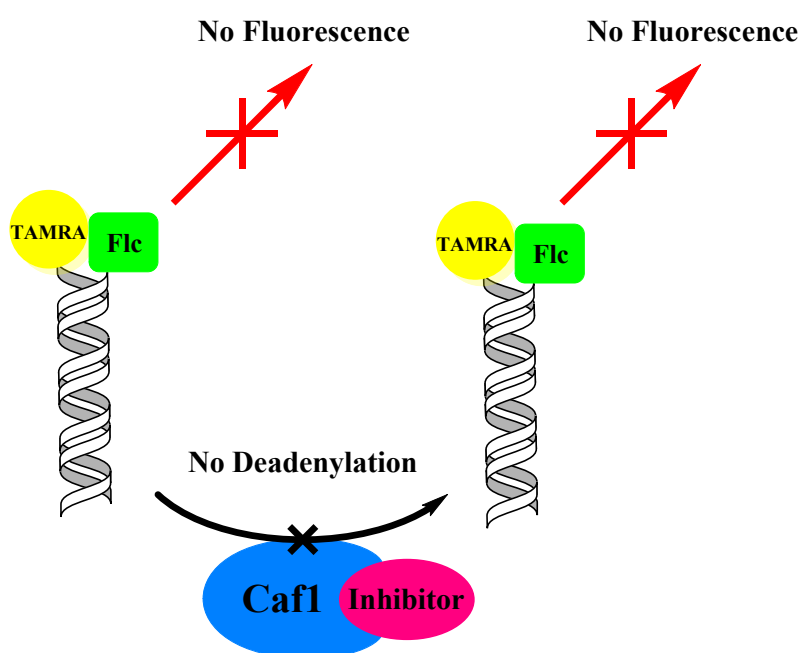


Figure. 13 Diagram of Caf1 inhibition preventing Flc fluorescence

The reaction is conducted in the presence of the Caf1 inhibitor at a range of concentrations to give an activity curve from which an IC_{50} can be determined. This demonstrates the feasibility of a small molecule Caf1 inhibitor and the possibility of measuring its potency by FRET assay.

1.6.3 Promega AMP-GLO Assay

An alternative luminescence-based assay was also shown to be effective for assessing compounds inhibitory activity towards Caf1. This assay measures the concentration of AMP present in a reaction mixture and can therefore quantify the rate of AMP production by deadenylation. This assay has been shown to produce consistent IC_{50} values to those of the FRET assay.^[96] The assay uses the same 5' fluorescein labelled RNA oligonucleotide as the substrate for deadenylation. After a period of incubation, a reagent is added to terminate the deadenylation reaction, and convert the produced AMP into ADP. A second reagent that converts ADP to ATP is added along with a luciferase enzyme and luciferin. The resultant ATP fuels the luciferase reaction generating bioluminescence in proportion to the original amount of AMP cleaved from the RNA strand.

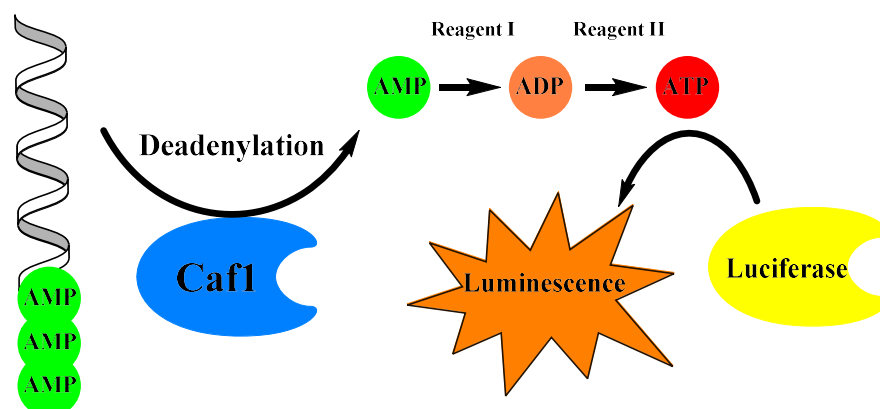


Figure. 14 Diagram of AMP-Glo assay emitting luminescence as a result of deadenylation.

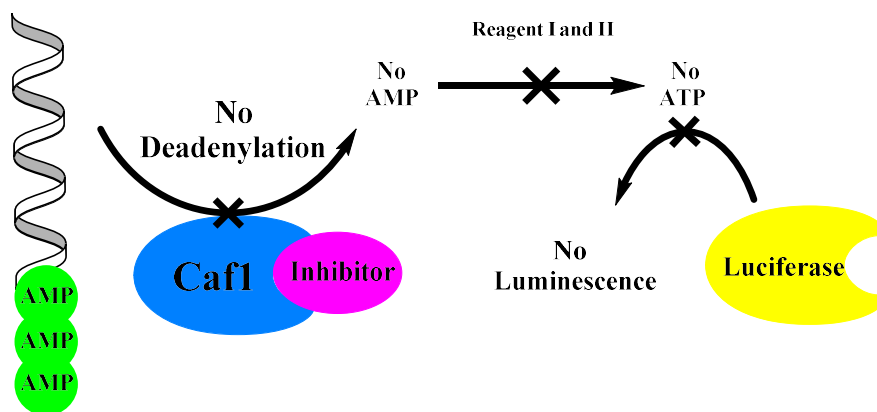


Figure. 15 Diagram of AMP-Glo assay failing to emit luminescence due to a lack of deadenylation.

1.7 Prior Work on *N*-hydroxy-xanthines

1.7.1 Xanthine numbering system

For clarity, the xanthine ring is displayed, showing the numbering system that will be used through this thesis.

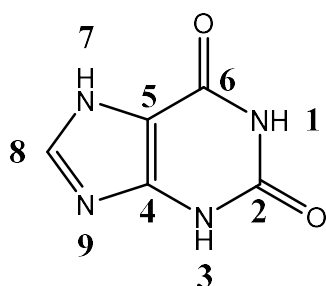
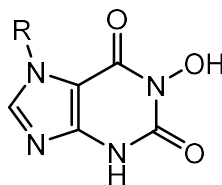


Figure. 16 Xanthine numbering system

1.7.2 Structure Activity Relationships

The structure activity relationship surrounding the 1-hydroxy xanthine has been somewhat explored.^[97] A range of substituents have been tested at the 7-position of the xanthine scaffold. It was discovered that the phenethyl group served as the best substituent. An aromatic ring was generally beneficial, with benzene outperforming pyridine and thiophene, the ideal spacer consisted of two carbons which increased potency ten-fold over a one or three carbon chain.

Table. 4 The IC₅₀ and cLogP (calculated using chemdraw) of 7-substituted 1-hydroxy-xanthines. Data collected by FRET assay. ^[97]

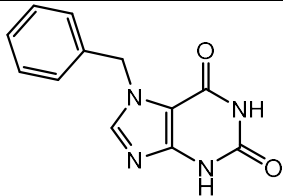
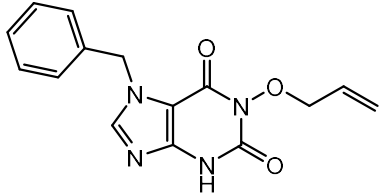
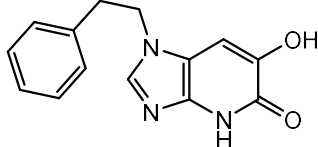


Compound	R	Caf1 IC ₅₀ (μM)	IC ₅₀ standard error (μM) (n=3)	cLogP
6a	Me	251.0	± 18.4	-3.05
6b		10.4	± 0.4	-1.53
6c		28.2	± 7.6	-1.02
6d		10.6	± 2.7	-3.03
6e		6.6	± 0.7	-3.03
6f		23.3	± 2.2	-3.03
6g		13.3	± 2.3	-0.57
6h		20.6	± 5.8	-1.38
6i		3.6	± 1.1	-1.30
6j		10.6	± 4.3	-1.28
6k		1.5	± 0.3	-0.95
6l		4.0	± 1.1	-2.45

The role of the *N*-hydroxyimide moiety in binding to the active site was demonstrated using compounds with modifications that compromise the binding of the *N*-hydroxyimide moiety, and consequently see a loss in activity. Removal or protection of the 1-hydroxy group results in compounds with no activity (table 5), and removal of the 6-carbonyl group reduced activity

tenfold. This shows the importance of the *N*-hydroxyimide to the binding of the inhibitors.

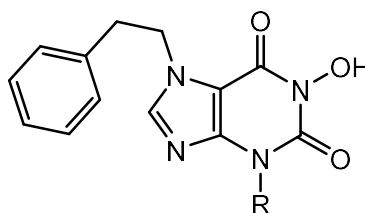
Table. 5 IC₅₀ and cLogP (calculated using chemdraw) of compounds with compromised hydroxyl imine moieties. Data collected by FRET assay. ^[97]

Compound	Structure	Caf1 IC ₅₀ (μ M)	Standard error (μ M) (n=3)	cLogP
7a		>1000	-	-0.85
7b		>1000	-	-0.05
7c		15.1	± 0.3	1.78

1.7.3 Lead Compound

Substituents were explored at the 3-position whilst maintaining the phenethyl substituent at the 7-position. A benzyl substituent or straight alkyl chains ranging from 1 to 12 carbons caused a reduction in potency, however isohexyl and propylphenyl substituents afforded similar potency to the 7-unsubstituted compound. This series of compounds all had a high degree of selectivity over Ccr4 with some compounds being selective over PARN. A dimethylamino propyl substituent (**8j**) gave the highest potency, as well as a high degree of selectivity over PARN and Ccr4, making compound **8j** a suitable lead compound.

Table. 6 IC₅₀ and cLogP (calculated using chemdraw) of 3-substituted 1-hydroxy-xanthines. Data collected by FRET assay ^[97]



Compound	R	Caf1 IC ₅₀ (μM)	Standard error (μM) (n=3)	cLogP
8a	CH ₃	9.3	± 1.7	0.40
8b	CH ₂ CH ₃	12.2	± 0.3	0.93
8c	CH ₂ (CH ₂) ₃ CH ₃	4.8	± 1.2	2.51
8d	CH ₂ (CH ₂) ₇ CH ₃	9.9	± 3.6	4.10
8e	CH ₂ (CH ₂) ₈ CH ₃	20.7	± 6.7	4.63
8f	CH ₂ (CH ₂) ₁₀ CH ₃	29.5	± 22.4	5.67
8g	CH ₂ Ph	14.5	± 0.9	2.17
8h	CH ₂ (CH ₂) ₂ Ph	2.1	± 0.3	2.87
8i		1.7	± 0.4	2.91
8j		0.59	± 0.11	1.02
8k		8.7	± 0.3	0.61

A hypothesised binding mode was generated for the lead compound using molecular modelling using an X-ray crystal structure of Caf1 complexed to Not1. ^[54] Several key interactions were proposed. The *N*-hydroxy imide moiety chelates the two Mg²⁺ ions in the active site as well as forming a key hydrogen bond interaction with Asp¹⁶¹ of the DDED domain. The phenethyl 7-substituent can then find the correct conformation to form a cation- π interaction with one of the magnesiums. The dimethyl aminopropyl 3-substituent (protonated at physiological pH) is thought to act as a hydrogen bond donor to the carbonyl backbone of Phe⁴³.

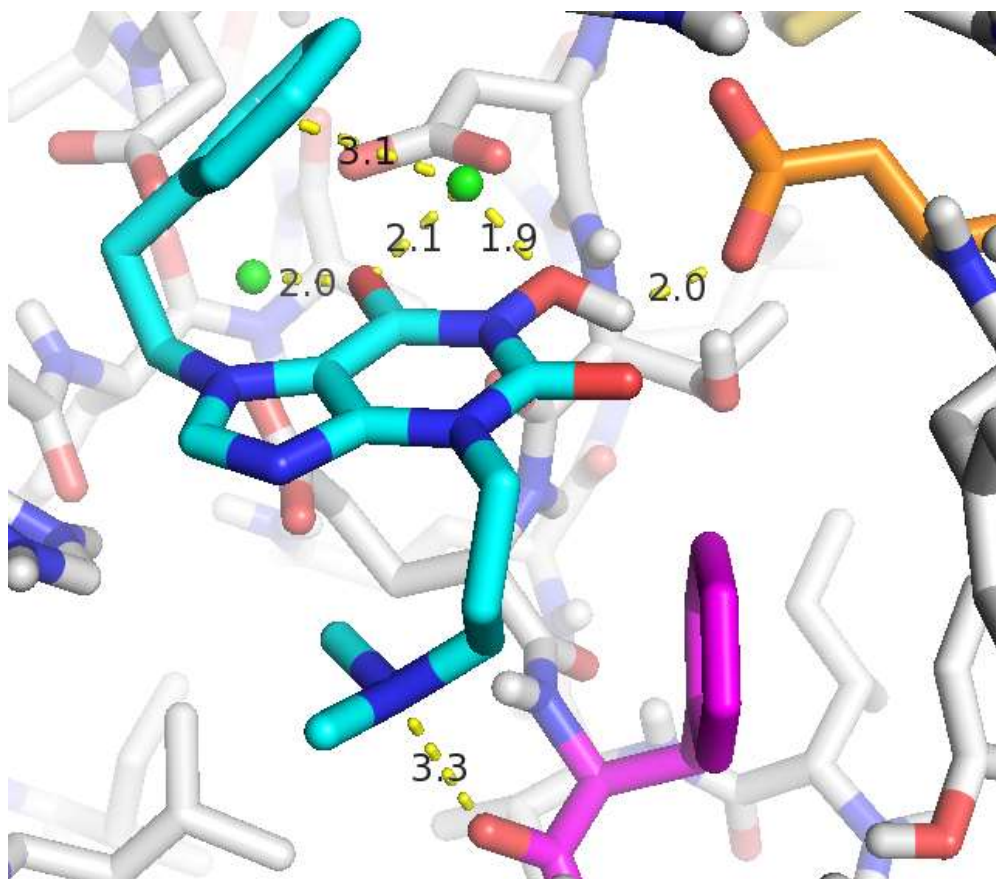


Figure. 17 Lead compound docked into the CafI active site displaying hypothesised binding mode. Distances are shown for the cation- π interaction, magnesium binding and hydrogen bonding to Asp¹⁶¹ (highlighted in orange). Also shown is the hydrogen bond formed between the 3-substituent and Phe⁴³ (highlighted in purple). Distances labelled in angstroms ^{[54][55][97]}

The role of the magnesium ions in this model of inhibitor binding has been demonstrated using thermal shift assays. Differential scanning fluorimetry based thermal shift assays show increased thermal stability of enzyme-inhibitor complex over the enzyme alone. The increase in stability was demonstrated with several inhibitors and was roughly proportional to the inhibitor IC₅₀, demonstrating the binding of the inhibitor to the enzyme. This increase in stability is not observed in the absence of magnesium, suggesting that magnesium is essential to inhibitor binding. ^[96]

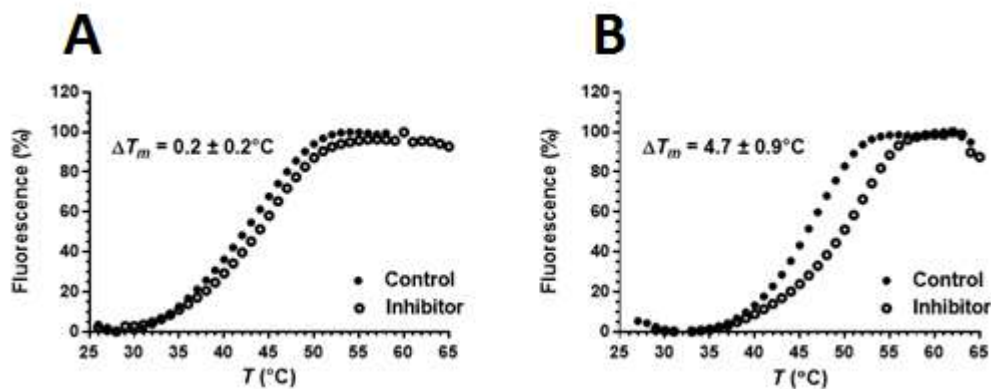


Figure. 18 Plots of enzyme stability (fluorescence %) vs temperature (°C). (A) in the absence of magnesium the inhibitor (**8j**) has negligible effect on the enzyme stability. (B) In the presence of magnesium the inhibitor affords extra thermal stability to the enzyme.^[96]

1.8 Hypothesis

Caf1 has been shown to reduce bone density by the negative regulation of osteoblast activity. It is hypothesised that Caf1 inhibition is a novel therapeutic approach to the improvement of bone density to assist the management of osteoporosis and other brittle bone diseases. This would fill a very large global unmet medical need. Therefore, the main aim of this project is to further the process of drug discovery of potent and selective, small molecule Caf1 inhibitors.

We hypothesise that due to the osteoblast inhibitory activity of CNOT7, a small molecule Caf1 inhibitor, would serve to increase bone density and present a novel therapeutic approach in the treatment of osteoporosis.

1.9 Research Aims

This project will focus on the structure activity relationship (SAR) of the 1-hydroxy-xanthine series of compounds using **8j** as the lead compound. The main aims of this project are the following.

- To maximise activity towards Caf1 by exploring SAR around the 3, 7, and 8 positions
- Explore derivatisation around the 3-position experimenting with the functional group, and properties of the linker
- Experiment with substitution pattern on the benzene ring on the 7-substituent
- Experiment with linker group and ring system at the 7-position
- Experiment with substitution at the 8-position
- Molecular modelling will be used, in combination with newfound SARs as evidence for the binding mode of the inhibitor towards Caf1
- The existing binding mode will be supported and utilised to design further iterations of compounds, or alternatively a new binding mode will be proposed and used for future compound design
- A library of compounds will be designed, synthesised and tested for activity towards Caf1

2. Derivatisation at the 3-Position

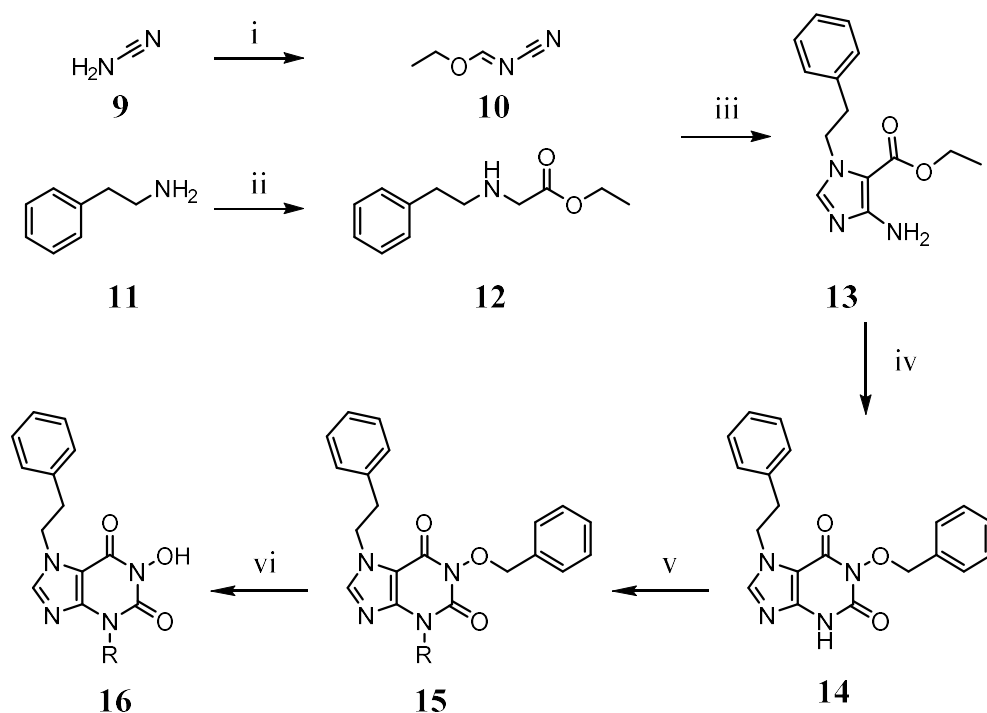
2.1 Relevance of 3-Substituent

The lead compound has a propyl dimethylamine substituent at the 3-position of the xanthine scaffold. This substituent has been demonstrated to afford a significant increase in potency over the unsubstituted analogue. The amine group has also shown to be necessary for this potency increase as the non-functionalised alkyl analogue does not display the same improvement.^[97] A series of compounds was designed and synthesised utilising a range of analogous 3-substituents. These substituents consisted of a range of functional groups and conformationally restrained carbon chains. The potency of the compounds to inhibit Caf1 was assessed and the resultant SAR relationships used to design further compounds and infer information about the mode of binding to the Caf1 active site.

The following chapter will describe the design, synthesis and biochemical profiling of a series of 3-substituted compounds analogous to the lead compound.

2.2 Synthetic Route to 3-Substituted Compounds

The synthesis of the lead compound and analogues at the 3-position has been previously reported.^[97] This synthetic route, with minor adaptations was utilised for the synthesis of 3-substituted analogues (scheme 1).



Scheme. 1 Synthetic route to 3-substituted analogues. Reagents and conditions: i) triethyl orthoformate, reflux, 73 %. ii) ethyl 2-bromoacetate, CHCl_3 , 0 °C, 70 %. iii) 1) THF, reflux. 2) potassium *tert*-butoxide, EtOH, reflux, 79%. iv) 1) carbonyl diimidazole, PhMe reflux. 2) O-benzyl hydroxylamine hydrochloride, triethylamine, PhMe, reflux. 3) sodium hydride, NMP, 80 °C, 83 %. v) alkyl halide (R-X), caesium carbonate, DMF, 80 °C, 30-95%. Or boronic acid, pyridine, copper (II) acetate, DCM, rt, 43 %. vi) hydrogen (1 atm), Pd/C, MeOH, DCM, rt, 82% - 94%.

Ethyl cyanoimidoformate (**10**) was synthesised by heating cyanamide (**9**) with triethoxy orthoformate which acts as both the electrophile and solvent.^{[98][99]}

10 must then be distilled from the reaction mixture under vacuum due to thermal decomposition occurring at a lower temperature (~ 90 °C) than the boiling point at 1 atm of pressure. **10** is formed in high yield with the cyclic trimer, melamine as a minor side product. **10** must be stored at low temperature and used as soon as possible as it will decompose over time.

An excess of phenylethylamine (**11**) was alkylated with ethyl 2-bromoacetate followed by column chromatography to give ethyl phenethylglycinate (**12**), the

excess phenylethylamine acts as a base to neutralise the HBr formed by the reaction and the product is easily purified by chromatography.

The imidazole ring (**13**) was synthesised by the alkylation of phenylethylamine (**11**) with the ethyl cyanoimidoformate (**10**) to form tertiary amine intermediate.^[99] After deprotonation with potassium *tert*-butoxide, enolate **13** forms and attacks the nitrile group in an intramolecular cyclisation to form imidazole **13**.^[100] The literature method for this reaction used a catalytic amount (0.15 eq) of base for enolate formation that would then begin a cascade of deprotonation.^[99] While this is possible it was found that a more stoichiometric amount (1.1 eq) gave higher yields (49% at 0.15 eq, 79% at 1.1 eq).

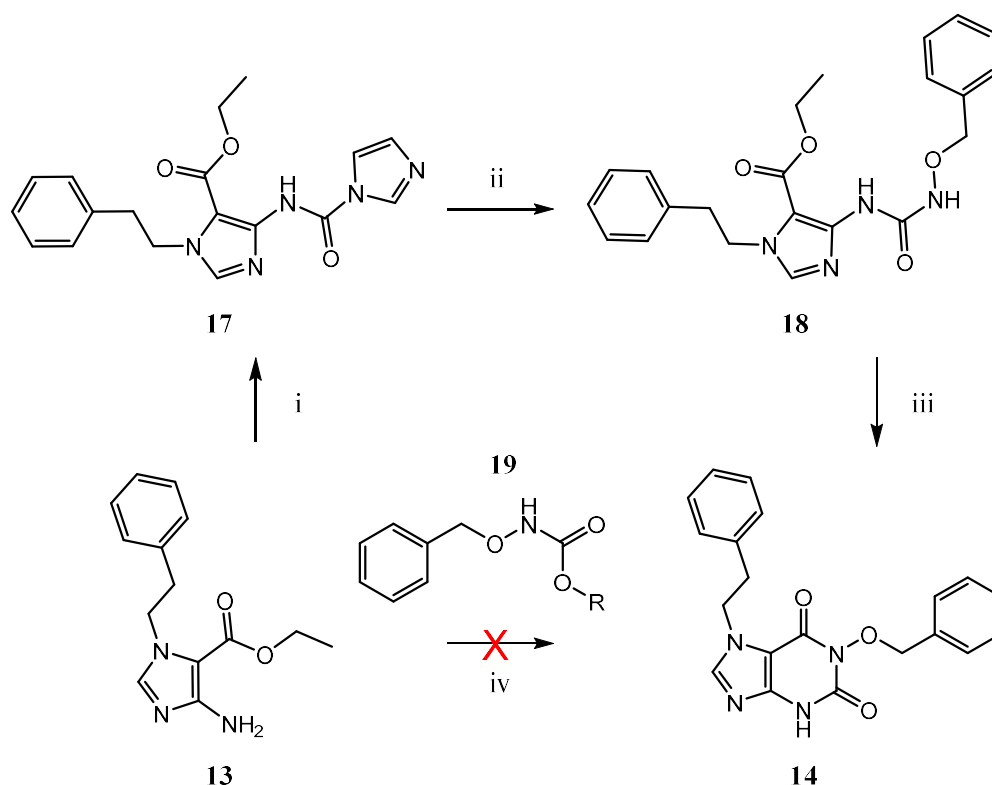
Formation of xanthine **14** was achieved by reacting **13** with CDI followed by O-benzyl hydroxylamine to form a urea intermediate (**18**). The intermediate was deprotonated, leading to intramolecular cyclisation to xanthine **14** (scheme 2). A range of conditions were tested for optimal xanthine cyclisation (table 7). The literature method of aqueous NaOH in EtOH is non-ideal due to the hydroxide mediated hydrolysis of both the intermediate urea back to the starting material, and the ethyl ester to the carboxylic acid thus preventing cyclisation. This problem can be avoided using a non-nucleophilic base in anhydrous conditions. Sodium hydride in polar aprotic solvent was found to be an optimal method in terms of yield, with NMP having a distinct safety advantage over DMF as sodium hydride DMF mixtures have the possibility of thermal runaway when used on sufficiently large scale.^{[101][102]}

Table. 7 attempted conditions for urea cyclisation.

Experimental conditions	Yield (%)
EtOH, aqueous sodium hydroxide, 80 °C	0 - 50
PhMe, sodium hydride, 80 °C	0
THF, EtOH, potassium <i>tert</i> -butoxide, 80 °C	60
DMF, sodium hydride, 80 °C	76
NMP, sodium hydride, 80 °C	83

An alternative method examined was the reaction of **13** with an isocyanate formed *in situ* from an appropriately substituted carbamate, (**19**) (scheme 2).^[103] After this method proved unsuccessful with either an aromatic or aliphatic group (labelled R in scheme 2) on the carbamate, it was attempted to synthesise and isolate the benzyloxy isocyanate.^[104] Attempts to do so also proved unsuccessful. Isocyanate based methods may be inappropriate for the

synthesis of 1-hydroxy xanthines as the corresponding protected hydroxyl isocyanate may be too unstable.



Scheme. 2 Xanthine cyclisation methods. Reagents and conditions: i) CDI, PhMe reflux. ii) O-benzyl hydroxylamine hydrochloride, triethylamine, PhMe, reflux. iii) various conditions and yields. See table 7. iv) potassium tert-butoxide, diglyme, 80 °C, 0 %.

The desired 3-substituent was incorporated into xanthine **14** using alkylation by the corresponding alkyl halide to give **15**. Potassium carbonate was originally used as a base but was changed to caesium carbonate which proved superior in the case of the less reactive alkyl chlorides due to increased solubility in DMF. When caesium carbonate was used the alkylation generally proceeded in high yields (80-95%) with lower yields sometimes occurring for alkyl halides containing basic amines. The alkyl halides used were either commercially available or were synthesised from equivalent alcohols by chlorination with thionyl chloride, and/or via the use of Boc or CBz protecting groups. This method of alkylation was however found to be limited to primary alkyl halides.

In the case of compound **16u** in which a phenyl group was directly attached at the 3-position. *N*-arylation was achieved by copper mediated Chan-Lam-

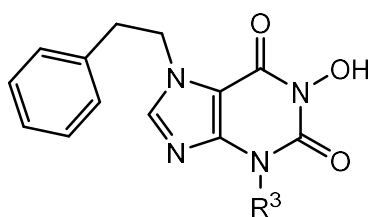
Evans coupling.^{[105][106][107]} The reaction conditions used were taken from successful *N*-arylation of nucleotides such as thymine and uracil^[108] which share a structural moiety with xanthine.

The benzyl protecting group was removed by Pd/C catalysed hydrogenation yielding **16**, purification was performed, if required using reverse phase column chromatography eluting with MeOH and H₂O.

2.3 Pharmacological Data

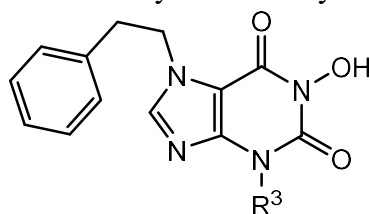
IC₅₀ values towards Caf1 were measured using a FRET assay^[95] The IC₅₀ values are half maximal inhibitory concentrations of the given compound towards the Caf1 enzyme, used as a measure of the potency of the compound to inhibit Caf1.

Table. 8 3-substituted compound IC₅₀ values and cLogP (calculated using chemdraw). IC₅₀ data collected by FRET assay.



Compound	R ³	Caf1 IC ₅₀ (μM)	SEM (μM n=3)	cLogP
8j		3.30	0.81	1.02
16a		6.59	1.33	0.45
16b		13.67	3.36	0.21
16c		21.22	3.66	0.12
16d		53.80	2.73	0.87
16e		2.49	0.79	2.07
16f		8.65	1.08	0.89
16g		58.60	10.54	-0.02
16h		31.76	8.96	-3.50
16i		7.34	1.12	1.54
16j		13.33	2.65	3.59

Table. 8 3-substituted compound IC₅₀ values and cLogP (calculated using chemdraw). IC₅₀ data collected by FRET assay.



Compound	R ³	CafI IC ₅₀ (μM)	SEM (μM n=3)	cLogP
16k		10.78	1.07	1.31
16l		21.19	1.63	0.67
16m		41.64	6.91	2.17
16n		14.95	3.85	3.40
16o		7.70	1.22	2.61
16p		51.81	8.72	1.28
16q		15.11	3.11	-0.03
16r		34.00	5.16	1.47
16s		169.04	14.74	0.57
16t		15.24	2.34	0.15
16u	-Ph	59.48	4.53	2.14

2.4 Structure Activity Relationships

2.4.1 Hydrogen Bond to Phenylalanine⁴³

The main pharmacophore of the 3-substituent was proposed to be the protonated dimethyl amine donating a hydrogen bond to the backbone of Phe⁴³ in the CafI active site.^[97] This hypothesis was supported by a positive correlation between the substituent's hydrogen bond donating ability and observed IC₅₀. Potency was reduced 10-20 fold in compounds with no hydrogen bond donating ability. This was achieved by, substitution of the amine group for an ether (**16d**), quarterisation of the amine (**16h**) or introduction of a piperazine group to reduce protonation at the appropriate

nitrogen (**16g**). Substituents with an alcohol group (**16c**), or amines with mildly reduced basicity (**16a**, **16b** and **16f**) suffered from smaller drop offs in potency whilst the more basic diethyl group (**16e**) was the only such compound to show improved potency over the lead. However, the binding efficiency index ^[109] for the primary (**16a**), monomethyl (**16b**), dimethyl (**8j**) and diethyl (**16e**) amines are all very similar at 14.7, 15.0, 15.3 and 14.6 respectively. This implies the variation in potency between these groups is largely caused by lipophilicity rather than hydrogen bond strength.

2.4.2 Phenyl Rings and Conformational Restraints on the 3-Substituent

Compounds **16u**, **16m** and **16n** all contain a phenyl ring with a carbon linker of 0, 2 and 4 carbons, respectively. These compounds were synthesised to complete a series of similar compounds including two previously reported compounds which have the 1 and 3 carbon linkers. ^[97] However none of the phenyl group containing compounds showed good potency compared to the lead.

Compounds **16i**, **16k** and **16l** all contain conformationally restrained linkers of 3 carbon chain length connected to a basic nitrogen. These compounds have fewer rotatable bonds which reduces conformational entropy with the intention of potentially increasing the binding affinity. ^{[110][111]} None of the above compounds successfully achieved increased potency over the lead. The likely reason for this is that the ideal binding conformation is prevented by the conformational restraint. The diversity of this group of compounds was limited by the availability/complexity of starting materials, however it is feasible that with more detailed information about the binding pose a suitable conformationally restrained substituent could be designed.

Compound **16u** served both these purposes as the phenyl ring could also act as a conformational restrained 3 carbon linker if substituted at the 3-position. However, this route was not explored due to the phenyl ring being poorly tolerated.

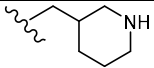
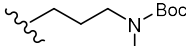
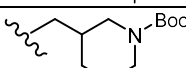
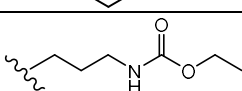
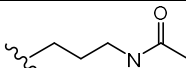
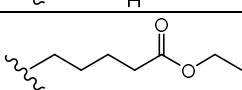
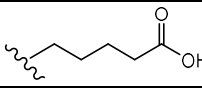
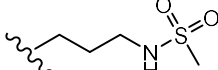
2.4.3 Boc Protected Amines on the 3-Substituent

It was observed that with compounds **16o** and **16j** that the presence of the Boc protecting group did not significantly detriment potency compared to the deprotected analogue even though the possibility of hydrogen bond donation from the amine was removed. This was contrary to the binding hypothesis and therefore implies some other binding interaction is responsible for the potency. It was hypothesised that the Boc group must be involved in some positive

interaction with the enzyme active site. A range of different carbonyl containing functional groups (**16p**), (**16q**), (**16r**), (**16s**), and (**16t**) were explored at this position to test this hypothesis and the surrounding SARs.

Unfortunately, none of the other carbonyl containing functional groups could recreate the high potency of the Boc groups. An alternative explanation for the high potency of the Boc containing compounds is the additional lipophilicity afforded by the Boc group, resulting in an increase of 1.09 units of Clog P. ^[112] The effect of this additional lipophilicity can be shown by calculating the lipophilic efficiency (LiPE). ^[r-113] This is a parameter used measure of the drug compound and differs from measuring potency alone as it subtracts lipophilicity from pIC₅₀ to give a measure of binding interactions other than non-specific hydrophobic forces. The Boc containing compounds have much lower LiPE than their deprotected analogues, as well as failing to outperform the other carbonyl functional group containing compounds. The LiPE analysis suggests that the deprotected amine containing compounds strongly outperform their Boc protected analogues.

Table. 9 Comparison of LiPE of Boc containing compounds, their deprotected analogues, and other carbonyl functional group containing compounds.

Compound	Structure	LiPE
8j		4.72
16a		4.68
16k		3.92
16o		2.95
16j		2.17
16p		3.13
16q		4.27
16r		2.53
16s		2.67
16t		4.39

2.5 Conclusion

None of the compounds with varied substituents at the 3-position afforded significant increases in potency, this was even more apparent when parameters such as BEI or LiPE were considered. The tertiary amine remained the optimum choice of substituent at the 3-position and was conserved during further substitution at differing positions. The SARs of the compounds synthesised supported the 3-substituent's proposed role in the binding hypothesis.

One possible explanation for discrepancies between the observed and expected SAR is that the Caf1 X-ray crystal structure used for molecular modelling and compound design is co-crystallised with a NOT1 MIF4G domain.^[54] The enzyme used in pharmacological testing was not part of a complex. It is possible that the tertiary structure of the co-crystallised Caf1 differs from that of free Caf1 in a way that would mislead any compound design based off of the X-ray crystal structure.

3. Derivatisation at the 7-position

3.1 Relevance of the 7-Position

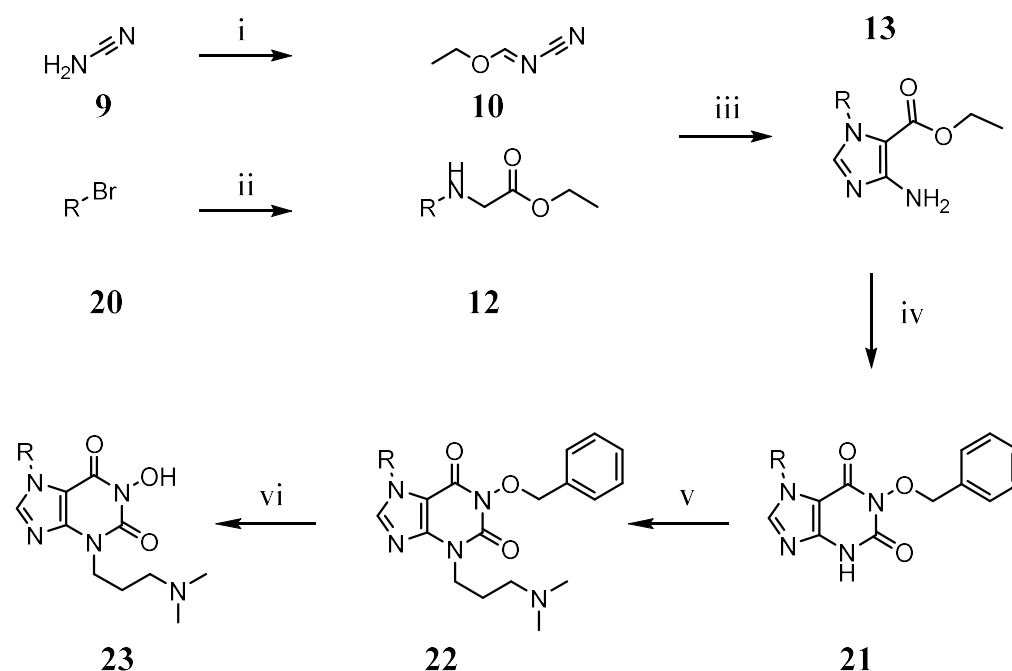
The lead compound has a phenethyl substituent at the 7-position. This substituent is believed to form a cation- π interaction towards one of the magnesium ions in the Caf1 active site and has been shown to contribute strongly towards the inhibitor's activity.^[97] Cation- π interactions are an important non-covalent bond in a number of biological systems,^[114] and is a prime target for optimisation. A further series of compounds varied at the 7-position were designed and synthesised to test this binding hypothesis and improve potency by optimising the structure of the substituent. A range of substitution was explored on the benzene ring, in addition to alternative ring systems and changes to the 2-carbon chain connected to the benzene ring. This series of compounds maintained the propyl dimethylamino substituent present at the 3-position.

The following chapter will describe the design, synthesis and biochemical profiling of a series of analogues to the lead compound, derivatised at the 7-position. It will also outline the SARs surrounding these compounds and the consequential changes made to the proposed mode of binding based on these new findings.

3.2 Synthetic Route to 7-Substituted Compounds

The synthesis of 1-hydroxy xanthines with varied 7-substituents has been previously reported using the same synthesis as with the 3-substituents (scheme 3) with a varied starting amine.^[97] This method however has disadvantages. The alkylation of the starting amine with ethyl 2-bromoacetate presented difficulties. In the literature method, the starting amine is used in excess to act as a base as well as reactant. This works well with a cheap, readily available amine. However, many of the desired starting amines were substituted phenethylamines which were often commercially unavailable and required prior synthesis, some were also controlled substances that were illegal to purchase or synthesise.^[115] An alternative step was used where the starting amine was replaced by an alkyl bromide (**20**) which tended to be more readily available. The alkyl bromide was reacted with ethyl glycinate in the presence of a potassium iodide catalyst, this forms the more reactive alkyl iodide by *in-situ* Finkelstein reaction which then undergoes S_N2 reaction with

the ethyl glycinate to give **21**.^[116] The same modifications to the literature method discussed in chapter 2 were also used to improve yields and reliability (scheme 3).

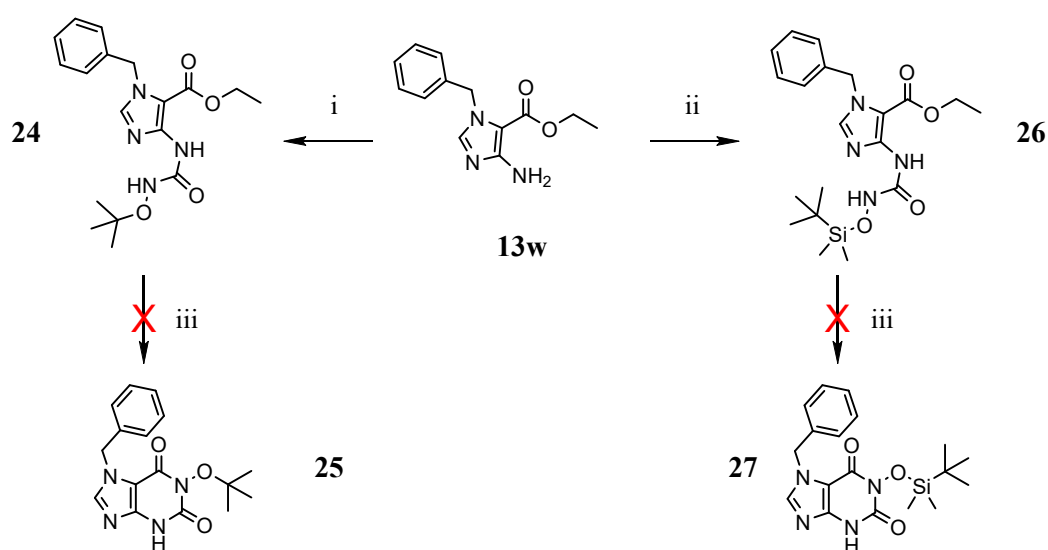


Scheme. 3 Synthetic route to 7-substituted analogues. Reagents and conditions: i) triethyl orthoformate, reflux, 73 %. ii) ethyl glycinate HCl, KI, MeCN, reflux, 87 %. iii) 1) THF, reflux. 2) potassium *tert*-butoxide, EtOH, reflux, 79%. iv) 1) carbonyl diimidazole, PhMe reflux. 2) O-benzyl hydroxylamine hydrochloride, triethylamine, PhMe, reflux. 3) sodium hydride, NMP, 80 °C, 83 %. v) alkyl halide (R-X), caesium carbonate, DMF, 80 °C, 30-95%. Or boronic acid, pyridine, Copper (II) acetate, DCM, rt, 43 %. vi) hydrogen (1 atm), Pd/C, MeOH, DCM, rt, 82% - 94%.

3.2.1 Late Stage Derivatisation

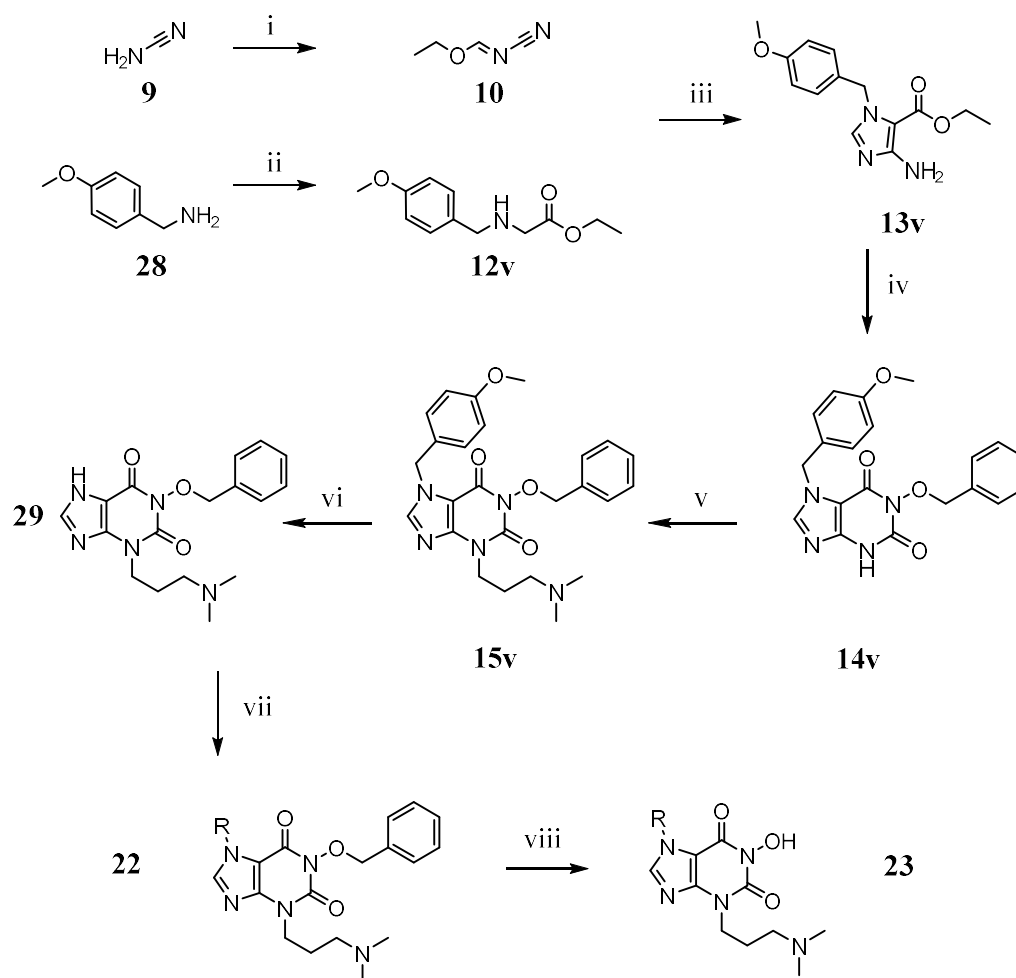
The other problem with this synthetic pathway (scheme 3) is the early stage derivatisation. The derivatisation is introduced in the first step which necessitates a 5-step synthesis for every analogue. Whilst this synthesis was employed successfully for some compounds, a more efficient synthetic pathway was designed in which the derivatised 7-substituent is introduced as the penultimate step. While the overall number of steps was increased, the presence of a common late-stage precursor (**29**) greatly reduced the number of reactions necessary to synthesise a series of 1-hydroxy xanthines derivatised at the 7-position.

This was achieved using a protecting group at the 7-position that could be removed independently from the protecting group at the 1-position. Initially a synthesis was designed where the 7-position was protected by a benzyl group to later be removed by hydrogenation^[117] and the 1-hydroxy group protected by an alternative protecting group stable to hydrogenation. The benzyl protected imidazole was synthesised from commercially available *N*-benzyl-glycine ethyl ester. However, the xanthine cyclisation step was not successful with any other suitable O-protected hydroxylamine. The synthesis failed under a range of cyclisation conditions, using both *O*-*tert*-butyl hydroxylamine or *O*-*tert*-butyldimethylsilyl hydroxylamine. The *tert*-butyl urea failed to cyclise, whilst the silyl ether consistently cleaved under cyclisation conditions.



Scheme. 4 Unsuccessful protecting group strategies. Reagents and conditions: i) 1) carbonyl diimidazole, PhMe, reflux. 2) *O*-*tert*-butyl hydroxylamine hydrochloride, triethylamine, PhMe, reflux. (not isolated). ii) 1) carbonyl diimidazole, PhMe, reflux. 2) *O*-*tert*-butyldimethylsilyl hydroxylamine hydrochloride, triethylamine, PhMe, reflux. (not isolated). iii) sodium hydride, NMP, 80 °C, 0 %. Or potassium *tert*-butoxide, THF, reflux, 0%. Or sodium ethoxide, EtOH, reflux, 0%. Or sodium hydride, THF, reflux, 0%.

The successful method used a *para*-methoxy benzyl (PMB) protecting group was placed at the 7-position by alkylating *para*-methoxy benzylamine with ethyl 2-bromoacetate to form **12v**. After steps iii, iv and v, which proceed in the same way as the prior route, the PMB group is removed to yield the 7-unsubstituted xanthine (**21**). **21** is then alkylated using the Mitsunobu reaction and the *O*-benzyl group removed by hydrogenation or by TFA cleavage in the case of substituents labile to hydrogenation.



Scheme. 5 Revised synthetic route utilising late stage derivatisation. Reagents and conditions: i) triethyl orthoformate, 95 °C, 73 %. ii) ethyl 2-bromoacetate, THF, 0 °C, 68 %. iii) 1) THF, reflux. 2) potassium *tert*-butoxide, EtOH, reflux, 79%. iv) 1) carbonyl diimidazole, PhMe, reflux. 2) O-benzyl hydroxylamine hydrochloride, triethylamine, PhMe, reflux. 3) sodium hydride, NMP, 80 °C, 83 %. v) 3-dimethylamino propyl chloride hydrochloride, caesium carbonate, DMF, 80 °C, 78 %. vi) TFA, 70 °C, microwave radiation, 57 % vii) PhMe, DIAD, PPh₃, corresponding alcohol (ROH), rt, 22% - 48 %. viii) hydrogen (1 atm), Pd/C, MeOH, DCM, rt, 82 % - 94 %. Or TFA, 120 °C, 33-98 %.

The PMB group at this position proved resistant to most classic PMB deprotection methods. Oxidative methods such as the use of DDQ^[118] or cerium ammonium nitrate^{[119][120]} failed to remove the PMB group. Acidic conditions often resulted in either no debenzylation, or undesired O-debenzylation of the benzyl ether protecting the 1-hydroxy group. TFA alone

at any temperature from rt to reflux failed to cause PMB deprotection and the addition of an anisole nucleophile afforded undesired O-debenzylation selectively whereas stronger acids such as sulphuric or triflic acid removed both protecting groups. Selective PMB debenylation was only achieved when the compound was heated in TFA by microwave radiation in a sealed vessel, these conditions were not 100 % selective against O-debenzylation, but after optimisation, 70 °C for 3.5 hours were found to be the ideal conditions to produce the desired product (**29**) in a reasonable yield, which can then be separated from the side product and starting material by column chromatography.

3.2.2 7-Selective Alkylation

Standard alkylation conditions using an alkyl halide and base resulted in alkylation at the 9-position as well as the 7-position in ratios ranging from 1:1 to 9:1 depending on solvent and base. Mitsunobu conditions however gave 100 % selectivity for the 7-position, the position of the substituent was confirmed using hetero nuclear multiple bond correlation (HMBC) NMR (figure 20).

HMBC is used to detect correlations between different atoms in a structure through multiple bonds. This can be used to demonstrate the adjacency of certain atoms in a molecule for the purposes of structural elucidation.^[121] The HMBC NMR spectrum of compound **23j** (which was the first compound to be synthesised and isolated using the Mitsunobu method) was used to demonstrate the introduction of the substituent at the desired 7-position rather than the 9-position.

Figure 19 shows the structure of the compound with the relevant carbon atoms labelled. Carbon **A**, which is part of the added substituent appears at 47.1 ppm on a ¹³C NMR spectrum. The protons attached to carbon **A** are observed as a triplet at 4.51 ppm by ¹H NMR. On the HMBC spectrum the proton peak corresponding to carbon **A** correlates to both carbon **B** which appears at 106.4 ppm (correlation shown in blue, figure 20) and carbon **C** which appears at 141.6 ppm (correlation shown in red, figure 20). No correlation can be observed between carbon **A** and carbon **D** which appears at 149.4 ppm. If the substituent had reacted at the 9-position rather than the 7-position, carbon **A** would be two bonds away from carbon **D**. The protons on carbon **A** would then be likely show a correlation carbon **D**. A similar such correlation is however observed between the carbon **D** and the protons on carbon **E** which

appear as a triplet at 4.20 ppm (correlation shown in black, figure 20). The protons of carbon **E** also lack correlation to carbon **B**.

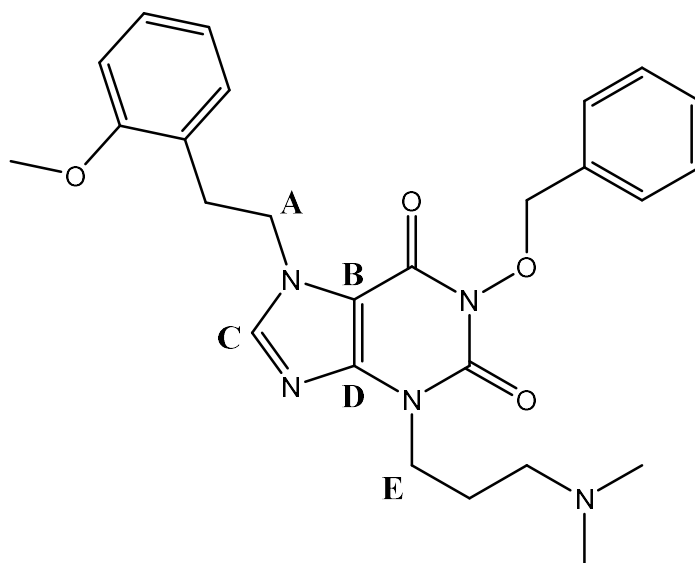


Figure. 19 Structure of **23j** with relevant carbons labelled.

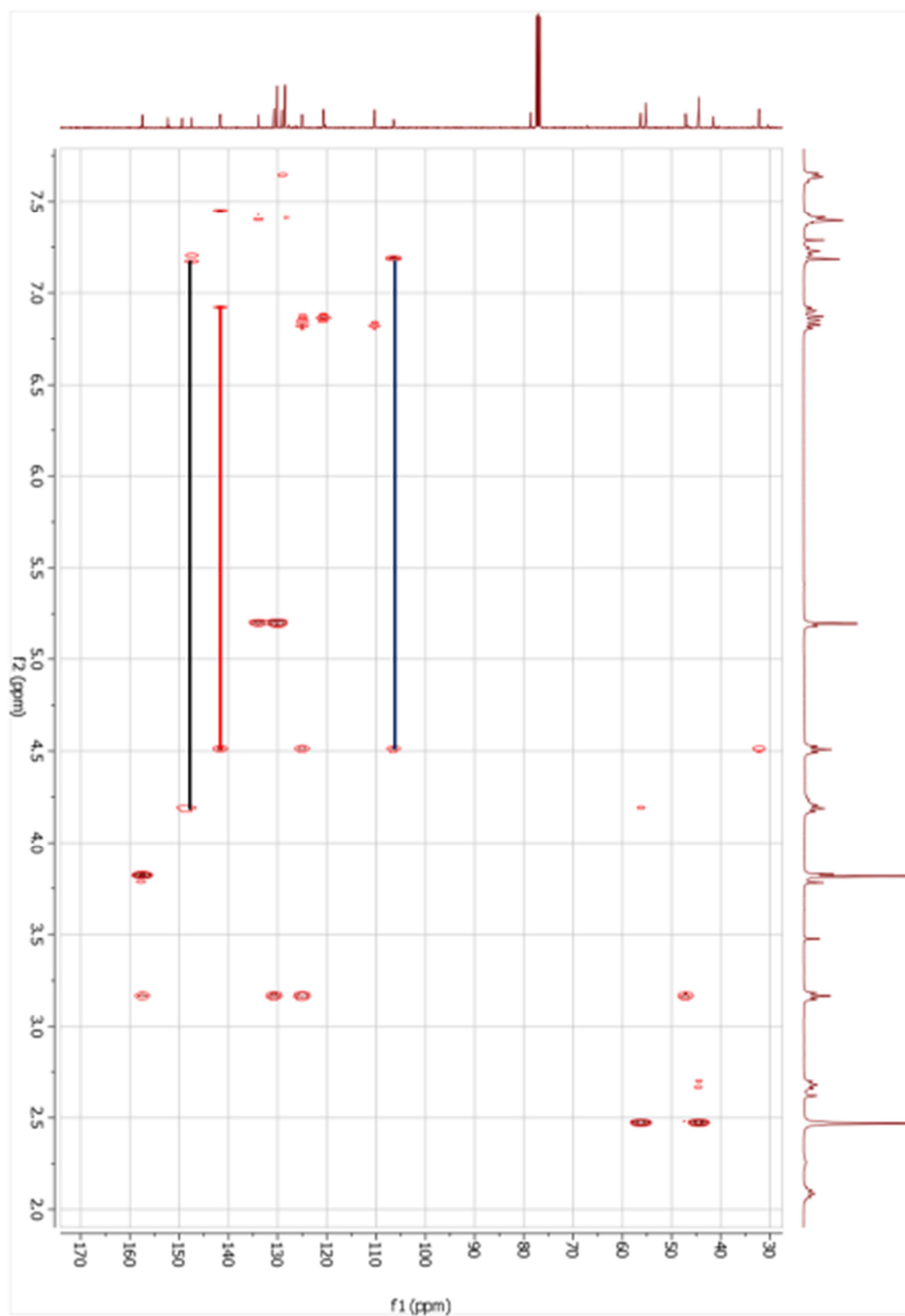
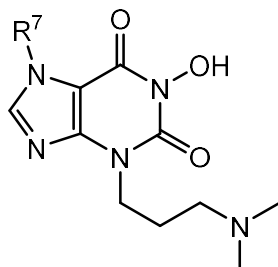


Figure. 20 HMBC NMR spectrum of compound **23j** with specific heteronuclear correlations highlighted.

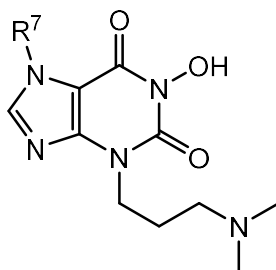
3.3 Pharmacological Data

Table. 10 7-substituted compound IC₅₀ values and cLogP values (calculated using chemdraw). *IC₅₀ data collected by FRET assay. **IC₅₀ data collected by AMP Glo assay.



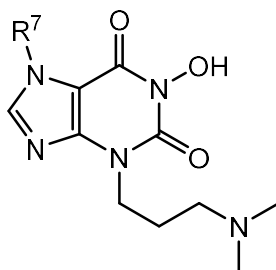
Compound	R ⁷	Caf1 IC ₅₀ (μM)	SEM (μM n=3)	cLogP
8j		3.30*	0.81	1.02
23a		12.28*	2.51	1.51
23b		9.54*	0.29	1.51
23c		5.84*	2.02	1.51
23d		2.69*	0.89	1.16
23e		8.44*	0.52	1.16
23f		13.07*	3.27	1.16
23g		4.77*	0.81	1.90
23h		5.36*	1.02	1.90
23i		7.18*	0.95	1.90
23j		12.22*	1.61	0.93
23k		0.71*	0.02	0.93

Table. 10 7-substituted compound IC₅₀ values and cLogP values (calculated using chemdraw). *IC₅₀ data collected by FRET assay. **IC₅₀ data collected by AMP Glo assay.



Compound	R ⁷	Caf1 IC ₅₀ (μM)	SEM (μM n=3)	cLogP
23l		26.11*	2.79	0.93
23m		5.21*	0.78	0.35
23n		5.67*	0.50	1.46
23o		1.23*	0.23	3.11
23p		1.79**	0.04	1.73
23q		2.36**	0.43	1.73
23r		3.22**	0.78	-0.21
23s		1.54**	0.23	0.76
23t		5.66*	1.01	1.23
23u		10.96**	1.42	1.30
23v		3.55*	0.57	1.02
23w		3.09*	0.68	1.22

Table. 10 7-substituted compound IC₅₀ values and cLogP values (calculated using chemdraw). *IC₅₀ data collected by FRET assay. **IC₅₀ data collected by AMP Glo assay.



Compound	R ⁷	Caf1 IC ₅₀ (μM)	SEM (μM n=3)	cLogP
23x		16.56**	0.70	1.32
23y		15.79**	1.86	1.41
23z		3.94**	2.00	1.45
23aa		4.89**	1.28	0.96
23ab		11.76*	0.28	2.19
23ac		5.76*	0.87	2.19
23ad		5.27**	0.56	1.22
23ae		45.82**	9.01	1.54
23af		20.88**	4.08	0.42
23ag		-**	-	-0.71
23ah	-H	-**	-	-1.10

3.4 Structure Activity Relationships

3.4.1 Benzene Ring Substituents

Two strategies were initially employed to probe the SAR of simple substitution on the benzene ring and consequently support or discourage the proposed binding hypothesis. The Topliss decision tree ^[122] was followed with the aim of discovering high potency compounds. A more generalised set of 2,

3 or 4 ring substituted compounds of various electronic characteristics were prepared to test the effects of electron donation/withdrawal.

The Topliss tree is a flowchart designed by John G Topliss to find the optimum substitution on a benzene ring as early as possible whilst minimising the synthesis of unsuccessful compounds. ^[122] This is achieved using the Hansch method ^[123] of using a small group of compounds to identify how the electronic and steric parameters affect the SAR. ^[124]

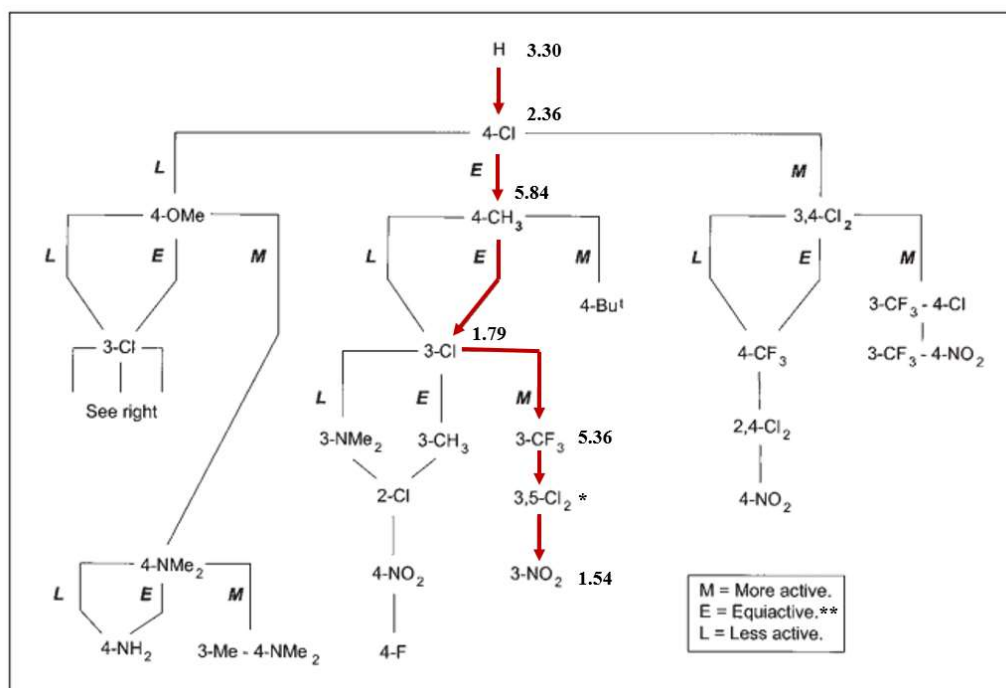


Figure. 21 Topliss decision tree for aromatic substituents ^[122] with route highlighted in red and IC₅₀ of relevant compounds displayed in μM . * compound not synthesised as the dichloro moiety was unstable to both hydrogenation and acid mediated benzyl deprotection. ** compounds are considered equiactive if the IC₅₀ is within two-fold range.

The Topliss tree resulted in compound **23s**, 3-nitro was one of the most beneficial ring substituents, showcasing the success of the Topliss tree method. The success of the nitro group which is strongly electron withdrawing, and the progression down the electron withdrawing substituent route was a surprising outcome. It was hypothesised that the cation- π interaction between the benzene ring and enzymatic magnesium would be strengthened by electron donating groups and weakened by electron withdrawing groups. Therefore, it was expected that the Topliss experiment was predicted to result in a more electron rich ring rather than an electron poor one as substituent electronics have been shown to influence cation- π bond

strength. ^[124] This outcome did not support the cation- π binding hypothesis as a strongly electron withdrawing group such as a nitro would be expected to reduce potency. ^[125]

3.4.2 Benzene Ring Electronics

The main finding from the ring substituted compounds was that the expected positive correlation between ring electron density and potency was not observed. Most substituents decreased potency somewhat but electron withdrawing groups did not necessarily perform worse than electron donating groups. Strongly electron withdrawing CF_3 groups (**23g**), (**23h**) and (**23i**), caused a modest decrease in potency at each position but weakly electron withdrawing groups had more mixed results. Fluorine at the 2-position (**23d**) gave a mild increase in potency as did 3-chloro (**23p**) and 4-chloro (**23q**). Fluorine(s) at other positions such as 3 (**23e**), 4 (**23f**), 3,4 (**23t**) or 2,5 (**23u**) all reduced the potency. In addition to the highly potent compound **23s** with a strongly electron withdrawing nitro group, no immediate relationship can be seen between electronics and potency.

The same outcome was found for groups of an electron donating nature. A (weakly electron donating) methyl group at the 2, 3 or 4 position (**23a**), (**23b**) and (**23c**) decreased potency. Additionally, a (strongly electron donating) methoxy group proved even less beneficial at the 2 or 4-position (**23j**) and (**23l**) but greatly increased potency at the 3-position (**23k**). **23k** was the most potent of the ring substituted compounds although the reason for this increase in potency is not clear. The homologous 3-ethoxy (**23m**) and 3-hydroxy (**23n**) compounds both had ten-fold higher IC_{50} s. 3,5-methoxy di-substitution (**23v**) showed no improvement over the lead (**8j**) and neither did the use of a (more strongly electron donating) amine group (**23r**). The unique potency of **8j** implies that the 3-methoxy group increases binding by a specific interaction rather than increasing ring electron density.

The overall relationship between potency and electronics can be observed by plotting pIC_{50} against the Hammett substituent constant of the ring substituent. The Hammett substituent constant is a measure of the polarisation exerted on a benzene ring by a substituent. ^[126] Because the Hammett substituent constants are determined by change in rate of reaction at the *meso*-position, *meta* and *para* substituents constants have different values due to the position's different mesomeric directing effects. Consequently, the substituents effects on the *meso*-position are not identical to their effects on cation- π bonding. The *para* substituent constant is more influence by the mesomeric (π) effect than the

meta substituent constant. The positive relationship between electron donation into the π system and cation- π bond strength has been demonstrated.^[127]

However, it has been shown that the Hammett substituent constants for the *meta* position, which are dominated by inductive (σ) effects better represent the substituent's effects on cation- π bond strength in all but the most powerful π -donating/accepting substituents.^{[128][129]}

Nevertheless, neither *meta* or *para* substituent constants showed any significant correlation with potency. This observation further discredits the cation- π bond and implies that the benzene ring must be involved in some other interaction.

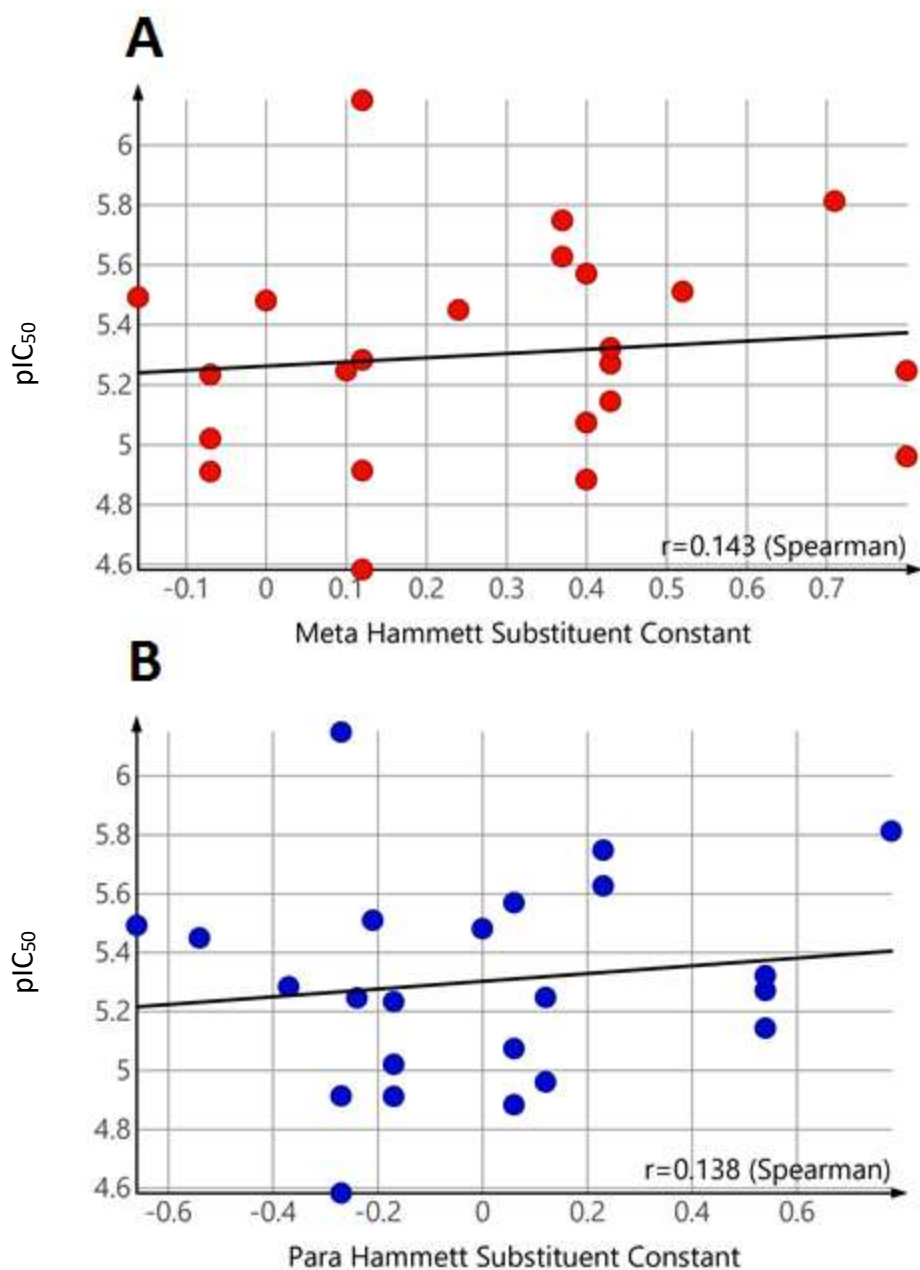


Figure. 22 (A)(red) pIC_{50} vs meta Hammett substituent constants. (B)(blue) pIC_{50} vs para Hammett substituent constants. Correlations calculated using Spearman's rank correlation coefficient. Hammett substituent constants for di-substituted rings were combined additively. Reference for Hammett substituent constant values used. ^[130]

3.4.3 Alternative Aromatic Rings and Steric Bulk on the 7-Substituent

Several alternative cyclic systems were tested on the 7-substituent in the place of benzene. These compounds were designed to further examine the nature of the interaction that this substituent is involved in.

Aromatic systems that are more electron rich than benzene would be expected to form stronger cation- π interactions. This hypothesis was supported by previous work in which more electron poor rings (pyridine and thiophene) in place of benzene were shown to reduce potency.^[97] This prediction was again, not observed in the cases of indole (**23ad**) and naphthalene (**23ab**) and (**23ac**), all of which gave a decrease in potency. These findings could potentially be explained by the steric bulk of the indole and naphthalene rings compared to the benzene ring. It is clear that the sterics have some influence on binding as the 1-ethylnaphthalene substituent (**23ac**) had double the activity of the 2-ethylnaphthalene substituent (**23ab**). However, overall there was no obvious relationship between steric bulk around the 7-substituent and potency. The substituted phenyl ring containing compounds can have their steric bulk quantified using the Taft substituent steric parameter. The Taft substituent steric parameter is a measure of a substituent's steric bulk.^[131] When the Taft steric parameters for the substituents on the benzene rings are plotted against the corresponding compounds pIC₅₀s, no relationship is observed between steric bulk and potency. This analysis along with the high potency of the 3-phenoxy compound (**23o**) show that steric bulk at the 7-substituent does not necessarily reduce binding.

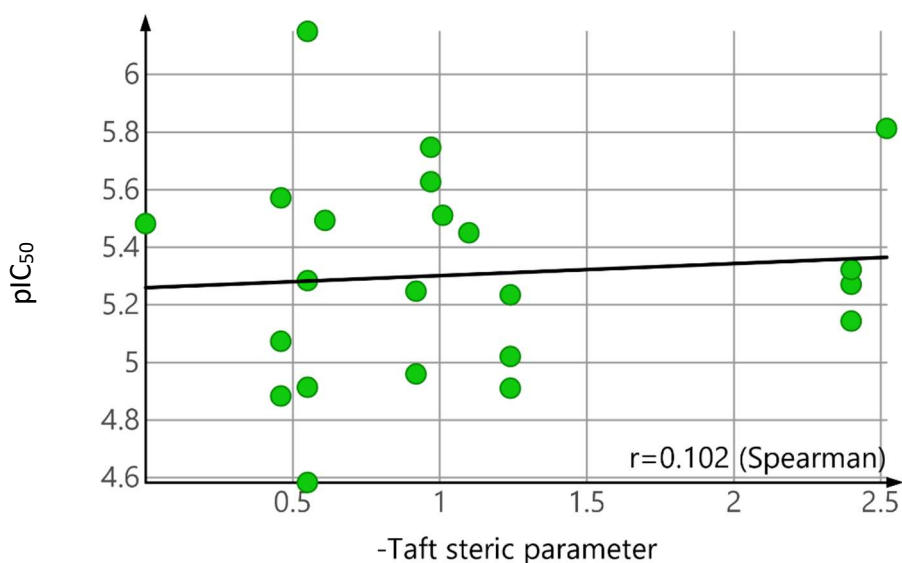


Figure. 23 pIC_{50} vs -Taft steric parameter. Taft steric parameters are negative values, they are here converted into positive values. Correlations calculated using Spearman's rank correlation coefficient. Taft substituent steric parameter for di-substituted rings were combined additively. Reference for Taft substituent steric parameter values used. ^[130]

3.4.4 Aliphatic Rings on the 7-Substituent

When the benzene ring was replaced with a cyclopentane (**23ae**), the potency was reduced 15-fold but not eliminated entirely, as it is when the 7-substituent is removed completely (**23ah**). Cyclopentane was chosen as a benzene bioisostere because it has a comparable size and lipophilicity but is saturated so can not form a cation- π interaction. This result shows that a saturated, unsubstituted substituent still contributes towards, and facilitates binding and therefore likely fits into a lipophilic pocket in the active site. When the benzene is replaced with a cyclopropane (**23af**), the potency is reduced by only 7-fold, a significantly smaller drop off than that observed with cyclopentane. The cyclopropane ring, whilst saturated has some aromatic character due to the C-C σ bonds having some degree of π character. ^[132] This shows a positive relationship between binding and aromaticity as the benzene ring had the strongest binding, the cyclopentane ring had the weakest and the cyclopropane was somewhere in the middle. If these compounds share the same binding pose, the position of the 7-substituent must be one in which can form strong lipophilic interactions, but also benefit from the presence of a π -system. This means the aromatic ring likely fits into a lipophilic pocket in the

active site that contains some aromatic residues that provide π - π stacking interactions.

3.4.5 Glide Docking and New Binding Pose

The results from the new generation of 7-substituted compounds were not consistent with the previously proposed binding pose.^[97] Using the observed SARs and Glide Docking, a new binding pose was proposed. Glide docking is a computational method of predicting the 3D orientation, and strength of an intramolecular interaction such as the binding of a small molecule ligand to a protein.^{[133][134][135]} Glide docking was used to predict the binding pose of 1-hydroxy-xanthine compounds to Caf1. Constraints were utilised to force the *N*-hydroxyimide moiety to chelate the magnesiums in a bidentate fashion where each magnesium is coordinated to by two oxygens (figure 24), similarly to that seen in X-ray crystal structures of *N*-hydroxyimide-FEN1 complexes.^[89]

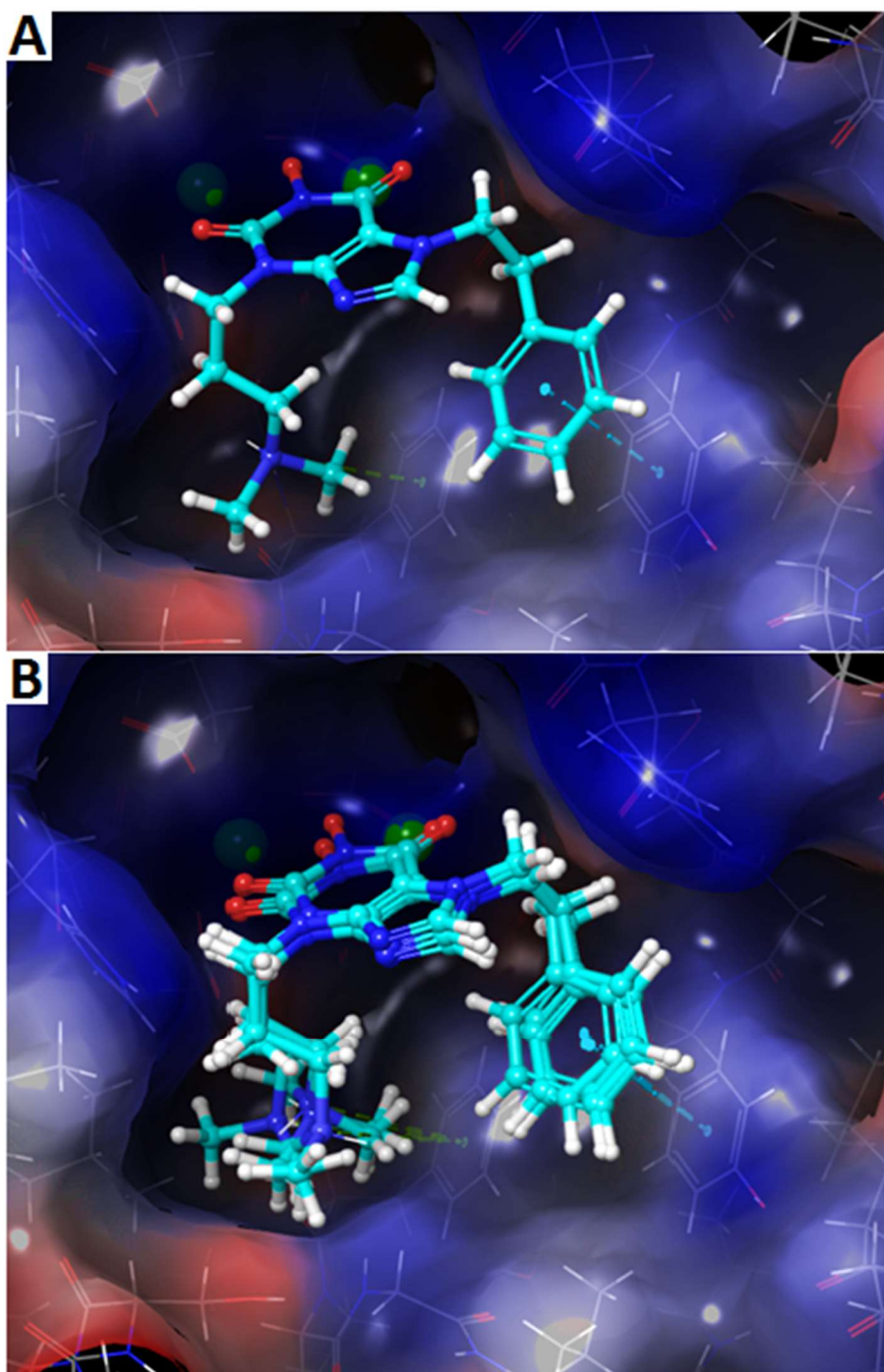


Figure. 24 (A) proposed binding mode generated using Schrödinger suite. The top-rated binding pose. (B) an overlay of the five highest rated poses, all of which are very similar. ^{[54][136]}

This new binding mode involves bidentate coordination to each magnesium (green) and the benzene ring of the 7-substituent π - π stacking with Tyr¹⁶⁰ (turquoise dashed line). The protonated tertiary amine group of the 3-substituent is thought to hydrogen bond (yellow dashed line) to the backbone Phe⁴³. A cation- π interaction also appears to be possible between Phe⁴³ and the

positively protonated amine (green dashed line). The hydrogen bond has been shown to be more essential than the charge as the potency is reduced tenfold when the amine is quaternised (**16h**).

This pose can also explain the high potency of certain compounds such as **23s**, **23o** and **23k** due to the substituent on the benzene ring potentially forming a hydrogen bond to the hydroxy group of the tyrosine.

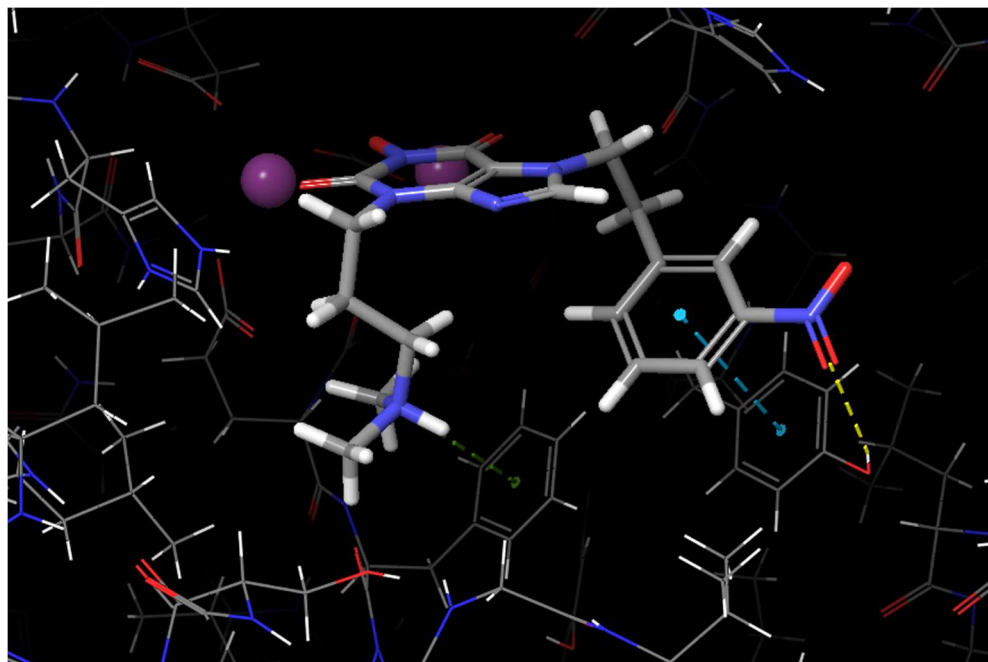


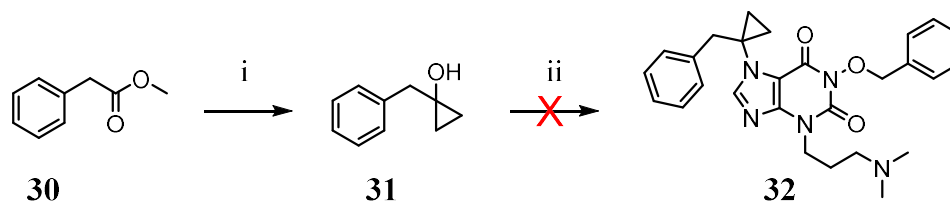
Figure. 25 Computationally predicted pose of compound (**23s**) with the nitro substituent accepting a hydrogen bond from Phe⁴³ (yellow dashed line). Also noteworthy is the π - π stacking interaction between the 7-substituent and Phe⁴³.
[54][136]

3.4.6 Conformational Restraint

Like the 3-substituent, conformational restraint was explored as a strategy for increasing potency. The simplest method of achieving this was methyl substitution at each position on the 2-carbon chain giving compounds (**23x**) and (**23y**). Methyl substitution on carbon linkers has been shown to greatly increase potency in up to as many as 8 % of cases by influencing the chain conformation and favouring a conformation complimentary to the active site.
[137][138]

This effect can also be achieved with a cyclopropane ring. [139][140] A cyclopropane alpha to the benzene ring on the 7-substituent was synthesised from the commercially available corresponding alcohol to yield (**23z**). However, the beta spirocyclopropane substituted phenethyl alcohol had to be

synthesised using the Kulinkovich reaction.^[141] The resulting tertiary alcohol however was not successfully attached to the scaffold using Mitsunobu conditions without causing cyclopropane ring opening, even when specialised conditions suited to tertiary alcohols were used.^{[142][143]}



Scheme. 6 Unsuccessful route to the compound containing a spirocyclopropane ring beta to the benzene ring on the 7-substituent. Reagents and conditions: i) Titanium isopropoxide, ethyl magnesium bromide, THF, 89 %. ii) phenoxy diphenylphosphine, 2,6-dimethylcyclohexa-2,5-diene-1,4-dione, toluene, 0 %.

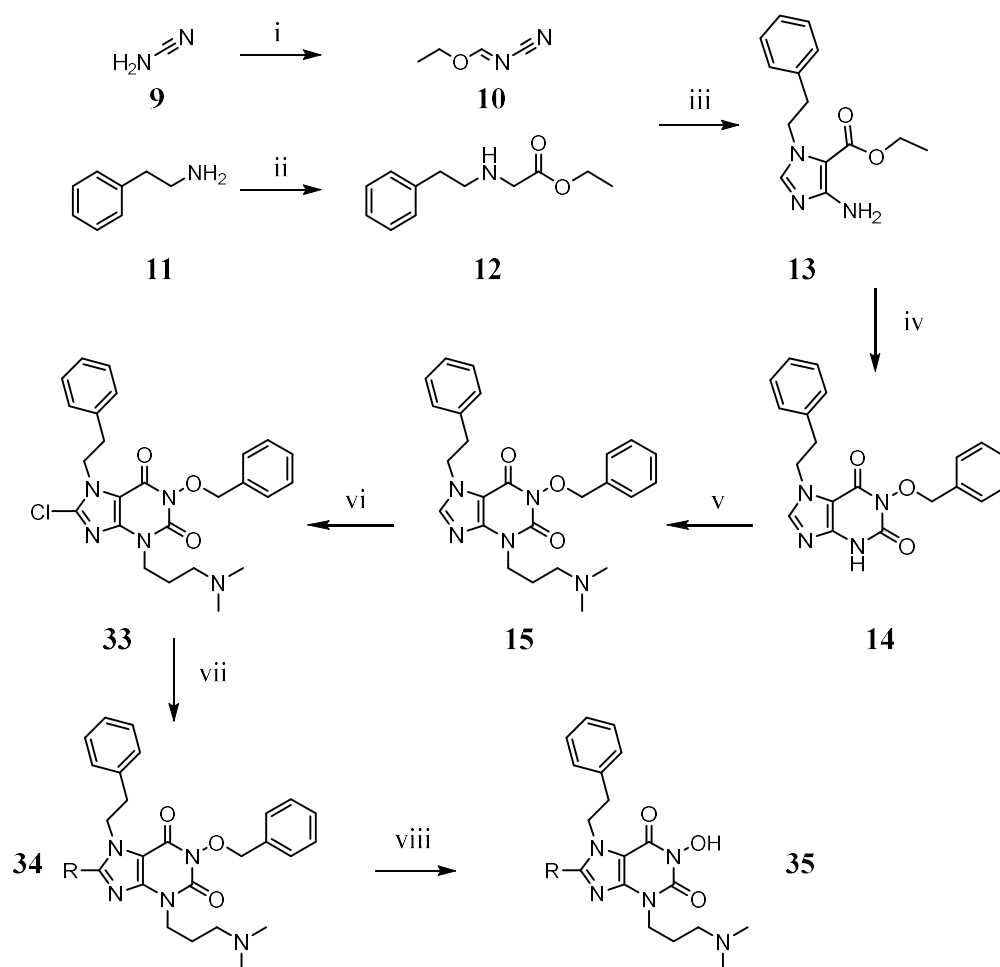
Another compound was made with a cyclobutene ring fused to the benzene ring and carbon linker (**23aa**), this fused ring system reduces the number of rotatable bonds and therefore conformational degrees of freedom. Therefore, reducing the number of possible non-bioactive conformations and the entropic penalty incurred through the compound existing in the biologically active conformation rather than the global minimum energy conformation.^[144]

Unfortunately, none of these strategies were successful in increasing potency, this also does not give any information as to the nature of the binding pose as conformational restraints could be beneficial to either pose.

4. Derivatisation at the 8-Position and Synergistic Derivatisation

4.1 Synthetic Route to 8-Substituted Compounds

It was discovered that the 8-position of the 1-hydroxy xanthine scaffold could be chlorinated using *N*-chloro succinimide. This is a method that is known for other xanthines.^[145] The 8-chloro position on the scaffold then acts as a useful site of reactivity to further functionalise the compound by nucleophilic aromatic substitution.



Scheme. 7 Synthetic route to 8-substituted analogues. Reagents and conditions: i) triethyl orthoformate, reflux, 73 %. ii) ethyl 2-bromoacetate, CHCl_3 , 0 °C, 70 %. iii) 1) THF, reflux. 2) potassium *tert*-butoxide, EtOH, reflux, 79%. iv) 1) carbonyl diimidazole, PhMe reflux. 2) O-benzyl hydroxylamine hydrochloride, triethylamine, PhMe, reflux. 3) sodium hydride, NMP, 80 °C, 83 %. v) dimethylamino propyl chloride HCl, caesium carbonate, DMF, 80 °C, 30%-95%. vi) *N*-chloro succinimide, THF, 75 %. vii) various conditions*, 77-100 %. viii) hydrogen (1 atm), Pd/C, MeOH, DCM, rt, 19% - 81%.

The synthetic pathway was the same as that used for the 3-substituted series until the chlorination step takes place at intermediate **15** (step vi scheme 7). Chlorination proceeded in good yields and the only purification necessary was partitioning between EtOAc and aqueous 1 molar sodium hydroxide solution to wash out the succinimide side product. This method of purification is not possible if the chlorination takes place prior to the 3-alkylation step (scheme 7). The exposed proton at the 3-position is somewhat acidic and can be deprotonated giving negative charge to the scaffold, solubilising it in the

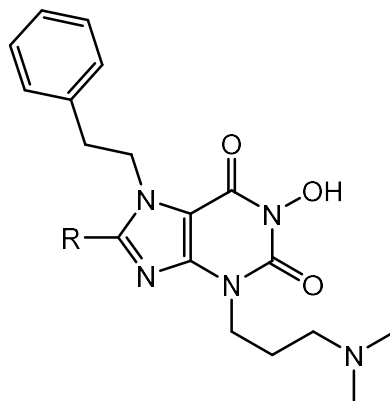
aqueous layer rather than the organic. If the chlorination takes place on intermediate **14**, whilst the reaction is successful, column chromatography is necessary as a method of purification.

*secondary and tertiary amine substituents were added using the corresponding amine freebase in methanol or ethanol. ^[146] The primary amine was introduced using sodium azide in DMF, the azide was then reduced to the primary amine during the subsequent hydrogenation. The phenolic ether was introduced using phenol and caesium carbonate in DMF.

In the case of 8-amino compounds the catalytic hydrogenation caused some removal of the amine group. Fortunately, the amine was cleaved slower than the benzyl protecting group. This necessitated as short reaction times as possible to maximise yields. This means that the corresponding 8-unsubstituted compound was formed as a side product and the 8-amino compounds had to be purified using reverse phase column chromatography or HPLC, depending on the difference in elution time between the two compounds. Consequently, percentage yields for the 8-amino compounds were often very low. This side reaction was not observed with the 8-phenolic ether compound (**35f**).

4.2 Pharmacological Data

Table. 11 8-substituted compounds IC₅₀ values, and cLogP values (calculated by chemdraw). Data collected by AMP-Glo assay



Compound	R ⁸	Caf1 IC ₅₀ (μM)	SEM (μM n=3)	cLogP
35a		1.53	0.24	0.45
35b		0.65	0.12	1.03
35c		1.85	0.41	1.62
35d		1.01	0.17	2.04
35e		1.98	0.18	1.63
35f		2.27	0.42	2.27

4.3 Structure Activity Relationships

Substitution at the 8-position proved very beneficial. Compound **35b**, with a dimethyl amine at the 8-position was the most potent compound prepared, and to the best of our knowledge the most potent small molecule Caf1 inhibitor to date. Other similar substituents also resulted in potent compounds. All the other amine substituents were in a similar area of high potency.

According to the proposed binding mode, this section of the molecule does not interact with the active site but protrudes into the solvent. Consequently, it is hypothesised that the substituent contributes towards binding by influencing the electronic character of the xanthine ring and providing steric bulk that restrains the rotation of the 7-substituent, reducing its conformational degrees of freedom (shown in figure 26). An electron donating group such as an amine would increase the electron density of the xanthine ring system and increase the negative charge of the coordinating oxygens, thus strengthening their interaction with the positively charged magnesium. The pKa of the 1-hydroxy group on the lead compound with no 8-substituent was calculated to be 5.20. ^[147] The amine substituents at the 8-position were all calculated to increase the pKa to 5.23 from 5.20. This represents a 7 % ^[148] increase to the ability of the negatively ionised 1-hydroxy groups tendency to attract positive charge. This electronic effect may be very small/negligible so is likely to be only partially responsible for the increased potency. It is also hypothesised using molecular modelling that the 8-substituent blocks the free rotation of the 7-substituent, reducing its conformational degrees of freedom and consequently the entropy loss of binding. This would explain the lower potency of less bulky primary, (**35a**) and secondary (**35e**) amine substituted compounds. The pyrrolidine substituent present in **35c**, may also be less effective at blocking rotation than the piperidine substituent in **35d** due to the reduced C-N-C bond angle and consequential reduction in steric bulk in pyrrolidine (105.2°) compared to piperidine (109.8°). ^[149] The predicted binding pose also suggests that intramolecular lipophilic forces reward the bioactive pose.

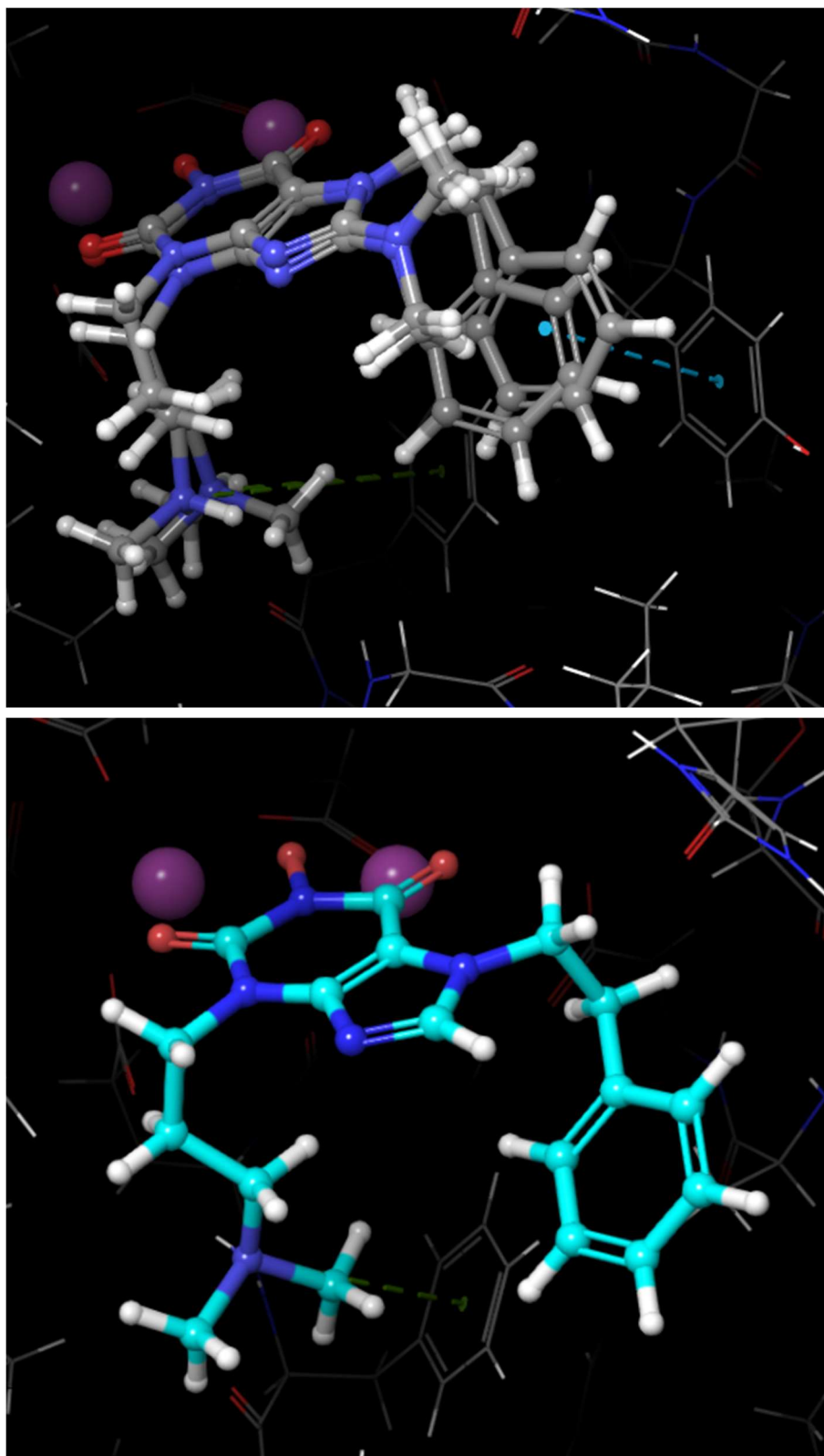


Figure. 26 Overlay of two very similar computationally predicted binding poses for **35b** (top) compared to that of the lead compound (**8j**) (bottom). In the predicted **35b** pose the 8-dimethylamino substituent is extending out of the active site and restricting the rotation of the 7-substituent. ^{[54][136]}

4.4 Unsuccessful 8-Substitution

The series of 8-substituted compounds was the most successful at increasing potency. This made the 8-position a highly desirable source of derivatisation. The only functional groups utilised at the 8-position were amines and a phenolic ether which were incorporated by nucleophilic aromatic substitution. A more diverse range of substituents would be of value as they would answer important SAR questions. The following paragraphs will describe a range of reactions that were used to attempt to attach other functional groups at this position, however none proved successful.

4.4.1 Halogenation

The effects of the electron density of the xanthine ring could be examined by placing an electron withdrawing group at the 8-position. There is a strong precedent for lithiation of purine-based ring systems at this position as well as subsequent reaction with various electrophiles.^[150] One such example was lithiation followed by fluorination using *N*-fluorobenzenesulfonimide (NFSI).^[151] Fluorination proved unsuccessful so an 8-chloro substituted compound was synthesised by hydrogenation of **33**.

However, this 8-chlorinated compound was unstable once the benzyl protecting group was removed from the 1-hydroxy position. The isolated compound decomposed when exposed to aqueous or methanolic environment making chemical and biological characterisation impossible. This implies that good leaving groups are not appropriate at this position, therefore further attention was directed only towards carbon-carbon bond formation at this position.

4.4.2 Metal Catalysed Reactions

Various metal catalysed reactions were attempted to install aromatic rings at the 8-position to form a bi-aryl system. This was a desirable outcome because the additional aromatic ring would not only perform the same steric function as the amine group but also serve as a potential site for further derivatisation.

A variety of methods were used to try to couple a benzene group to the xanthine ring. Suzuki coupling using 8-chloro xanthines is known but yields are low, and negatively influenced by substituents.^[152] Yields can be improved by using the an 8-bromo xanthine instead of 8-chloro,^[152] so after the Suzuki reaction was unsuccessful using intermediate **33**, it was attempted to synthesise the bromo analogue. Known methods of xanthine bromination

such as *N*-bromo succinimide ^[153] or elemental bromine ^[154] were also unsuccessful for this scaffold as they caused debenzylation and undesired bromination of benzene rings respectively. Several other organometallic reactions with **33** were unsuccessfully attempted, including nucleophilic reaction with phenyl lithium, Grignard reaction with phenyl magnesium bromide, and Kumada coupling. ^[155]

4.4.3 Radical Methods

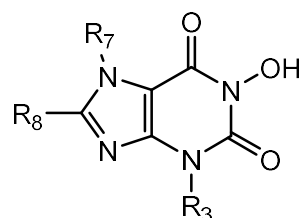
Reactions utilising free radicals have been used to introduce substituents at the xanthine 8-position. ^{[156][157]} Two particularly attractive radical methods were attempted. The first such method was a trifluoromethylation using zinc trifluoromethanesulphonate, ^[158] and the other was an isopropylation. ^[159] The trifluoromethyl group is a desirable 8-substituent as it is strongly electron withdrawing so would provide information as to the effects of the xanthine ring electronics on potency, and is a poor leaving group so is less likely to suffer from stability issues. An isopropyl substituent would also give valuable information on the importance of the steric and electronic contributions of the 8-substituent. An 8-isopropyl compound would be homologues to the 8-dimethylamino compound (**35b**) and would elucidate the relative importance of the 8-substituent's steric and electronic effects on potency.

4.5 Synergistic Derivatisation

Some beneficial substituents were examined in combination to test for synergistic effects and if increases in potency could be combined to yield more potent compounds. It was found that substituents that increased potency, when used in combination, did not combine their beneficial effects.

4.5.1 Pharmacological Data

Table. 12 multiply substituted compound IC₅₀ values and cLogP values (calculated by chemdraw). *IC₅₀ data collected by FRET assay. **IC₅₀ data collected by AMP Glo assay.



Compound	R ₃	R ₇	R ₈	Caf1 IC ₅₀ (μM)	SEM (μM n=3)	cLogP
36			-H	2.35*	0.54	2.21
37			-H	1.87*	0.07	1.99
38				2.98**	0.50	2.93

The propyl diethylamine substituent at the 3-position was the only 3-substituent to lower the IC₅₀ in the presence of the lead molecule's phenethylamine substituent at the 7-position. This substituent however was not as beneficial when combined with other 7-substituents. **36** with a 2-fluoro on its 7-substituent had a very similar IC₅₀ to its propyl dimethylamino homologue (**23d**) and (**37**) with a 3-methoxy on its 7-substituent had a higher IC₅₀ than its propyl dimethylamino homologue (**23k**). The 8-dimethylamino substituent was demonstrated to not have the same beneficial effect on potency when the 7-substituent was different. The presence of the fluorine in compound **38** seemed to prevent the 8-dimethylamino substituent from having the same positive impact as it does in the absence of the fluorine (**35b**).

This discrepancy between the benefit to potency afforded by the 3 or 8-substituents depending on the nature of the 7-substituent implies the possibility of multiple bioactive binding modes which are favoured or disfavoured by compound variation. These poses would likely all include chelation of the magnesium ions but differ in the interactions made between the substituents and the active site.

4.5.2 The 8-dimethylamino, 7-(3-methoxy phenethyl) Compound

The hypothetical ideal combination was of the two substituents most beneficial to potency. The compounds bearing the 8-dimethylamino (**16k**), and 7-(3-methoxyphenethyl) (**35b**) substituents and these two compounds had the highest potency of all. A compound bearing both of these substituents would be a highly desirable candidate for synthesis and was predicted to potentially be of high potency.

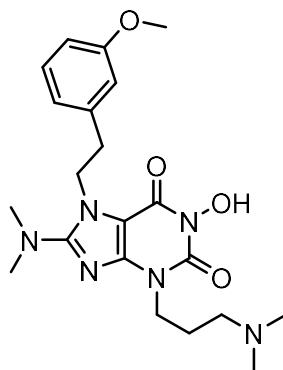
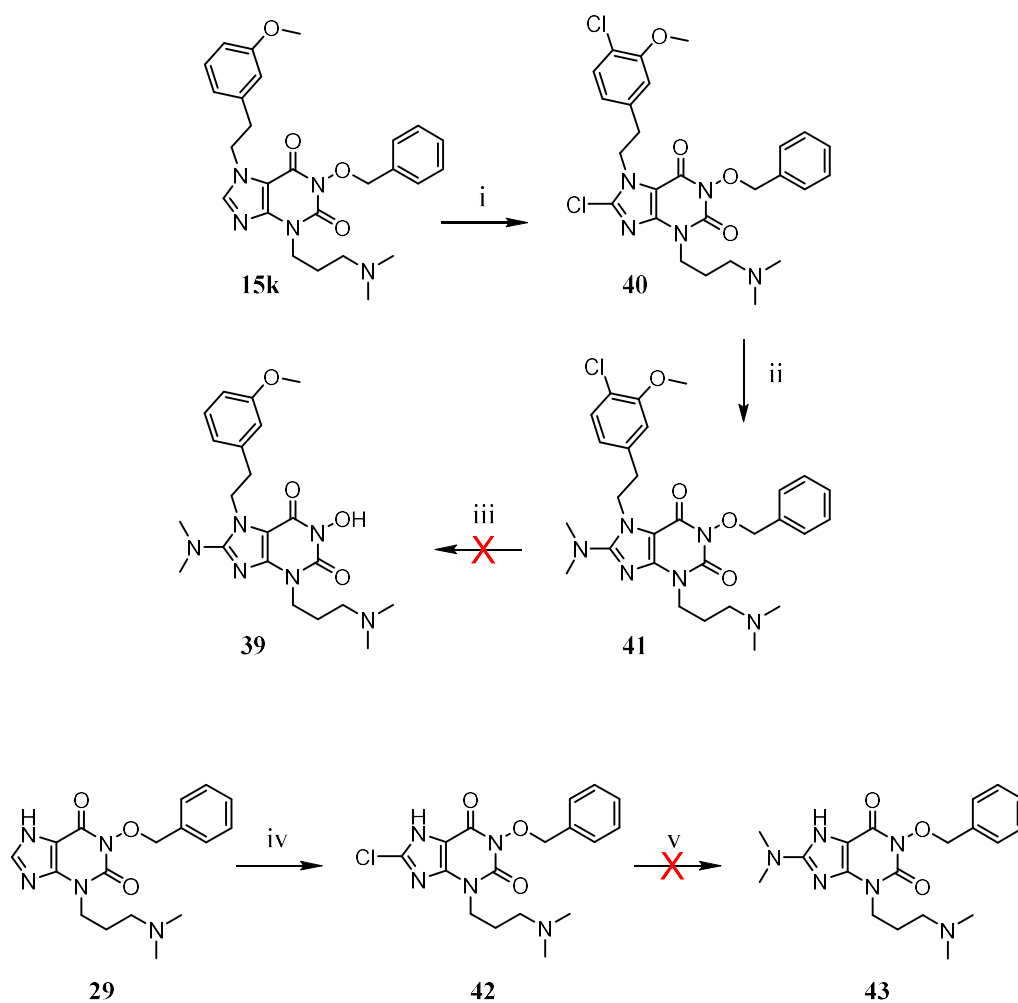


Figure. 27 Compound **39**

The synthetic pathway utilised to access the 8-substituted series was not successful for this compound. The chlorination reaction used to chlorinate the xanthine 8-position also chlorinated the 7-(3-methoxyphenethyl) substituent at the 4-position (scheme 8). An electron donating substituent on the benzene ring increases nucleophilicity causes reaction with the chlorine electrophile when the unsubstituted ring does not. The synthesis was continued with the plan that the extraneous chlorine would be removed during the hydrogenation step. The subsequent nucleophilic aromatic substitution proceeded as normal but the final hydrogenation deprotection step was not successful. The 8-amine substituent was labile to hydrogenation, and the C8-N bond was cleaved faster than the aryl chloride (scheme 8).

It was also attempted to attach the 8-substituent first, prior to the addition of the 7-substituent. The 7-unsubstituted precursor (**29**) was successfully chlorinated using *N*-chloro succinimide in acetonitrile at 100 °C under microwave radiation. The subsequent nucleophilic aromatic substitution using dimethylamine was not successful on the 7-unsubstituted 8-chloro xanthine **42** even when the reaction temperature was increased to reflux.



Scheme. 8 Synthetic routes to compound **37**. Reagents and conditions: i) *N*-chloro succinimide, THF, 68 %. ii) Dimethylamine, EtOH, 70 %. iii) hydrogen (1 atm), Pd/C, MeOH, DCM, rt, 0 %. iv) *N*-chloro succinimide, MeCN, 100 °C, microwave radiation, 57 %. v) Dimethylamine, EtOH, rt – reflux, 0 %.

Compound **39**, whilst still a highly desirable target for synthesis has yet to be successfully prepared.

5. Differential Scanning Fluorimetry

5.1 Thermal Shift Assays

Differential scanning fluorimetry (DSF) is a form of thermal shift assay that determines the thermal stability of an enzyme. The enzyme is incrementally heated in the presence of a lipophilic fluorescent dye, as the enzyme is thermally denatured, hydrophobic sites are exposed which bind to the lipophilic dye. The fluorescence of the dye is quenched by water so the exclusion of water caused by the dye binding to newly exposed lipophilic sites increases fluorescence. This means the fluorescence observed can be used to track the denaturation of the protein, the protein melting temperature (T_m) can be determined by the temperature which corresponds to half maximal fluorescence value. ^{[160][161][162]}

A protein complexed with a small molecule ligand with complimentary binding, such as a substrate, metal cofactor or competitive inhibitor adds additional stability to the protein. ^{[163][164]} The difference in melting temperature (ΔT_m) acts as a good approximation of the strength of the small molecules binding to the protein. ^[165] This method has been used as an assay to assess the potency of compounds for drug discovery purposes. ^{[166][167]}

DSF has been used in the past to demonstrate the binding of magnesium to CafI ($\Delta T_m = 3.90 \pm 0.19^\circ\text{C}$), and the binding of the lead compound (**8j**) to CafI in the presence of magnesium; ($\Delta T_m = 4.74 \pm 0.27^\circ\text{C}$). It was also shown that **8j** does not bind to CafI in the absence of magnesium, ($\Delta T_m = -0.21 \pm 0.25^\circ\text{C}$). ^[96]

5.2 Mutagenesis Studies for Analysis of Binding Mode

DSF studies on CafI mutants were used to examine the binding poses of a group of compounds and demonstrate the role of magnesium in their binding. Specific aromatic residues thought to be involved in compound binding were chosen to be substituted by alanine, thus removing their side chain.

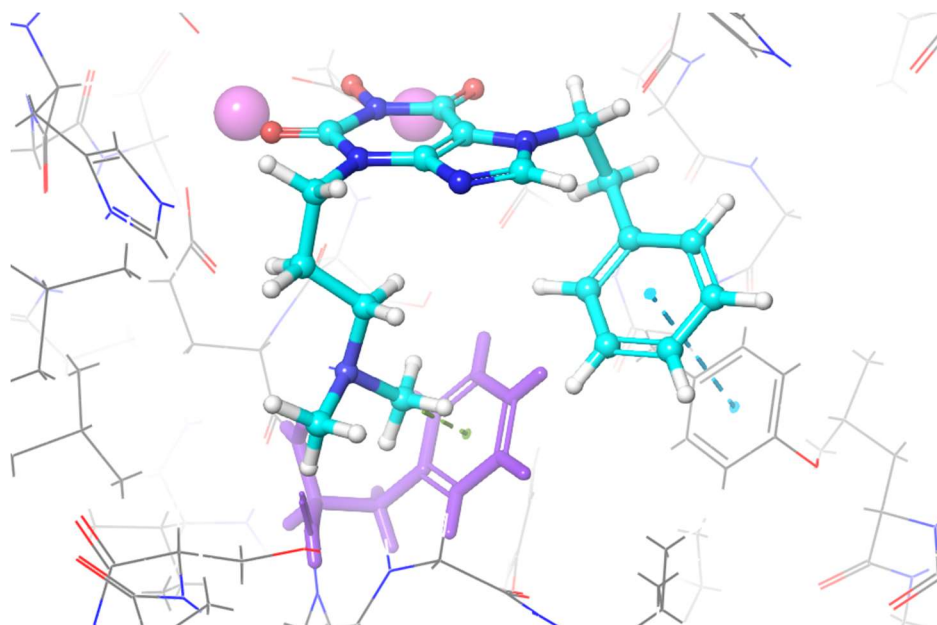


Figure. 28 Predicted binding pose of compound **8j** with Phe⁴³ highlighted in purple. [54][136]

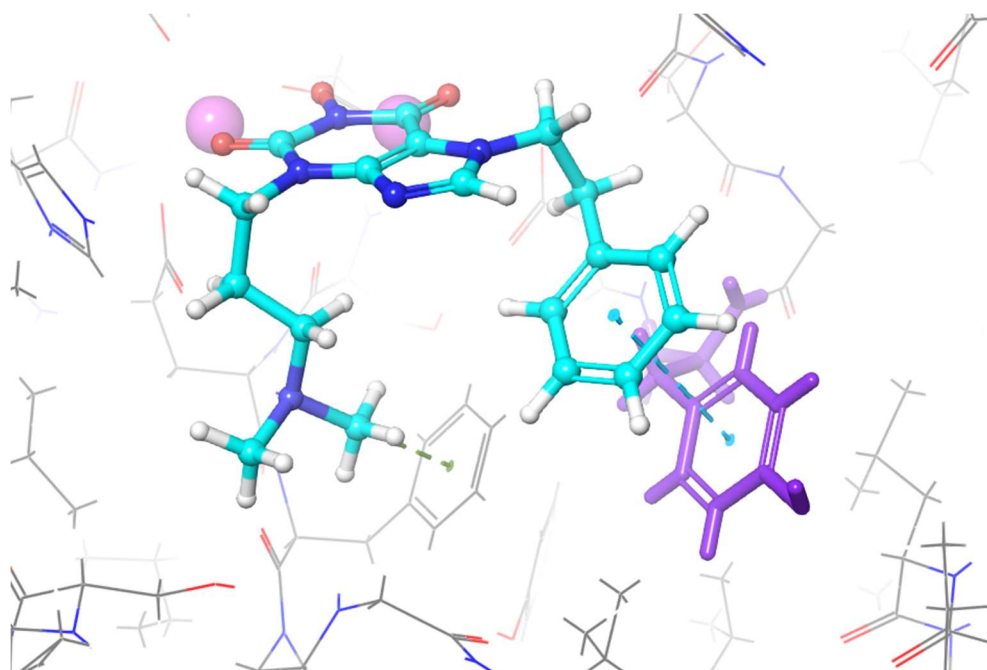


Figure. 29 Predicted binding pose of compound **8j** with Tyr¹⁶⁰ highlighted in purple. [54][136]

The importance of the selected residue to compound binding can be quantified by the difference in ΔT_m between a compound bound to the wild type CafI and between the CafI mutant. The importance of select residues to the binding of specific compounds was used to glean information about the binding pose.

The residues predicted to be involved in binding to the compound side chains were phenylalanine⁴³ and tyrosine¹⁶⁰ (figures 28 and 29).

Three CafI variants were prepared by Dr Sebastiaan Winkler. The first variant had Phe⁴³ substituted for alanine, the second had Tyr¹⁶⁰ substituted for alanine, and the final variant had both Phe⁴³ and Tyr¹⁶⁰ substituted for alanines. T_m values were determined for the wild type and each variant in both the presence and absence of magnesium (table 13). Also shown is the resulting ΔT_m of the binding of magnesium to each variant.

Table. 13 T_m values and SEM (n=3) for CafI mutants and wild type in both the presence, and absence of magnesium. ΔT_m Values for the binding of magnesium for CafI mutants and wild type. ΔT_m Values of mutation represent the loss in stability caused by the sequence mutation.

	Wild type	Phe ⁴³ -	Tyr ¹⁶⁰ -	Phe ⁴³ and Tyr ¹⁶⁰ -
T_m / °C Magnesium present	46.78 ± 0.17	46.36 ± 0.25	43.13 ± 0.18	44.29 ± 0.49
T_m / °C Magnesium absent	43.06 ± 0.63	42.83 ± 0.18	41.70 ± 0.49	42.36 ± 0.32
ΔT_m / °C	3.71 ± 0.78	3.53 ± 0.20	1.43 ± 0.66	1.93 ± 0.76

The observed ΔT_m value for the binding of magnesium to wild type CafI was consistent with the previously observed value. The wild type and Phe⁴³ - CafI enzymes appear to be similarly stabilised by complexing magnesium and have similar thermal stability in the absence of magnesium. Tyr¹⁶⁰ - and Phe⁴³Tyr¹⁶⁰ - are both afforded a smaller stabilisation by complexing magnesium and have a few degrees lower T_m in the absence of magnesium. This finding suggests that Tyr¹⁶⁰ is more important than Phe⁴³ to the structure of the protein and that Tyr¹⁶⁰ may be contribute to the binding of the magnesium ions. Each CafI mutant behaved similarly to the wild type in the DSF experiment and produced uniform melting curves of the expected shape (figure 30). This shows that the mutations did not remove the proteins ability to correctly fold, and to complex magnesium ions.

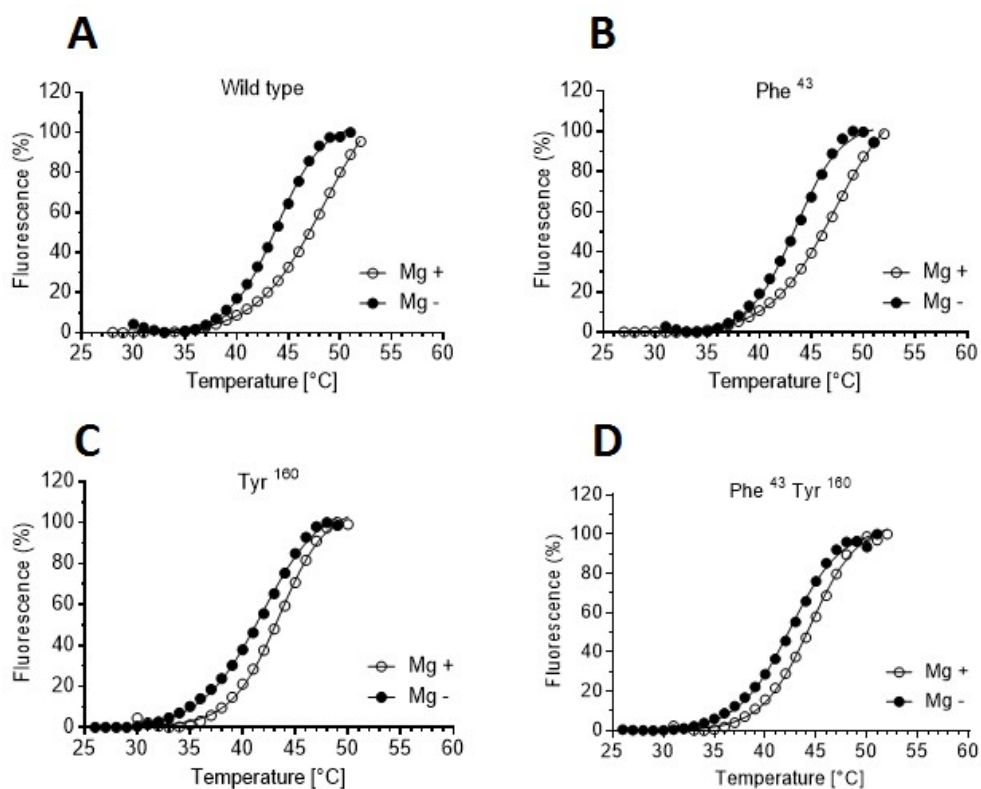


Figure. 30 Cafl mutant and wild type melting curves in the presence (white) and absence (black) of magnesium. Wild type (A), Phe⁴³ - (B), Tyr¹⁶⁰ - (C), Phe⁴³Tyr¹⁶⁰ - (D).

The variant Cafl proteins were shown to behave in a comparable manner to the wild type. The mutants were then compared to the wild type for their binding towards a 1-hydroxy xanthine inhibitor (**8j**). ΔT_m values for the protein binding to **8j** were calculated from the difference between the T_m in the presence and absence of **8j**. This was calculated for each protein in the presence, and absence of magnesium (table 14).

Table. 14 ΔT_m values and SEM (n=3) for the binding of **8j** to Caf1 mutants and wild type compared to with no compound in the presence, and absence of magnesium.

	Wild type	Phe ⁴³ -	Tyr ¹⁶⁰ -	Phe ⁴³ and Tyr ¹⁶⁰ -
(8j) ΔT_m / °C Magnesium present	4.11 ± 0.22	0.88 ± 0.18	2.43 ± 0.12	1.71 ± 0.42
(8j) ΔT_m / °C Magnesium absent	0.29 ± 0.14	-0.01 ± 0.21	-0.27 ± 0.17	-0.10 ± 0.11

The role of magnesium in each protein was demonstrated by the lack of any significant ΔT_m in the absence of magnesium. This shows that each of the proteins requires the magnesium to bind to **8j**.

The ΔT_m value for **8j** binding to the wild type, both with and without magnesium was consistent with previously observed values. In agreement with the proposed binding mode, the amino acid substitutions to the Caf1 active site negatively impacted the binding of the inhibitor when magnesium was present. This effect was the largest with the Phe⁴³ – mutant which was stabilised very little by **8j**. This suggests that the Phe⁴³ residue is strongly involved in the binding of **8j**. The Tyr¹⁶⁰ -mutant had a smaller, but still significant loss in ΔT_m compared to the wild type, which suggests that Tyr¹⁶⁰ is involved in binding to the inhibitor.

Once it had been demonstrated that the amino acid substitutions impact compound binding in agreement with the proposed binding mode, a diverse range of the 1-hydroxy xanthine compounds were tested using DSF to determine their ΔT_m values (table 15). The compounds were selected to represent a range of different functional groups and substituents, as well as a range of IC₅₀ values.

Table. 15 ΔT_m values and SEM (n=3) for the binding of a set of 1-hydroxy xanthine compounds. Compounds ΔT_m values were determined for the wild type Caf1 and each mutant in the presence of magnesium, as well as the wild type in the absence of magnesium.

Compound	Wild type $\Delta T_m / ^\circ\text{C}$	Phe ⁴³ – $\Delta T_m / ^\circ\text{C}$	Tyr ¹⁶⁰ – $\Delta T_m / ^\circ\text{C}$	Phe ⁴³ and Tyr ¹⁶⁰ – $\Delta T_m / ^\circ\text{C}$	Wild type (Mg-) $\Delta T_m / ^\circ\text{C}$
8j	4.11 ± 0.22	0.88 ± 0.18	2.43 ± 0.12	1.71 ± 0.42	0.29 ± 0.14
35b	6.52 ± 0.61	1.74 ± 0.48	2.43 ± 0.12	1.40 ± 0.63	-0.07 ± 0.71
23af	1.66 ± 0.59	1.07 ± 0.45	1.82 ± 0.82	0.54 ± 0.11	-0.11 ± 0.15
23ae	2.59 ± 0.69	0.67 ± 0.95	0.82 ± 0.08	-1.0 ± 0.60	0.25 ± 0.37
23k	5.20 ± 0.52	1.04 ± 0.62	1.79 ± 0.53	-0.74 ± 0.60	0.58 ± 0.58
23s	4.93 ± 0.49	0.32 ± 0.80	2.09 ± 0.31	1.61 ± 0.86	0.08 ± 0.38
23o	5.57 ± 0.16	1.81 ± 0.59	1.81 ± 0.17	1.22 ± 0.59	-0.20 ± 0.57
16b	0.66 ± 0.23	0.51 ± 0.48	3.06 ± 0.29	0.30 ± 0.66	0.11 ± 0.67
23ac	3.67 ± 0.70	0.83 ± 0.42	0.24 ± 0.69	1.18 ± 0.58	0.18 ± 0.71
16m	1.10 ± 0.22	1.16 ± 0.35	1.33 ± 1.41	1.31 ± 0.57	0.31 ± 0.64
35f	3.22 ± 0.41	0.97 ± 0.30	3.40 ± 0.37	2.68 ± 0.49	-0.52 ± 0.75
23ad	2.75 ± 0.31	0.28 ± 0.41	2.73 ± 0.17	1.84 ± 0.64	0.41 ± 0.92
23y	2.78 ± 0.38	-0.87 ± 0.43	1.66 ± 0.22	2.10 ± 0.51	1.07 ± 0.18
38	6.42 ± 0.16	0.43 ± 0.46	3.87 ± 0.11	2.29 ± 0.68	0.17 ± 0.48

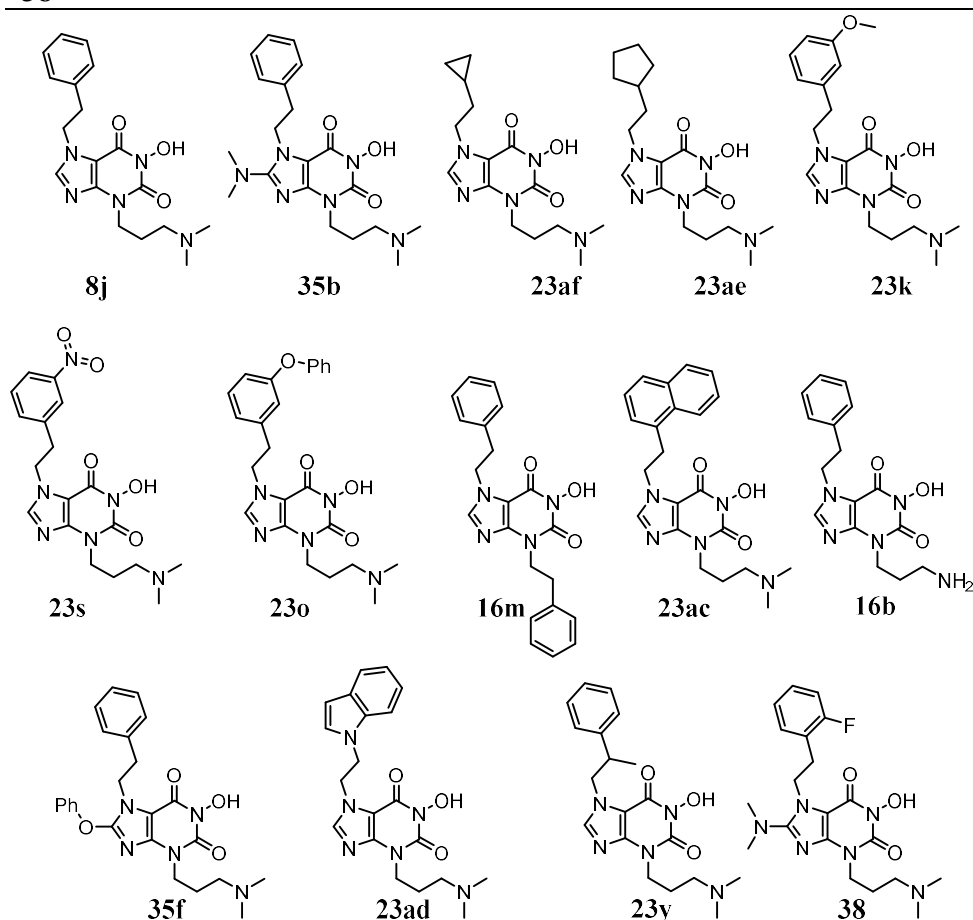


Figure. 31 compound structures for table 15

The ΔT_m values observed for the set compounds with regards to binding to the wild type Caf1 correlated strongly with the experimentally observed pIC_{50} s of the compounds ($r = 0.866$). The compound with the highest ΔT_m value observed was also the compound with the lowest IC_{50} value (**35b**). This supports the validity of DSF being used as an assay to determine compound potency towards Caf1. This is in agreement with Pantoliano *et al.* 2001 J Biomol Screen. [168]

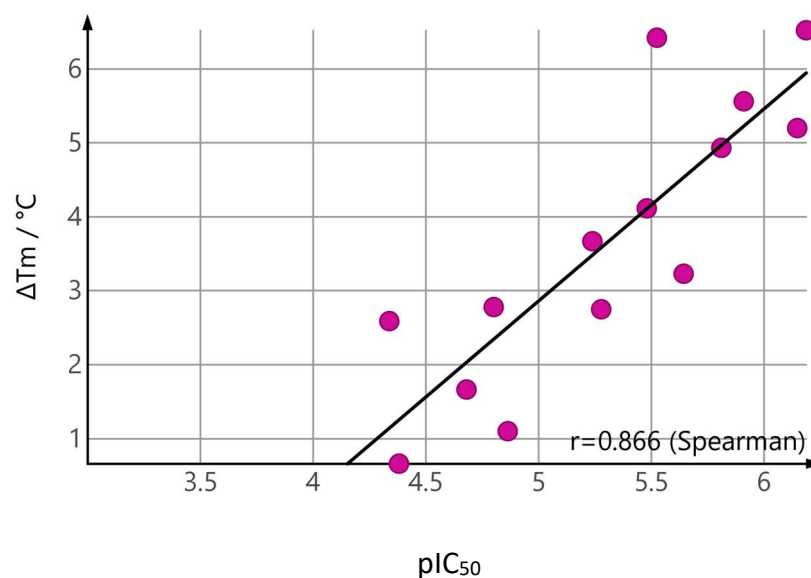


Figure. 32 ΔT_m values plotted against pIC_{50} values. Correlation calculated using Spearman's rank correlation coefficient.

5.3 Structure Activity Relationships

5.3.1 Phenylalanine⁴³

All compounds except **16b** and **16m** saw a decrease in ΔT_m when Phe⁴³ was replaced by alanine, suggesting that the Phe⁴³ side chain was integral to their binding. **16b** and **16m** both had different 7-substituents from the usual propyl dimethylamine and did not have their ΔT_m significantly impacted by this mutation. All the compounds with the propyl dimethylamine substituent at the 7-position had their binding reduced suggesting that this substituent binds to Phe⁴³. This supports the newly proposed binding hypothesis which includes this interaction. The exact nature of the binding between the 7-substituent and Phe⁴³ is still unclear. The amine group (protonated at physiological pH) can form a cation- π interaction with the aromatic Phe⁴³ side chain, however there is evidence against this interaction as the quaternised analogue (**16h**) was not

similarly potent, suggesting that the hydrogen is essential to the interaction. It was initially hypothesised that the amine donates a hydrogen bond to the backbone carbonyl, however the backbone is conserved in the Phe⁴³ variant Caf1. This suggests that the amine donates the hydrogen bond to the Phe⁴³ side chain rather than backbone. Aromatic rings, including the phenylalanine side chain are known to act as hydrogen bond acceptors towards amines forming hydrogen bonds of comparable strength to those between amines and water.^{[169][170]} An alternative possibility is that substituting Phe⁴³ for an alanine changes the tertiary structure of the protein in such a way that moves the backbone out of the appropriate hydrogen bonding range.

5.3.2 Tyrosine¹⁶⁰

The substitution of Tyr¹⁶⁰ for alanine also had a large negative impact on the ΔT_m values of most of the compounds. Other than **16b** which has been shown to bind in a different pose than the other compounds. The compounds whose ΔT_m values were not significantly reduced by the mutation (**23ae**), (**35f**), (**23ad**), and (**23y**) tended to have features that might prevent π - π stacking to Tyr¹⁶⁰, and increased lipophilicity that could facilitate alternative binding to the surrounding hydrophobic pocket. **23ae** has the benzene ring on the 7-substituent replaced by a saturated cyclohexane ring thus preventing π - π stacking. **35f** has a large lipophilic, aromatic phenol ring at the 8-position which is likely to impact the binding pose and change the position of the 7-substituent, preventing its binding to Tyr¹⁶⁰. **23ad** has the benzene ring on the 7-substituent replaced for an indole, and **23y** has a methyl group on the 2-carbon linker of the 7-substituent, these modifications to the sterics of the 7-substituent could impact its ability to bind to Tyr¹⁶⁰, this is supported by their increased IC₅₀s compared to **8j**. It is therefore hypothesised that the reason these compounds are not impacted by this mutation is that their 7-substituents bind to alternative hydrophobic regions. It was likely that the compounds that suffer the largest reductions in ΔT_m towards the Tyr¹⁶⁰ mutant in place of the wild type, were those in which 7-substituent binding towards Tyr¹⁶⁰ was the strongest. Reduction in ΔT_m was the greatest in compounds whose 7-substituent contained a 3-substituted benzene ring, (**23k**), (**23s**), and (**23o**), and compounds containing an 8-substituent (**35b**), and (**23ae**). These features are both hypothesised to strengthen the 7-substituents binding to Tyr¹⁶⁰. A hydrogen bond accepting group at the 3-position of this benzene ring is thought to form a hydrogen bond to the Tyr¹⁶⁰ phenolic side chain, and the xanthine 8-substituent is thought to restrict undesirable 7-substituent rotation. There exists a correlation ($r = 0.662$) between compound potency and the

difference in the compound's ΔT_m value with regards to the wild type and the Tyr¹⁶⁰ mutant (figure 33). This helps demonstrate that it is the most highly potent compounds that benefit from binding to Tyr¹⁶⁰ and shows the importance of Tyr¹⁶⁰ to compound binding. This supports the newly proposed binding mode in which the 7-substituent contributes towards binding by π - π stacking with Tyr¹⁶⁰.

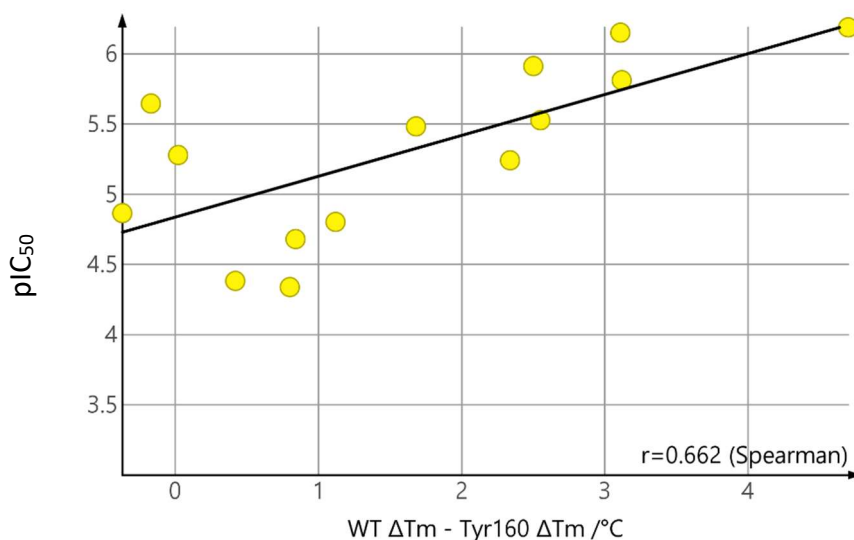


Figure. 33 Correlation between pIC₅₀ and wild type ΔT_m - Tyr¹⁶⁰ ΔT_m . Correlation calculated using Spearman's rank correlation coefficient.

5.3.3 Phenylalanine⁴³ and Tyrosine¹⁶⁰

When both Phe⁴³ and Tyr¹⁶⁰ were substituted for alanines, the effect on compound ΔT_m values was more complex. Some compounds lost most or all their binding, whilst others did not have significantly lower ΔT_m values than they did for the singular Phe⁴³, and Tyr¹⁶⁰ mutants. This suggests that some compounds are able to bind to the magnesium and have their side chains interact with alternative regions of the active site to retain some binding whereas others cannot. This supports the idea that there may more multiple possible modes of binding and that their viability is sensitive to changes in substituent properties.

5.4 Conclusions About Binding Mode

The use of CafI variants gave unique insight into inhibitor binding poses and the surrounding SARs. Both the Phe⁴³ residue and the Tyr¹⁶⁰ were shown to be involved in compound binding. Additional evidence has been generated in

agreement with the proposed specific interactions of these residues with compound substituents supporting the newly proposed binding mode. The magnesium dependent nature of the binding of these compounds to Csf1 has also been demonstrated.

6. Conclusions and Future Work

6.1 General Conclusions

Using **8j** as a lead compound a library of 1-hydroxy xanthine compounds were synthesised based on derivatisation at the 3, 7 and 8-positions. The compounds were chemically characterised using NMR and HRMS. IC₅₀ values towards CafI were determined using either a FRET or luminescent AMP detection assays.

Improvements were made to the literature synthetic pathway to access 3-substituted 1-hydroxy xanthines. A novel synthetic pathway was developed to utilise late stage derivatisation to more efficiently synthesise a library of 7-substituted 1-hydroxy xanthine compounds from a common late stage precursor. A route was also developed to incorporate substituents onto the xanthine 8-position which tended to be highly beneficial towards potency.

Two novel nanomolar potent compounds were discovered, representing a 5-fold increase in potency over the lead as well as several compounds other compounds with increased potency over the lead. The observed SARs of the compounds, combined with computational modelling lead to the proposal of a new alternative binding mode. DSF was used to demonstrate compound binding to CafI and the necessity of magnesium to compound binding. Mutagenesis of the CafI enzyme was used to substitute specific residues predicted to be involved in compound binding. DSF was used to measure specific compound's interaction with these mutants. The findings supported the newly proposed binding hypothesis.

LiPE plotted graphically is a useful way of visualising the library of compounds. ^[171] cLogP is plotted against pIC₅₀ with diagonal lines dividing the graph into sections that represent ranges of LiPE values (figure 34). Many conclusions can be drawn from this analysis. The correlation between cLogP and pIC₅₀ (not shown graphically on figure 34) was low ($r = 0.174$, Spearman's rank). This validates the observed SARs in showing that generally the observed changes in potency are caused by more specific and polar interactions rather than purely non-specific lipophilic binding. Compounds with the highest LiPE values make for the best drug candidates. Compound **16h** has the highest LiPE value of 6.68 although this is an outlying value because the compound contains a quaternary amine with permanent formal charge giving it a much lower cLogP than all the other compounds which are

treated as neutral, this cLogP value of -2.2 is also inappropriately low for a drug-like compound and is likely to have a negative impact on membrane permeability.^[172] This combined relatively low pIC₅₀ makes **16h** of little interest. The four compounds in the 5-6 region, (**23r**), (**35a**), (**23k**), and (**35b**) from left to right can be identified as the most successful compounds by this metric. These compounds have two recurring features that seem to contribute to high LiPE. They either have a low molecular weight amine substituent at the xanthine 8-position or an electron donating substituent at the 3-position of the 7-phenethyl benzene ring.

The bulk of the remaining compounds have LiPE of 3-5 with the lead compound (**8j**) at the high end of this group with a LiPE of 4.7. A small number of compounds are in the 1-3 region, these are mostly compounds with various counterproductive functional groups present on the 3-substituent.

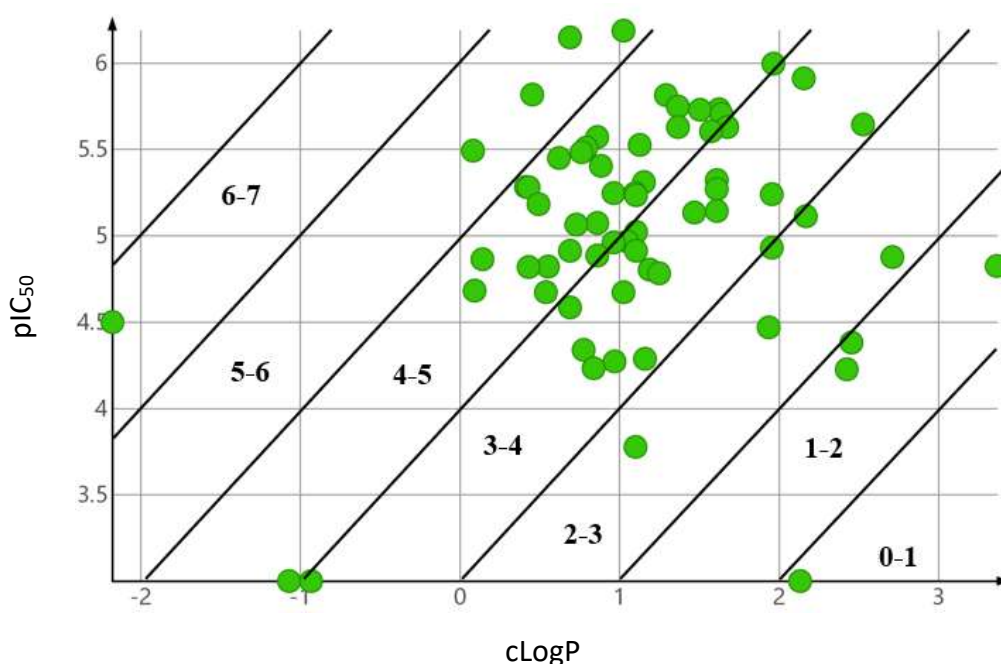


Figure. 34 LiPE plot of all Caf1 inhibitor compounds.

6.1.1 3-Substituents

A range of substituents utilising different functional groups, and conformationally restrained linkers were tested in place of the propyl dimethylamino substituent. This was achieved with minor modifications on the published synthetic pathway to **8j**. None of the alternative functional groups proved useful, however changing the dimethylamine group to a diethylamine group did afford a slight increase in potency from 3.30 μ M to 2.49 μ M. This was part of an observed correlation between the functional

group's hydrogen bond donating ability and potency. This trend supported suggests that the role of the propyl dimethylamino 3-substituent in binding is hydrogen bond dependent.

6.1.2 7-Substituents

The 7-substituent was initially the starting point for a multistep synthesis. An alternative synthetic pathway was developed to allow for late stage derivatisation of an advanced common precursor. This greatly reduced the total number of reactions required to synthesise a varied set of 7-substituted compounds.

The 7-substituent contained a benzene ring that was previously thought to be involved in a cation- π interaction. This hypothesis was tested using electron withdrawing, and electron donating substituents on this ring. The positive relationship between aromatic ring electron density and bond strength expected in the case of a cation- π interaction was not observed. The electronic properties of the ring appeared to have no correlation. Aromatic systems of increased electron density also failed to increase potency. Saturated rings in the place of benzene had a somewhat negative effect on potency but much less so than removing the substituent entirely, in the case of a cation- π bond the saturated ring would not display any bonding as it lacks the π -electron system. This evidence all contradicts the cation- π hypothesis.

The observed SARs and molecular modelling was used to generate a new proposal for bioactive binding pose of the lead compound (**8j**). This pose involved *N*-hydroxyimide moiety bi-dentally chelating each magnesium while both the 3 and 7-substituents forming binding interactions with the surrounding Cafl active site. The 7-substituent was proposed to be involved in π - π stacking with a tyrosine residue in a lipophilic pocket of the active site. Specific functional groups on the 7-substituent's benzene ring that contribute to increases in potency were thought to also be able to hydrogen bond to this tyrosine.

6.1.3 8-Substituents

A new synthetic strategy was developed to incorporate functionalisation at the xanthine 8-position. This tended to have very beneficial effects on potency with the entire 8-substituted series of compounds all having increased potency over the lead as well as compound **35b** being to the best of our knowledge the most potent small molecule Cafl inhibitor to date.

Currently the 8-substituted compound library is limited due to synthetic restrictions and stability issues. The synthetic pathway is currently limited to adding substituents *via* nucleophilic aromatic substitution, this is only possible with certain functional groups. Certain other functional groups are also unstable at the 8-position. Many organometallic methods were tried to couple substituents to the 8-position, so far without success.

6.1.4 Synergistic Substitution

Some beneficial substituents were combined with other substituents at another position to see if they would affect potency in a synergistic manner. These compounds did not achieve increased potency and the beneficial effects of the substituents were not additive. This suggests that there are multiple bioactive binding poses available and that the substituents present change the optimum binding pose achieved by the compound.

6.1.5 DSF and Mutagenesis Studies

DSF experiments were used to demonstrate the binding of compounds to CsfI and the necessity of magnesium for this interaction to occur. The validity of DSF as a means of assessing compound potency was demonstrated by the close correlation between the experimentally observed ΔT_m values and IC_{50} values. Mutated CsfI enzymes were prepared to substitute specific residues that were predicted to be involved in compound binding. These mutant enzymes were then used in DSF experiments with a range of compounds to examine the role of these residues in binding. Evidence was produced that strongly supports the newly proposed binding mode and offers explanations of the observed SARs.

6.2 Future Work

6.2.1 8-Amino Series

There remain many opportunities for the library of 1-hydroxy xanthine compounds to be expanded. The series of 8-amino compounds were all of higher potency than the lead compound (**8j**) and are therefore a very attractive source of future compounds to yield further increases in potency. The addition of the amine group at this position is clearly beneficial to potency and this series of compounds represents the most attractive avenue of future exploration. This warrants future studies into the role of this substituent in binding, the optimisation of this substituent, and improved synthetic methods of accessing this series of compounds.

We have proposed an explanation for the role of the 8-amino substituent in compound binding but there is still potential for exploration of the surrounding SAR. The preparation of a more electronically neutral 8-substituent of similar steric dimensions would provide important information on the relevance of the steric *vs* electronic effects of the 8-substituent. The Alkyl chains on the amine group offer high potential to build further functionalisation into the molecule. Additional functionalisation can be incorporated onto the amine alkyl chains that could offer the potential for further binding as well as the modulation of the compounds drug-like properties such as aqueous solubility or lipophilicity.

The synthetic pathway used to access the 8-amino series of compounds was low yielding, and not compatible with certain functional groups, thus preventing the synthesis of highly desirable targets such as **39**. An improved synthetic route may involve alternative protecting group strategy at the 1-hydroxy position, removing the need for the low yielding hydrogenation step. It would also be desirable to develop a synthetic pathway that allows for the 8-chlorination to take place prior addition of 7-substituents. This would allow for the addition of 7-substituents containing groups incompatible with the chlorination step such as the 3-methoxy phenethyl substituent.

Addition of an 8-amino substituent prior to the 7-substituent would give a late stage common precursor to more rapidly generate a set of compounds combining the 8-amino group with a large variety of 7-substituents. This is another important set of future compounds as it is predicted by the newly proposed binding mode that the 7, and 8-substituents could synergise beneficially.

6.2.2 Other 8-Substituents

It has been demonstrated that a phenol can be used as a nucleophile to form a phenolic ether at the 8-position. To date only one such compound was synthesised (**35f**), but it was shown increased potency *vs* the lead compound **8j**. The role of the phenolic ether group in binding is unclear but much like the alkyl chains of the 8-amine substituents, the additional benzene ring had many options for potentially beneficial derivatisation. The benzene ring gives the opportunity to use a Topliss decision tree to quickly assess the optimum ring substituents. ^[122]

Many synthetic methods were examined with the aim of directly attaching aryl groups at the 8-position. Whilst no successful methods were reported in this thesis, many options remain unexplored and it is unlikely that this is

impossible. The successful synthesis of such compounds would provide another large set of potential compounds.

6.2.3 Selectivity Studies

Caf1 is one of several closely related magnesium dependent deadenylase enzymes, some of which have been shown to be essential to survival.^[70] This means that selectivity towards Caf1 over other deadenylases will be an essential feature of any side effect free Caf1 inhibiting drug, as off target pharmacology at these deadenylases is likely to cause toxicity. The 1-hydroxy xanthine series has been found to be selective for Caf1 over Ccr4 (another deadenylase subunit of the Ccr4 Not complex). Some compounds including **8j**, showing selectivity to Caf1 over PARN (another essential deadenylase enzyme).

Some preliminary work was done on the selectivity of the reported compounds towards Caf1 over PARN. None of the published X-ray crystal structures of PARN are co-crystallised with their active site magnesium ions.^{[173][174]} This makes these not appropriate for use in the molecular modelling of compounds dependent on magnesium for binding (based on the assumption that the compounds bind to PARN in a similar manner as they do to Caf1). A homology model was produced by superimposing the two conserved DEDD domains of the PARN X-ray crystal structure (PDB 2A1R)^[174] and the Caf1 X-ray crystal structure (PDB 4MGJ.B).^[54] The backbone of the Caf1 X-ray crystal was removed leaving behind the magnesium ions in PARN DEDD domain. This model was then used to glide dock the library of compounds to predict their activity towards PARN. A small number of assays were carried out to measure the IC₅₀ values of a few compounds selected based on the homology model.

Table. 16 select compound's IC₅₀ values towards PARN their selectivity towards their preferred deadenylase. Selectivity values calculated by higher IC₅₀ / lower IC₅₀

Compound	IC ₅₀ /μM	SEM /μM	(n)	Caf1 selectivity	PARN selectivity
8j	28.61	2.39	2	8.7	-
35b	1.35	0.33	2	2.1	-
23s	1.84	0.03	2	1.2	-
16f	1.90	-	1	-	21.9
16u	2.67	-	1	-	22.3
16m	0.79	-	1	-	10.9

The lead compound (**8j**) was found to have an IC₅₀ consistent with its literature value and to be selective towards Caf1. ^[97] This selectivity towards Caf1 was greatly reduced with more potent Caf1 inhibitors such as **35b** and **23s** which had higher IC₅₀s towards PARN. The Modelling predicted that **16f**, **16u** and **16m** would be highly active compounds towards PARN as they have lipophilic groups at the 3-position which fit into a lipophilic pocket in the PARN active site. These compounds showed high activity and selectivity towards PARN, both validating the computational model and providing a starting point for any future search for selective PARN inhibitors should they ever be desired.

Determining the selectivity of these compounds for Caf1 and using the resulting SARs to design increasingly selective compounds represents an important piece of future work. Selectivity over other deadenylases will be an essential feature of a potential Caf1 inhibitor drug candidate compound.

6.2.4 Membrane permeability

CNOT7 mediated deadenylation is a cytoplasmic process. ^[175] This means that for a Caf1 inhibitor to be efficacious *in vivo*, cell permeation must be achieved. No experiments have so far been performed to measure the membrane permeability of the 1-hydroxy-xanthine series.

An *initial* concern was that the ionisable *N*-hydroxy-imide group would impede membrane permeability. However, there is a literature precedent for hydroxy-pyrimidine based successfully showing activity against cellular targets. ^[89] The polar surface area (PSA) of **8j** was calculated to be 79.69 Å². ^[176] This PSA is well below the generally accepted limit of 140 Å² for cell permeant compounds. ^[177] The PSA of **8j** is calculated to be low enough (< 90 Å²) to potentially cross the blood-brain barrier which may be undesirable

source of toxicity. ^[178] Permeation of Cafl inhibitors through the blood-testis barrier will likely cause toxicity due to the role of Cafl in spermatogenesis. ^[1-59]

Experimentation to determine and optimise the membrane permeability of this series is clearly an important future project.

7 Experimental

7.1 Modelling

7.1.1 Autodock

ChemBioDraw professional v15 was used to create a 2D structure cdx file. ChemBio3DUltra v14 was then used generate a minimum energy 3D conformation as a PDB file using an MM2 calculation. Grid preparation and docking was performed using AutoDock 4.2 and AutoDockTools 4.2. The receptor was PDB 4MGJ chain C which was protonated, and partial charges were assigned (including for the two Mg^{2+} ions). Results were visualised in the PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.

7.1.2 Schrödinger Suite Glide Docking

The receptor was PDB 4MGJ chain C which was prepared using the protein preparation wizard. The Receptor grid was prepared using the grid preparation tool, gridbox contained an area 26 Å from the central Mg^{2+} ions, and spacial constraints were used to require at least one oxygen atom within 2.75 Å of one magnesium atom. Compounds were docked using the glide docking tool using standard settings with enhanced planarity, and Epik calculated protonation states at pH 7.4 +/- 2.0.

7.2 Materials and Methods

7.2.1 Materials

All chemicals used were purchased from commercial suppliers and used without further purification unless otherwise specified or were synthesised and are listed below. Compounds were characterised using a combination LCMS, HRMS, ^1H NMR and ^{13}C NMR.

7.2.2 Liquid Chromatography Mass Spectrometry

The LCMS used was a Shimadzu UFLCXR HPLC system equip with Biosystems MDS SCIEX API2000 electrospray ionisation mass spectrometer. The column was a Gemini 3 μm C18 110 Å, liquid chromatography column 50 x 2 nm. The eluent was a mixture MeCN and H_2O , with 0.1% formic acid

with a gradient of 1:19 v/v to 19:1 v/v over either 5 or 15 minutes. The flow rate was 0.5 ml/min and UV detection was 220 nm.

7.2.3 High Resolution Mass Spectrometry

High resolution mass spectrometry was performed with a Bruker microTOF machine using MS electrospray ionization operating in positive ion mode and also negative ion mode in the case of any compounds with acidic groups.

7.2.4 Nuclear Magnetic Resonance

NMR spectra were recorded using a Bruker Advance 400 instrument using either CDCl₃, MeOD-D₄, DMSO-D₆, or DMF-D₇ as solvents. ¹H NMR was recorded at 400.13 MHz and ¹³C NMR at 101.6 MHz. Chemical shifts are recorded in ppm by reference to the solvent residual peaks. J values are quoted in Hz.

7.2.5 Reverse Phase Column Chromatography

Reverse phase column chromatography was performed on a C₁₈ Puriflash 50 μM 20 g flash column. The eluent was a mixture of MeOH and H₂O, with 0.01% trifluoroacetic acid with a gradient of 1:19 v/v to 19:1 v/v.

7.2.6 High Performance Liquid Chromatography

High performance liquid chromatography was performed using a Waters Millennium 995 LC system. The eluent was monitored using photodiode array detection. Mobile phases were solvent A, 0.1% TFA in water, and solvent B, acetonitrile, degassed by helium bubble and sonication, respectively.

7.3 Pharmacology

7.3.1 FRET Assay

Compounds were diluted to 3000 μM, 1000 μM, 300 μM, 100 μM, 30 μM, 10 μM, 3 μM and 1 μM. 1 μl of each dilution was added to a well in a greiner 384 fluotrac plate, followed by 4 μl of master mix # 1 (25% CafI enzyme 50 - 100 nM in 20 mM Tris-HCl pH 7.9, 500 mM NaCl, 10% glycerol, 1 mM β-M, 25 % MgCl₂ 20 mM and 50 % reaction buffer (20 mM Tris-HCl pH 7.9, 50% glycerol, 5 mM β-mercaptoethanol). The plate was centrifuged at 1200 RPM for 15 seconds, shaken for 20 seconds and incubated at rt for 15 minutes. To each well was then added 5 μl of master mix # 2 (20 % Fluorescent RNA substrate (5'-Fluorescein-CCUUUCCAAAAAAA-3') 10 μM in TE buffer. 80 % reaction buffer (20 mM Tris-HCl pH 7.9, 50% glycerol, 5 mM β-

mercaptoethanol)) and the plate was centrifuged at 1200 RPM for 15 seconds, shaken for 20 seconds and incubated at 30 °C for 1 hour. To each well was then added 10 µl of master mix # 3 (30 % Fluorescent DNA probe (5'-TTTTTTTTTTGGAAAGG-tetramethylrhodamine-3') 10 µM in TE buffer. 10 % (SDS 10% solution in water). 60% TE buffer. The plate was centrifuged at 1200 RPM for 15 seconds, shaken for 20 seconds and incubated at rt for 2-3 hours. The plate was read using a synergy plate reader to measure fluorescence. Temperature: 25 °C Excitation: 485/20 Emission: 528/20 Optics position: Top Sensitivity: 90. ^[86] All compounds tested had a purity of > 95% as tested by HPLC.

7.3.2 AMP-GLO Assay

Compounds were diluted to 3000 µM, 1000 µM, 300 µM, 100 µM, 30 µM, 10 µM, 3 µM and 1 µM. 1 µl of each dilution was added to a well in a low volume 96 well plate, followed by 4 µl of master mix # 1 (25% CalfI enzyme 50 - 100 nM in 20 mM Tris-HCl pH 7.9, 500 mM NaCl, 10% glycerol, 1 mM β-M, 25 % MgCl₂ 20 mM and 50 % reaction buffer (20 mM Tris-HCl pH 7.9, 50% glycerol, 5 mM β-mercaptoethanol)). The plate was centrifuged at 1200 RPM for 15 seconds, shaken for 20 seconds and incubated at rt for 15 minutes. To each well was then added 5 µl of master mix # 2 (20 % Fluorescent RNA substrate (5'- Fluorescein -CCUUUCCAAAAAAA-3') 10 µM in TE buffer. 80 % reaction buffer (20 mM Tris-HCl pH 7.9, 50% glycerol, 5 mM β-mercaptoethanol)) and the plate was centrifuged at 1200 RPM for 15 seconds, shaken for 20 seconds and incubated at 30 °C for 1 hour. 3 µl of this mixture was transferred to a new plate in duplicate, 3 µl of AMP-GLO reagent 1 was added to each well and incubated at rt for 1 hour. 3 µl of 1% solution reagent 2 in Kinase GLO was added to each well and incubated at rt for 1 hour. The plate was read using a GloMax Navigator Microplate Luminometer. All compounds tested had a purity of > 95% as tested by HPLC.

7.3.3 Differential Scanning Fluorimetry

Differential scanning fluorimetry was carried out using an Agilent MX3005p machine and the fluorescent dye SYPRO Orange (5000X in DMSO). Standard assays (20 µl) contained: 2 µM purified enzyme, 20 mM Tris-HCl pH 7.8, 50 mM NaCl, 10% glycerol, 1 mM β mercaptoethanol, 5x SYPRO Orange dye, 100 µM compound, 5% DMSO. Assays carried out in the presence of magnesium also contained 2 mM MgCl₂. Reactions were performed in black 96-well thin-wall plates with V-shaped wells. After sealing, reactions were

collected at the bottom of the wells by centrifugation. Fluorescence scanning was carried out using a temperature gradient from 25°C to 95°C at 1°C per minute using the FAM (492 nm) filter for excitation and the ROX (610 nm) filter for detection. Data was acquired using MxPro QPCR software (Agilent), exported to Microsoft Excel and analysed using publically available macros for Microsoft Excel 2013 and templates for Graphpad Prism. ^[179]

7.4 Chemistry

7.4.1 Standard Procedures

Standard procedure. 1 –Alkylation of xanthines at the 3-position.

A stirred solution of appropriate substituted xanthine (1 eq), appropriate alkyl halide (1.5 eq) and caesium carbonate (2 eq, or 3.5 eq in the case of HCl salt containing alkyl halides) in DMF (0.08 M) was heated at 80 °C for 24 hours. The solvent was removed *in vacuo*, the residue was partitioned between saturated aqueous sodium bicarbonate solution (10 ml) and EtOAc (10 ml). The aqueous layer was extracted into EtOAc (3 x 10 ml). Combined organic fractions were dried (MgSO₄), filtered and the solvent was removed *in vacuo* to afford the appropriate alkylated xanthine which was used in the next step without further purification.

Standard procedure. 2 – Chlorination of alcohols to appropriate alkyl chlorides.

To a stirred solution of the appropriate alcohol (1 eq) in anhydrous CHCl₃ (0.5 M) at 0 °C was added thionyl chloride (1.5 eq) dropwise and the solution allowed to warm to rt and stirred for 24 hours. The solvent was removed *in vacuo* and the residue dissolved in minimum volume of EtOH. Et₂O was added until a precipitate formed which was collected by filtration to afford the appropriate alkyl chloride (as an HCl salt in the case of presence of a basic amine) which was used in the next step without further purification.

Standard procedure. 3 – Deprotection of the benzyl protecting group by hydrogenation.

To a stirred solution of benzyl protected compound (1 eq) in DCM (0.1 M) was added Pd/C (10 % w/w) followed by MeOH (0.01 M) and the mixture stirred under hydrogen atmosphere (1 atm) for 18 hours. The mixture was filtered through celite filter aid and the solvent was removed *in vacuo* to afford the appropriate debenzylated compound. Purification by flash column reverse phase chromatography when required eluting with H₂O:MeOH.

Standard procedure. 4 – Alkylation of glycine ethyl ester with substituted phenylethyl bromide.

To a stirred mixture of, glycine ethyl ester hydrochloride (2.2 eq), potassium carbonate (3 eq), potassium iodide (0.2 eq) and MeCN (0.5 M) at reflux was added dropwise a solution of the appropriate phenylethyl bromide (1 eq) in MeCN (5 M) and the mixture was heated at reflux for 24 hours. The solvent was removed *in vacuo*, the residue partitioned between saturated aqueous sodium bicarbonate solution and EtOAc. The aqueous layer was extracted into EtOAc. Combined organic fractions were dried (MgSO₄), filtered and the solvent was removed *in vacuo* to afford the appropriate alkylated ethyl glycinate. Purification by column chromatography eluting with EtOAc:hexane.

Standard procedure. 5 – Substituted imidazole synthesis from alkylated ethyl glycinate.

A stirred solution of alkylated glycine ethyl ester (1 eq) and freshly distilled ethyl cyanoimidoformate (1.2 eq) in anhydrous THF (0.3 M) was heated at reflux for 3 hours. The solvent was removed *in vacuo* followed by the addition of anhydrous EtOH (0.3 M) and potassium *tert*-butoxide (1.1 eq). The solution was stirred at reflux for 24 hours, quenched with saturated aqueous ammonium chloride solution, EtOH was removed *in vacuo* and extracted into DCM. Combined organic fractions were dried (MgSO₄), filtered and the solvent was removed *in vacuo* to afford the appropriate substituted imidazole. Purification by column chromatography eluting with EtOAc:hexane.

Standard procedure. 6 – Protection of amines with BOC or CBz groups

To a stirred mixture of CHCl_3 (0.1 M) and an equal volume of saturated aqueous sodium carbonate solution at 0 °C was added appropriate amine (1 eq) and either benzyl chloroformate or di-*tert*-butyl carbonate (1 eq) and the mixture allowed to warm to rt and stirred for 4 hours. Aqueous phase was extracted into DCM. Combined organic fractions were dried (MgSO_4), filtered and the solvent was removed *in vacuo* to afford the appropriate protected amine which was used in the next step without further purification.

Standard procedure. 7 – deprotection of BOC groups

A solution of BOC protected compound (1 eq) in 2:1 DCM:TFA (0.1 M) was stirred for 4 hours. The solvent was removed *in vacuo* to afford the appropriate BOC deprotected compound. Purification by flash column reverse phase chromatography when required eluting with H_2O :MeOH.

Standard procedure. 8 – Substituted Xanthine synthesis from substituted imidazole.

To a stirred solution of Carbonyldiimidazole (1.3 eq) in anhydrous toluene (0.02 M) was added appropriate substituted imidazole (1 eq) and heated at reflux for 3 hours. *O*-Benzylhydroxylamine hydrochloride (1.7 eq) and triethylamine (1.7 eq) were added and the solution heated at reflux for a further 3 hours. The solvent was removed *in vacuo* and residue redissolved in anhydrous NMP (0.04 M). Sodium hydride (4.5 eq) was added and solution was stirred at 80 °C for 18 hours. The solution was allowed to cool to rt, acidified to pH ~ 5 with aqueous HCl (2 M) and H_2O added until a precipitate formed which was collected by filtration to afford the appropriate substituted xanthine. Purification by recrystallization from MeOH.

Standard procedure. 9 – Mitsunobu conditions for alkylation of xanthines at the 7- position A solution of appropriate xanthine (1 eq), appropriate alcohol (3 eq) and triphenylphosphine (2 eq) in anhydrous toluene (10 mM) was stirred at 50 °C for 5 minutes, diisopropyl azodicarboxylate (2.25 eq) was added and the solution stirred at 50 °C for 15 minutes. The solvent was removed *in vacuo* to afford the appropriate substituted xanthine. Purification by column chromatography eluting with MeOH:DCM.

Standard procedure. 10 –conditions for chlorination of xanthines at the 8-position

A solution of appropriate xanthine (1 eq), and *N*-chlorosuccinimide (2 eq) in anhydrous THF (50 mM) was stirred 24 hours. The solvent was removed *in vacuo*, the residue was partitioned between aqueous sodium hydroxide solution (5 ml, 1 M) and DCM (10 ml). The aqueous layer was extracted into DCM (3 x 10 ml). Combined organic fractions were dried (MgSO₄), filtered and the solvent was removed *in vacuo* to afford the appropriate chlorinated xanthine.

Standard procedure. 11 –conditions for substitution of 8-chloro xanthines with amines

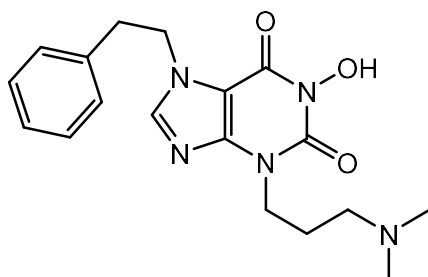
The appropriate 8-chloro xanthine (1 eq) was dissolved in a 2:1 v/v solution of EtOH:appropriate amine (0.1 M) and stirred until LCMS indicated completion. The solvent was removed *in vacuo*, the residue was partitioned between water (5 ml, 1 M) and DCM (10 ml). The aqueous layer was extracted into DCM (3 x 10 ml). Combined organic fractions were dried (MgSO₄), filtered and the solvent was removed *in vacuo* to afford the appropriate 8-substituted xanthine.

Standard procedure 12 -Conditions for reduction of carboxylic acids or esters into primary alcohols

To a stirred solution of appropriate carboxylic acid or ester (1 eq) in anhydrous THF (0.5 M) was added dropwise a solution of lithium aluminium hydride in THF (2M, 2 eq) and the solution stirred at rt for 2 hours. The reaction was quenched by adding isopropyl alcohol dropwise until no further effervescence was observed, followed by the addition of methanol and then saturated aqueous sodium potassium tartrate solution. The resulting mixture was stirred for 2 hours and extracted into DCM (3 x 10 ml). Combined organic fractions were dried (MgSO₄), filtered and the solvent was removed *in vacuo* to afford the corresponding primary alcohol. Purification by column chromatography when required eluting with hexane:Et₂O 1:1.

7.4.2 Compound Synthesis

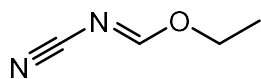
3-(3-(Dimethylamino)propyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (8j)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (70 mg, 0.16 mmol) to afford the title compound as an orange solid (54 mg, 94 %). ^1H NMR δ_{H}

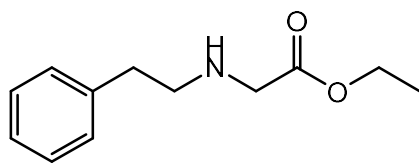
(400.13 MHz; DMSO): 10.56 (1H, s), 7.88 (1H, s), 7.32-7.15 (5H, m), 4.48 (2H, t, J 7.2), 4.02 (2H, t, J 7.2), 3.12 (2H, t, J 7.2), 2.25 (6H, s), 1.84 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 152.9, 149.9, 147.3, 142.9, 138.1, 129.1, 128.1, 127.1, 105.8, 56.7, 47.9, 45.6, 42.0, 36.8, 26.0. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{18}\text{H}_{24}\text{N}_5\text{O}_3$, 358.1874; found 358.1868. [M+Na] expected for $\text{C}_{18}\text{H}_{23}\text{N}_5\text{O}_3\text{Na}$, 380.1693; found 380.1692.

ethyl (E)-N-cyanoformimidate (10)



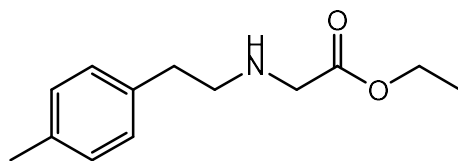
A stirred solution of cyanamide (5.08 g, 121 mmol) in triethyl orthoformate (18.27 ml, 110 mmol) was heated at reflux for 2 hours. EtOH was removed *in vacuo* to afford a yellow suspension. Purification by vacuum distillation 50-60 °C at 100 mm Hg to afford the title compound as a colourless liquid (7.85 g, 73 %, b.p. decomposes 80-90 °C). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 8.41 (1H, s), 4.41 (2H, q, J 8.0), 1.43 (3H, t, J 9.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 171.4, 114.2, 66.2, 13.6.

Ethyl phenethylglycinate (12)



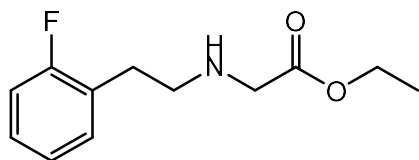
To a stirred solution of phenylethylamine (13.90 ml, 110 mmol) in anhydrous THF (25 ml) at 0 °C was added dropwise, a solution of ethyl 2-bromoacetate (5.54 ml, 50 mmol) in anhydrous THF (25 ml). The solution was allowed to warm to rt and stirred for 2.5 hours. The solvent was removed *in vacuo* and Et₂O (20 ml) added. The mixture was filtered, and the solvent was removed *in vacuo* to give a yellow oil. Purification by column chromatography eluting with 1:1 Et₂O:hexane to afford the title compound as a yellow oil. (7.28 g, 70%). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.35-7.21 (5H, m), 4.20 (2H, q, *J* 7.2), 3.44 (2H, s), 2.94-2.90 (2H, m), 2.85 (2H, t, *J* 7.0), 1.29 (3H, t, *J* 7.2). ¹³C NMR δ_C (101.6 MHz; CDCl₃): 172.3, 139.8, 128.7, 128.5, 126.2, 60.8, 51.8, 50.8, 36.5, 14.3.

Ethyl (4-methylphenethyl)glycinate (12c)



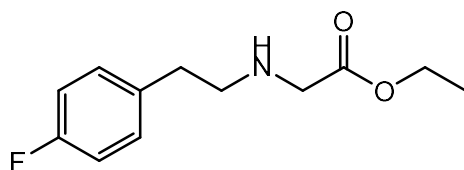
Prepared according to standard procedure 4 using 4-methyl phenethyl bromide (766 μl, 5.03 mmol) to afford the title compound as a yellow oil (971 mg, 87 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.15-7.11 (4H, m), 4.20 (2H, q, *J* 7.1), 3.43 (2H, s), 2.89 (2H, t, *J* 7.1), 2.80 (2H, t, *J* 6.9), 2.34 (3H, s), 1.29 (3H, t, *J* 7.1). ¹³C NMR δ_C (101.6 MHz; CHCl₃): 172.6, 136.6, 135.7, 129.2, 128.6, 60.7, 51.0, 50.7, 36.0, 21.1, 14.3.

Ethyl (2-fluorophenethyl)glycinate (12d)



Prepared according to standard procedure 4 using 2-fluoro phenethyl bromide (687 μl, 4.93 mmol) to afford the title compound as a light yellow oil (842 mg, 76 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.28-7.01 (4H, m), 4.20 (2H, q, *J* 7.1), 3.46 (2H, s), 2.93-2.85 (4H, m), 1.29 (3H, t, *J* 7.2). ¹³C NMR δ_C (101.6 MHz; CHCl₃): 172.4, 162.3, 131.0, 128.0, 126.4, 124.1, 115.4, 60.9, 50.9, 50.0, 35.7, 14.3.

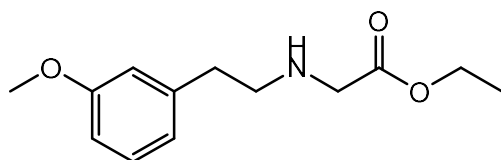
Ethyl (4-fluorophenethyl)glycinate (12f)



Prepared according to standard procedure 4 using 4-fluoro phenethyl bromide (1034 μ l, 7.39 mmol) to afford the title compound as a light yellow oil

(1318 mg, 79 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.20 (2H, dd, J 5.3 and 8.4), 7.03-6.98 (2H, m), 4.20 (2H, q, J 7.1), 3.43 (2H, s), 2.89 (2H, t, J 7.0), 2.80 (2H, t, J 7.0), 2.34 (3H, s), 1.29 (3H, t, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 172.5, 160.2, 135.3, 130.2, 115.2, 60.9, 50.9, 50.8, 35.7, 14.3.

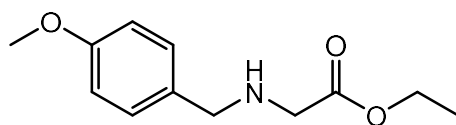
Ethyl (3-methoxyphenethyl)glycinate (12k)



Prepared according to standard procedure 4 using 3-methoxy phenethyl bromide (729 μ l, 4.65 mmol) to afford the title compound

as a yellow oil (665 mg, 60 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.24 (1H, t, J 7.8), 6.86-6.76 (3H, m), 4.20 (2H, q, J 7.2), 3.83 (3H, s), 3.44 (2H, s), 2.92 (2H, t, J 6.6), 2.82 (2H, t, J 6.6), 1.29 (3H, t, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 172.3, 159.8, 141.4, 129.5, 120.1, 114.5, 60.7, 51.0, 50.7, 36.0, 14.2.

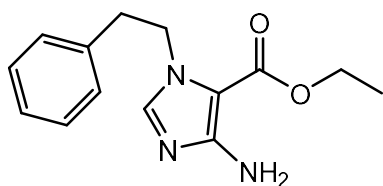
ethyl (4-methoxybenzyl)glycinate (12v)



To a stirred solution of 4-methoxy benzylamine (23.8 ml, 182.5 mmol), and triethylamine (50.8 ml, 365 mmol) in

THF (100 ml) was added dropwise ethyl bromoacetate (20.4 ml, 182.5 mmol) at 0 °C and allowed to warm to rt over 4 hours. The reaction mixture was filtered and washed with ether (2 x 20 ml) and solvent removed *in vacuo* giving a yellow oil. Purified by chromatography eluting with 3:1 hexane:Et₂O to afford the title compound as a yellow oil (9.2g, 23%). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.27 (2H, d, J 8.3), 6.89 (2H, d, J 8.3), 4.21 (2H, q, J 6.2), 3.82 (3H, s), 3.76 (2H, s), 3.48 (1H, s), 3.41 (2H, s), 1.30 (3H, t, J 8.3). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 172.4, 158.9, 131.6, 129.5, 113.9, 60.9, 55.3, 52.7, 50.0.

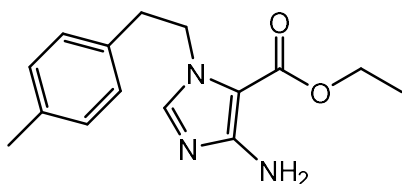
Ethyl 4-amino-1-phenethyl-1*H*-imidazole-5-carboxylate (13)



Prepared according to standard procedure 5, using ethyl phenethylglycinate (8.46 g, 40.9 mmol) to afford the title compound as orange crystals (8.42 g, 79 %, m.p. 77-79 °C). ¹H

NMR δ_{H} (400.13 MHz; CDCl₃): 7.34-7.09 (6H, m), 6.87 (1H, s), 4.87 (2H, brs), 4.40 (2H, q, *J* 7.2), 4.35 (2H, t, *J* 7.2), 3.04 (2H, t, *J* 7.2), 1.44 (3H, t, *J* 7.2). ¹³C NMR δ_{C} (101.6 MHz; CDCl₃): 167.7, 161.7, 139.8, 139.7, 128.8, 128.7, 126.6, 101.4, 60.0, 49.2, 38.0, 15.0.

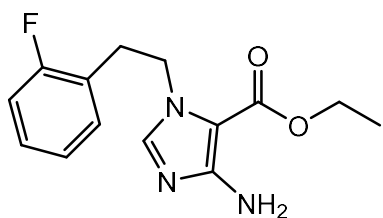
ethyl 4-amino-1-(4-methylphenethyl)-1*H*-imidazole-5-carboxylate (13c)



Prepared according to standard procedure 8, using ethyl 4-amino-1-(4-fluorophenethyl)-1*H*-imidazole-5-carboxylate (389 mg, 1.4 mmol) to afford the title compound as a

white solid. (402 mg, 76 %). ¹H NMR δ_{H} (400.13 MHz; MeOD): 7.11-6.95 (5H, s), 4.36 (2H, q, *J* 7.1), 4.33 (2H, t, *J* 6.6), 3.44 (2H, t, *J* 7.1), 2.29 (3H, s), 1.37 (3H, t, *J* 7.1). ¹³C NMR δ_{C} (101.6 MHz; MeOD): 169.8, 162.3, 161.1, 139.3, 136.0, 134.8, 128.8, 128.4, 62.9, 59.6, 48.8, 36.4, 13.6.

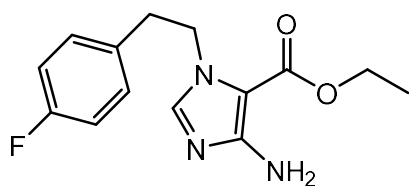
ethyl 4-amino-1-(2-fluorophenethyl)-1*H*-imidazole-5-carboxylate (13d)



Prepared according to standard procedure 5, using Ethyl (2-fluorophenethyl)glycinate (842 mg, 3.74 mmol) to afford the title compound as an orange solid (831 mg, 80 %). ¹H NMR

δ_{H} (400.13 MHz; MeOD): 7.30-7.03 (5H, m) 4.37 (2H, q, *J* 7.1), 4.28 (2H, t, *J* 7.1), 3.38 (2H, t, *J* 7.1), 2.29 (3H, s). ¹³C NMR δ_{C} (101.6 MHz; MeOD): 168.9, 162.3, 160.3, 139.3, 136.1, 134.8, 131.0, 128.0, 126.4, 124.1, 115.4, 59.7, 48.8, 36.7, 14.6.

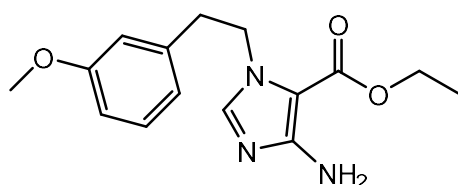
ethyl 4-amino-1-(4-fluorophenethyl)-1H-imidazole-5-carboxylate (13f)



Prepared according to standard procedure 5, using Ethyl (4-fluorophenethyl)glycinate (1.3 g, 5.8 mmol) to afford the title compound as a yellow solid (778 mg, 48 %).

^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.07-6.95 (1H, s), 6.85 (1H, s), 4.88 (2H, brs), 4.39 (2H, q, J 6.8), 4.32 (2H, t, J 7.1), 3.01 (2H, t, J 6.9), 1.43 (3H, t, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 168.2, 162.4, 160.1, 139.3, 133.5, 130.3, 130.2, 115.6, 59.8, 49.1, 36.6, 14.6.

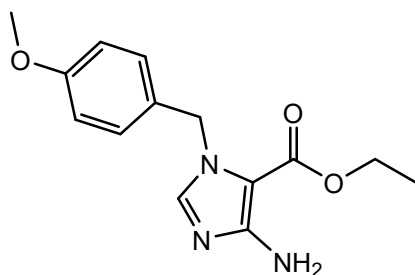
ethyl 4-amino-1-(3-methoxyphenethyl)-1H-imidazole-5-carboxylate (13k)



Prepared according to standard procedure 5, using Ethyl (3-methoxyphenethyl)glycinate (665 mg, 2.8 mmol) to afford the title compound

as a yellow oil (337 mg, 42 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.20 (1H, t, J 7.8), 7.06-6.60 (4H, m), 4.38 (2H, q, J 7.2), 4.33 (2H, t, J 7.0), 3.77 (3H, s), 3.00 (2H, t, J 7.0), 1.42 (3H, t, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 168.2, 161.4, 161.0, 159.8, 139.3, 139.1, 129.7, 121.4, 114.1, 112.2, 59.8, 55.1, 49.0, 37.4, 14.8.

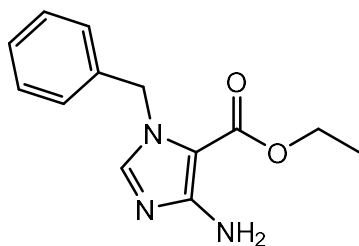
ethyl 4-amino-1-(4-methoxybenzyl)-1H-imidazole-5-carboxylate (13v)



Prepared according to standard procedure 5, using ethyl (4-methoxybenzyl)glycinate (2.75 g, 12.3 mmol) to afford the title compound as an orange solid (2.45 g, 72 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.21 (1H, s), 7.13 (2H, d, J 8.5), 6.88 (2H, d, J

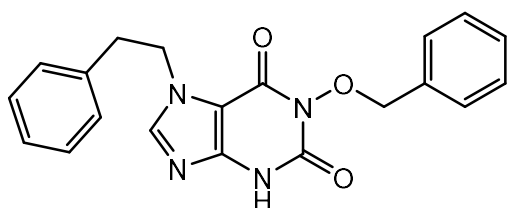
8.5), 5.29 (2H, s), 4.89 (2H, brs), 4.29 (2H, q, J 7.3), 3.81 (3H, s), 1.32 (3H, t, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 168.1, 161.0, 159.3, 139.0, 128.9, 128.7, 18.4, 114.2, 59.7, 56.3, 15.2, 14.5.

ethyl 4-amino-1-benzyl-1H-imidazole-5-carboxylate (13w)



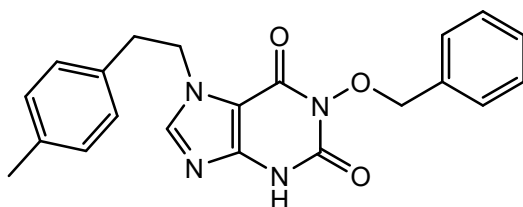
Prepared according to standard procedure 5, using ethyl benzylglycinate (5.90 g, 30.6 mmol) to afford the title compound as an orange solid (5.73 g, 78 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.21 (1H, s), 7.29-7.07 (5H, m), 5.29 (2H, s), 4.01 (2H, q, J 7.3), 3.77 (3H, s), 1.31 (3H, t, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 167.5, 161.7, 139.7, 139.4, 128.8, 128.5, 126.1, 18.4, 114.2, 59.5, 53.1, 14.3.

1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (14)



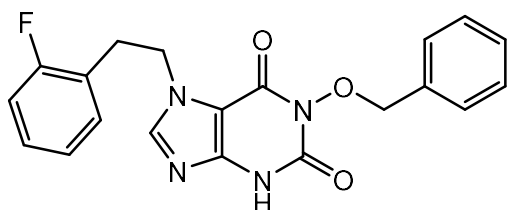
Prepared according to standard procedure 8, using ethyl 4-amino-1-phenethyl-1H-imidazole-5-carboxylate (950 mg, 3.7 mmol) to afford the title compound as a white solid. (979 mg, 74 %, m.p. 168-169 °C). ^1H NMR δ_{H} (400.13 MHz; DMSO): 12.14 (1H, s), 7.78 (1H, s), 7.60-7.14 (10H, m), 5.07 (2H, s), 4.46 (2H, t, J 7.3), 3.10 (2H, t, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 152.9, 149.2, 147.2, 143.4, 138.1, 135.1, 130.0, 129.3, 129.2, 128.9, 128.8, 127.1, 106.1, 77.9, 47.9, 36.8.

1-(benzyloxy)-7-(4-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (14c)



Prepared according to standard procedure 8, using ethyl 4-amino-1-(4-methylphenethyl)-1H-imidazole-5-carboxylate (240 mg, 0.9 mmol) to afford the title compound as a white solid. (71 mg, 22 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.65-6.92 (10H, m), 5.21 (2H, s), 4.47 (2H, t, J 7.1), 3.14 (2H, t, J 7.1), 2.33 (3H, s). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 152.3, 149.3, 147.7, 141.7, 135.7, 133.9, 134.2, 130.1, 129.8, 129.0, 128.6, 128.6, 106.1, 78.3, 41.9, 37.4, 21.3.

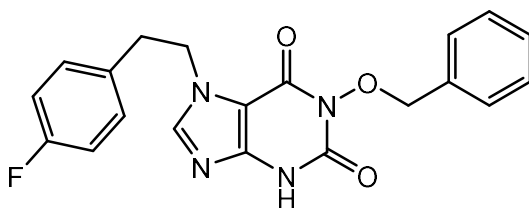
1-(benzyloxy)-7-(2-fluorophenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (14d)



Prepared according to standard procedure 8, using ethyl 4-amino-1-(2-fluorophenethyl)-1*H*-imidazole-5-carboxylate (831 mg, 3.0 mmol) to afford the title compound as a white

solid. (482 mg, 42 %). ¹H NMR δ_H (400.13 MHz; MeOD): 12.15 (1H, s), 7.80 (1H, s), 7.60-7.08 (9H, m), 5.05 (2H, s), 4.48 (2H, t, *J* 6.8), 3.16 (2H, t, *J* 6.8). ¹³C NMR δ_C (101.6 MHz; MeOD): 162.4, 160.0, 152.8, 149.0, 147.2, 143.6, 135.0, 131.8, 129.9, 129.4, 129.3, 128.8, 124.9, 115.7, 106.2, 77.9, 46.6, 30.1.

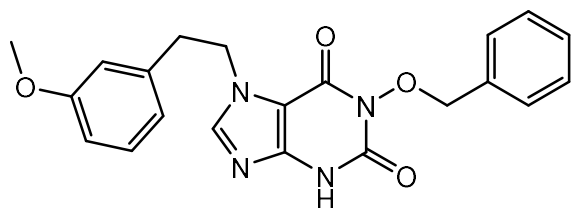
1-(benzyloxy)-7-(4-fluorophenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (14f)



Prepared according to standard procedure 8, using ethyl 4-amino-1-(4-fluorophenethyl)-1*H*-imidazole-5-carboxylate (389 mg, 1.4 mmol) to afford the title

compound as a white solid. (402 mg, 76 %). ¹H NMR δ_H (400.13 MHz; MeOD): 12.15 (1H, s), 7.64-7.38 (5H, m), 7.13 (2H, dd, *J* 8.6 and 5.4), 7.01 (2H, t, *J* 8.8), 5.15 (2H, s), 4.50 (2H, t, *J* 7.0), 3.13 (2H, t, *J* 7.0). ¹³C NMR δ_C (101.6 MHz; MeOD): 168.5, 161.6, 159.2, 151.6, 148.0, 145.2, 141.4, 132.7, 131.9, 128.8, 128.4, 127.3, 126.5, 113.3, 104.6, 76.5, 46.7, 34.3.

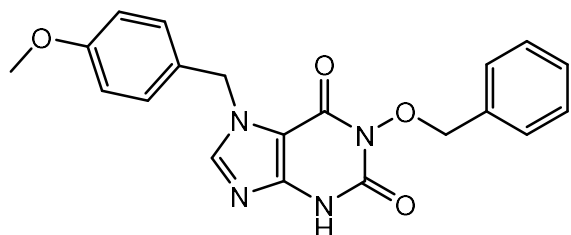
1-(benzyloxy)-7-(3-methoxyphenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (14k)



Prepared according to standard procedure 8, using ethyl 4-amino-1-(3-methoxyphenethyl)-1*H*-imidazole-5-carboxylate (337 mg, 1.16 mmol) to afford

the title compound as a white solid. (210 mg, 54 %). ¹H NMR δ_H (400.13 MHz; MeOD): 12.15 (1H, s), 7.78 (1H, s), 7.60-7.40 (5H, m), 7.20 (1H, t, *J* 7.8), 6.82-6.69 (3H, m), 5.06 (2H, s), 4.46 (2H, t, *J* 7.2), 3.73 (3H, s), 3.07 (2H, t, *J* 7.2). ¹³C NMR δ_C (101.6 MHz; MeOD): 159.7, 152.9, 149.2, 147.3, 143.5, 139.5, 135.1, 129.9, 129.9, 128.8, 121.4, 114.7, 112.6, 106.1, 78.0, 55.4, 49.1, 36.8.

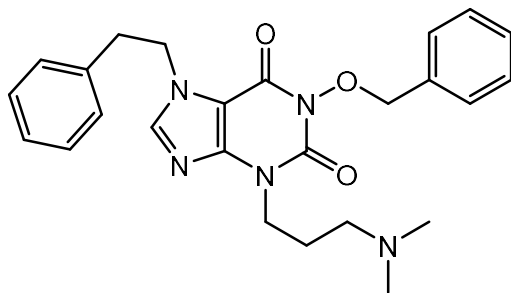
1-(benzyloxy)-7-(4-methoxybenzyl)-3,7-dihydro-1*H*-purine-2,6-dione (14v)



Prepared according to standard procedure 8, using ethyl 4-amino-1-(4-methoxybenzyl)-1*H*-imidazole-5-carboxylate (5.89 g, 21.4 mmol) to afford

the title compound as a white solid. (6.8 g, 85 %). ¹H NMR δ_H (400.13 MHz; DMSO): 12.18 (1H, s), 8.20 (1H, s), 7.57-7.39 (5H, m), 7.35 (2H, d, *J* 8.2), 6.92 (2H, d, *J* 8.2), 5.39 (2H, s), 5.05 (2H, s), 3.74 (3H, s). ¹³C NMR δ_C (101.6 MHz; DMSO): 159.5, 153.0, 149.1, 147.3, 143.4, 135.0, 129.9, 129.8, 129.3, 129.2, 128.8, 114.5, 106.0, 77.9, 55.6, 49.0.

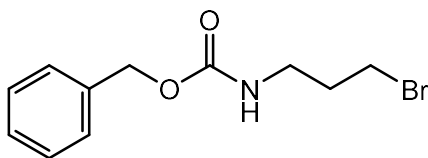
1-(Benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (15)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (125 mg, 0.35 mmol) and 3-dimethylamino-1-propyl chloride hydrochloride (82 mg, 0.52 mmol) to afford the title compound as a

yellow oil (70 mg, 30 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.68-7.08 (11H, m), 5.23 (2H, s), 4.50 (2H, t, J 7.0), 4.19 (2H, t, J 7.0), 3.16 (2H, t, J 7.1), 2.39 (2H, t, J 7.3), 2.25 (6H, s), 1.96 (2H, quin, J 6.7). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 152.5, 149.3, 147.9, 141.5, 137.0, 133.9, 130.1, 129.1, 128.8, 128.7, 128.4, 127.1, 106.2, 78.5, 56.8, 48.9, 45.5, 42.2, 37.2, 26.1.

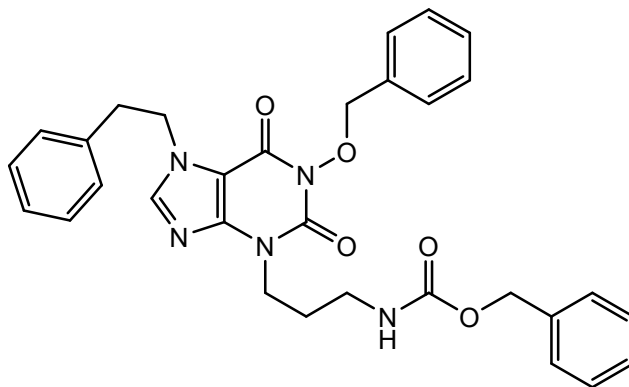
Benzyl (3-bromopropyl)carbamate



Prepared according to standard procedure 6 using 3-bromo propan-1-amine hydrobromide (1 g, 4.6 mmol) to afford the title compound as a colourless oil (1.26 g,

100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.42-7.34 (5H, m), 5.13 (2H, s), 4.95 (1H, s), 3.47 (2H, t, J 6.2), 3.39 (2H, dt, J 6.4 and 6.7), 2.11 (2H, quin, J 6.6). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 156.4, 136.4, 128.6, 128.2, 128.2, 66.9, 39.6, 32.6, 30.6.

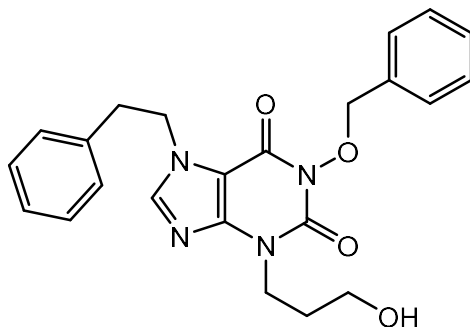
Benzyl (3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)carbamate (15b)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (100 mg, 0.27 mmol) and benzyl (3-bromopropyl)carbamate (110 mg, 0.4 mmol) to afford the title compound

as an orange oil. (140 mg, 93 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.65-7.06 (16H, m), 5.90 (1H, s), 5.21 (2H, s), 5.13 (2H, s), 4.49 (2H, t, J 7.1), 4.19 (2H, t, J 5.8), 3.18-3.12 (4H, m), 1.96 (2H, quin, J 5.8). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 156.5, 152.3, 149.6, 148.0, 141.6, 136.8, 133.8, 133.4, 130.2, 129.2, 128.9, 128.7, 128.5, 128.4, 128.1, 128.1, 127.2, 106.1, 78.6, 66.6, 49.0, 42.1, 37.3, 37.2, 28.3.

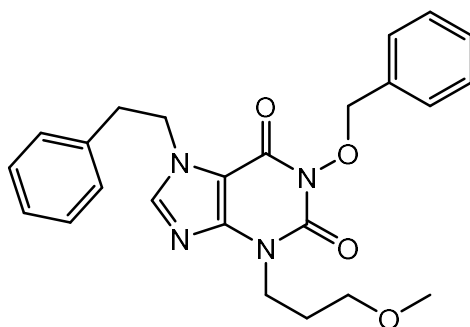
1-(Benzyloxy)-3-(3-hydroxypropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (15c)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (125 mg, 0.35 mmol) and 1-bromo-3-propanol (47 μl , 0.52 mmol) to afford the title compound as brown oil (140 mg, 95 %). ^1H NMR δ_{H}

(400.13 MHz; CDCl_3): 7.60-7.03 (11H, m), 5.20 (2H, s), 4.47 (2H, t, J 7.2), 4.27-4.17 (2H, m), 3.49 (2H, t, J 6.2), 3.12 (2H, t, J 7.2), 2.15 (2H, quin, J 6.2). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 152.3, 149.7, 148.1, 141.4, 136.8, 133.8, 130.2, 129.1, 128.9, 128.7, 128.5, 127.3, 106.0, 78.7, 57.9, 49.1, 40.1, 37.1, 30.9.

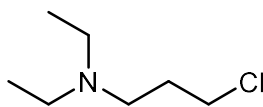
1-(Benzyloxy)-3-(3-methoxypropyl)-7-phenethyl-3,7-dihydro-1*H*-purine-2,6-dione (15d)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1*H*-purine-2,6-dione (125 mg, 0.35 mmol) and 1-bromo-3-methoxy-propane (59 μ l, 0.52 mmol) to afford the title compound as brown oil (131 mg, 82 %). ^1H NMR δ_{H}

(400.13 MHz; CDCl_3): 7.69-7.08 (11H, m), 5.23 (2H, s), 4.51 (2H, t, J 6.6), 4.25 (2H, t, J 6.6), 3.48 (2H, t, J 6.6), 3.34 (3H, s), 3.17 (2H, t, J 6.6), 2.07 (2H, quin, J 6.7). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 152.5, 149.3, 147.9, 141.4, 136.9, 134.0, 130.1, 129.1, 128.8, 128.7, 128.5, 127.2, 106.2, 78.6, 70.1, 58.7, 48.9, 41.3, 37.3, 28.2.

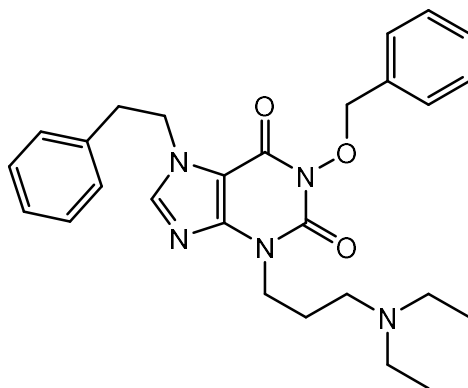
3-Chloro-*N,N*-diethylpropan-1-amine hydrochloride



Prepared according to standard procedure 2 using 3-(dimethylamino)propan-1-ol (628 mg, 4.8 mmol) to afford the title compound as the hydrochloride salt as

brown crystals (320 mg, 36 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.72 (1H, s), 3.77 (2H, t, J 6.6), 3.11 (6H, m), 2.16 (2H, tt, J 6.6 and 8.3), 1.23 (6H, t, J 6.7). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 48.5, 46.9, 42.9, 26.6, 9.0.

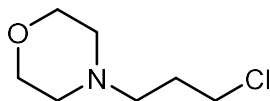
1-(Benzyloxy)-3-(3-(diethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (15e)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (100 mg, 0.27 mmol) and 3-chloro-*N,N*-diethylpropan-1-amine hydrochloride (74 mg, 0.4 mmol) to afford the title compound as an orange solid (117 mg, 91 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.71-7.08 (11H,

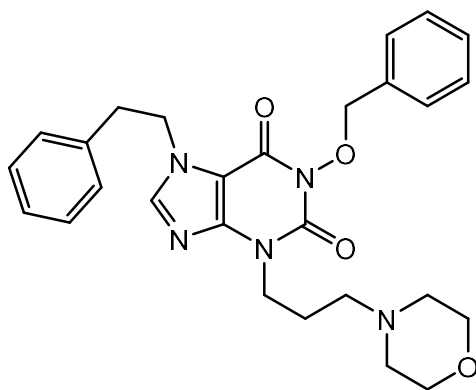
m), 5.23 (2H, s), 4.50 (2H, t, J 7.2), 4.17 (2H, t, J 7.1), 3.16 (2H, t, J 7.2), 2.57 (2H, t, J 7.1), 2.56 (4H, q, J 7.1), 1.96 (2H, quin, J 7.1), 1.03 (6H, t, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 152.5, 149.2, 147.9, 141.6, 137.0, 134.0, 130.1, 129.1, 128.8, 128.8, 128.2, 127.2, 106.2, 50.1, 49.0, 46.8, 42.4, 37.5, 25.5, 11.6.

4-(3-Chloropropyl)morpholine



Prepared according to standard procedure 2 using 3-morpholinopropan-1-ol (400 mg, 2.8 mmol) to afford the title compound as the hydrochloride salt as a pink solid (445 mg 97 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 11.76 (1H, s), 3.96-3.84 (4H, m), 3.77 (2H, t, J 6.8), 3.45-3.37 (4H, m), 3.18 (2H, dt, J 3.9 and 4.9), 2.24 (2H, tt, J 6.5 and 8.0). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 63.5, 54.1, 51.6, 42.9, 26.4.

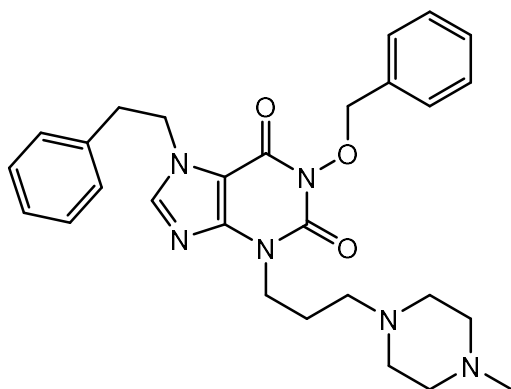
1-(benzyloxy)-3-(3-morpholinopropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (15f)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.22 mmol) and 4-(3-Chloropropyl)morpholine hydrochloride (66 mg, 0.33 mmol) to afford the title compound as a turquoise solid (104 mg, 96 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.68

(1H, s), 7.61-7.13 (10H, m), 5.14 (2H, s), 4.54 (2H, t, J 6.7), 4.31 (2H, t, J 6.5), 4.04 (4H, dd, J 13.1 and 2.9), 3.79 (2H, t, J 8.0), 3.47 (4H, d, J 12.70), 3.45 (2H, t, J 7.2), 2.23 (2H, quin, J 8.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 150.1, 148.3, 145.8, 141.1, 135.8, 132.7, 128.3, 127.4, 127.0, 126.9, 126.6, 125.1, 104.9, 76.6, 62.2, 53.0, 50.4, 46.9, 38.9, 35.2, 21.0.

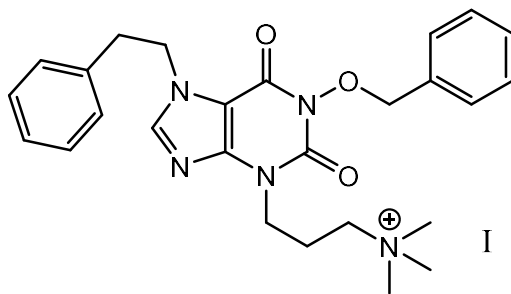
1-(benzyloxy)-3-(3-(4-methylpiperazin-1-yl)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (15g)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(phenethyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.22 mmol) and 1-(3-chloropropyl)-4-methylpiperazine dihydrochloride (84 mg, 0.34 mmol) to afford the title compound as a yellow oil (75 mg, 68 %). ^1H NMR δ_{H} (400.13

MHz; CDCl_3): 7.75-7.12 (11H, m), 5.19 (2H, s), 4.51 (2H, t, J 7.3), 4.22 (2H, t, J 6.7), 3.56-3.44 (4H, m), 3.18 (2H, t, J 6.4), 3.11 (2H, t, J 7.2), 2.82 (3H, s), 2.20 (2H, quin, J 6.3). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 152.2, 149.6, 147.2, 141.5, 136.8, 133.9, 130.1, 129.3, 128.9, 128.8, 128.6, 127.2, 106.4, 78.8, 54.5, 50.6, 48.9, 48.6, 43.2, 40.7, 37.2, 23.2.

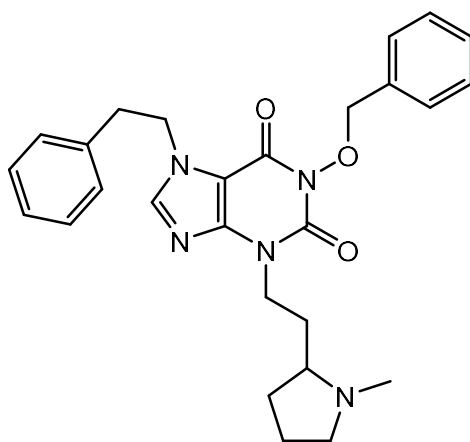
3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)-N,N,N-trimethylpropan-1-aminium iodide (15h)



To a solution of 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (30 mg, 0.07 mmol) in THF (3 ml) was added methyl iodide (20 μ l, 0.32 mmol) and stirred for 2 hours. Solvent

removed *in vacuo* to afford the title compound as a white solid (39 mg, 99 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 7.79 (1H, s), 7.45 (2H, dd, J 2.1 and 6.8), 7.43-7.38 (3H, m), 7.29-7.18 (3H, m), 7.12 (2H, d, J 7.2), 5.05 (2H, s), 4.46 (2H, t, J 7.0), 4.06 (2H, t, J 6.6), 3.32 (2H, t, J 7.0), 3.07 (2H, t, J 6.6), 2.99 (9H, s), 2.15-2.09 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 152.3, 149.3, 147.7, 143.3, 137.7, 134.5, 130.0, 129.2, 129.0, 128.9, 127.2, 106.3, 77.9, 52.9, 48.6, 41.2, 36.6, 21.9.

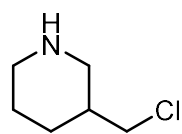
(RS) 1-(Benzyloxy)-3-(2-(1-methylpyrrolidin-2-yl)ethyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (15i)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (100 mg, 0.27 mmol) and (RS) 2-(2-chloroethyl)-1-methyl-pyrrolidine hydrochloride (74 mg, 0.4 mmol) to afford the title compound as an orange oil (117 mg, 87 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.67-7.06 (11H, m), 5.21 (2H, s), 4.49 (2H, t, J 7.2),

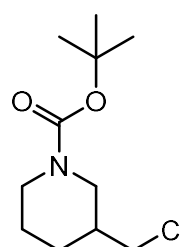
4.19-4.14 (2H, m), 3.15 (2H, t, J 7.2), 2.72-2.53 (1H, m), 2.33 (3H, s), 2.19-2.11 (2H, m), 1.84-1.59 (6H, m). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 152.5, 149.3, 147.8, 141.6, 136.9, 134.1, 130.1, 129.1, 128.8, 128.7, 128.5, 127.1, 106.2, 78.57, 63.9, 57.1, 49.0, 41.2, 40.5, 37.2, 30.7, 30.5, 29.7.

(RS) 3-(chloromethyl)piperidine hydrochloride



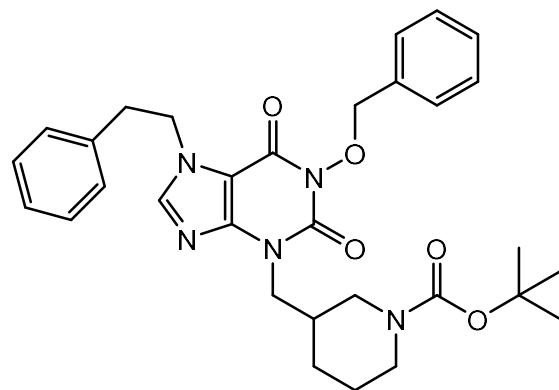
Prepared according to standard procedure 2 using (RS) 3-piperidinemethanol (333 mg, 2.9 mmol) to afford the title compound as brown crystals (465 mg, 86 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 9.29-8.82 (2H, m), 3.64 (1H, dq, J 9.9 and 5.6), 3.37-3.16 (5H, m), 2.77-2.64 (1H, m), 1.87-1.61 (2H, m), 1.35-1.10 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 63.5, 46.3, 43.7, 36.3, 25.3, 21.8.

(RS) *tert*-butyl 3-(chloromethyl)piperidine-1-carboxylate



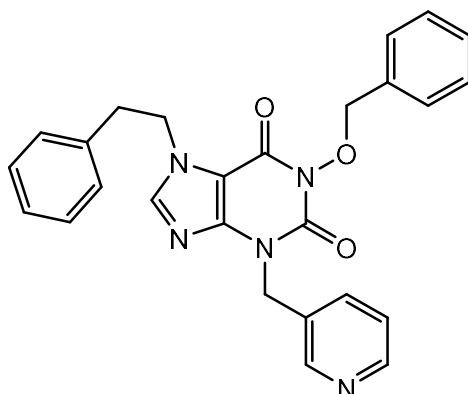
Made according to standard procedure 6 using (RS) 3-(chloromethyl)piperidine hydrochloride (233 mg, 1.25 mmol) to afford the title compound as a white solid (310 mg, 99 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 9.29-8.82 (2H, m), 3.64 (1H, dq, J 9.9 and 5.6), 3.28-3.11 (5H, m), 2.79-2.61 (1H, m), 1.76-1.58 (2H, m), 1.33-1.08 (2H, m), 1.49 (9H, s). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 154.8, 79.3, 60.1, 44.2, 40.9, 36.0, 28.2, 25.1, 21.6.

(RS) *tert*-butyl 3-((1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)methyl)piperidine-1-carboxylate (15j)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(phenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (80 mg, 0.22 mmol) and (RS) *tert*-butyl 3-(chloromethyl)piperidine-1-carboxylate (82 mg, 0.33 mmol) to afford the title compound as a yellow oil (64 mg, 52 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.67-7.09 (11H, m), 5.18 (2H, s), 4.54 (2H, t, J 6.4), 4.11-3.88 (4H, m), 3.55-3.22 (4H, m), 3.15 (2H, t, J 6.9), 1.72-1.60 (4H, m), 1.48 (9H, s). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 155.0, 152.4, 149.6, 147.8, 142.7, 137.3, 134.1, 130.0, 128.6, 128.3, 128.1, 126.6, 106.1, 79.5, 64.1, 49.5, 46.5, 38.4, 36.7, 35.3, 30.3, 28.1, 27.5, 26.9, 24.4.

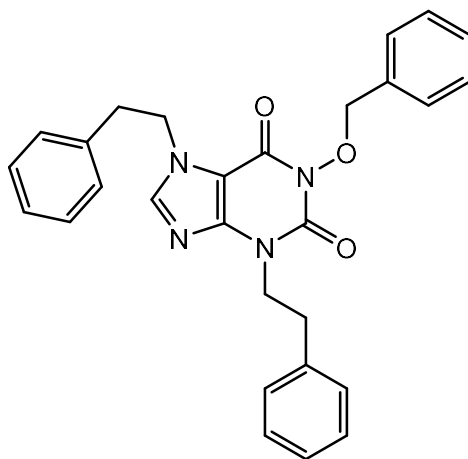
1-(benzyloxy)-7-phenethyl-3-(pyridin-3-ylmethyl)-3,7-dihydro-1H-purine-2,6-dione (15l)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(phenethyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.22 mmol) and 3-(chloromethyl)pyridine hydrochloride (54 mg, 0.33 mmol) to afford the title compound as a yellow oil (99 mg, 99 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 8.78 (1H, d, *J* 1.7), 8.55 (1H, dd, *J* 4.9

and 1.5), 7.85 (1H, dt, *J* 8.1 and 2), 7.63-7.60 (2H, m), 7.40-7.05 (10H, m), 5.29 (2H, s), 5.21 (2H, s), 4.48 (2H, t, *J* 7.3), 3.14 (2H, t, *J* 7.3). ¹³C NMR δ_C (101.6 MHz; CHCl₃): 152.2, 150.2, 149.4, 147.5, 141.6, 137.8, 133.7, 131.8, 130.1, 129.2, 129.0, 128.9, 128.8, 128.7, 128.5, 128.2, 127.2, 123.5, 106.3, 78.6, 49.0, 44.3, 47.2.

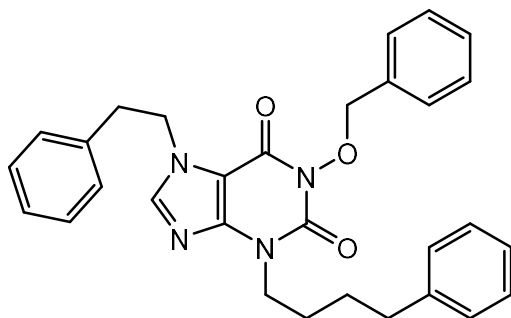
1-(Benzyloxy)-3,7-diphenethyl-3,7-dihydro-1H-purine-2,6-dione (15m)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.22 mmol) and phenethyl bromide (45 μ l, 0.33 mmol) to afford the title compound as a yellow oil (92 mg, 90 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.70-7.10 (16H, m), 5.21 (2H, s), 4.51 (2H, t, *J* 6.7), 4.39 (2H, t, *J* 7.3), 3.17 (2H, t, *J* 6.7), 3.13

(2H, t, *J* 7.3). ¹³C NMR δ_C (101.6 MHz; CHCl₃): 156.4, 149.1, 147.8, 141.6, 137.9, 137.0, 134.0, 130.2, 128.8, 128.8, 128.6, 128.5, 128.3, 127.2, 126.6, 125.4, 106.2, 78.6, 48.9, 44.8, 37.3, 34.1.

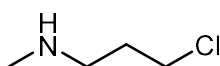
1-(Benzyloxy)-7-phenethyl-3-(4-phenylbutyl)-3,7-dihydro-1H-purine-2,6-dione (15n)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.22 mmol) and 1-bromo-4-phenylbutane (58 μ l, 0.33 mmol) to afford the title compound as a yellow oil (107 mg,

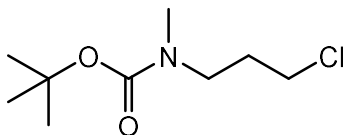
98 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.60-7.03 (16H, m), 5.19 (2H, s), 4.46 (2H, t, J 8.1), 4.17 (2H, t, J 6.4), 3.12 (2H, t, J 7.0), 2.68-2.61 (2H, m), 1.71-1.60 (4H, m). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 152.4, 149.3, 147.9, 141.9, 136.9, 137.0, 134.0, 130.1, 128.8, 128.7, 128.6, 128.4, 128.4, 127.1, 126.2, 125.9, 106.1, 78.5, 48.9, 43.7, 37.3, 34.9, 28.1, 27.6.

3-Chloro-N-methylpropan-1-amine



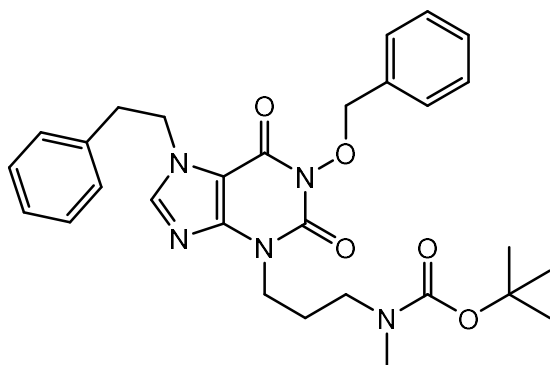
Prepared according to standard procedure 2 using 3-(methylamino)propan-1-ol (200 mg, 2.2 mmol) to afford the title compound as the hydrochloride salt as white crystals (314 mg, 97 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 9.66 (2H, s), 3.74 (2H, t, J 6.1), 3.17 (2H, tt, J 5.2 and 2.3), 2.74 (3H, t, J 5.7), 2.40 (2H, tt, J 6.3 and 1.9). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 47.1, 41.3, 33.1, 28.7.

***tert*-Butyl (3-chloropropyl)(methyl)carbamate**



Prepared according to standard procedure 6 using 3-chloro-N-methylpropan-1-amine hydrochloride (282 mg, 1.97 mmol) to afford the title compound as a colourless oil (401 mg, 98 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 3.57 (2H, t, J 6.6), 3.38 (2H, t, J 6.6), 2.90 (3H, s), 2.05-1.98 (2H, m), 1.49 (9H, s). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 155.5, 85.4, 53.5, 46.4, 42.6, 28.5, 27.4.

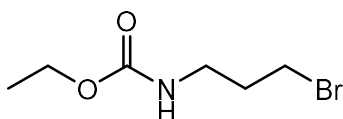
tert-butyl (3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)(methyl)carbamate (15o)



Prepared according to standard procedure 1 using 1-(Benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (120 mg, 0.33 mmol) and *tert*-Butyl (3-chloropropyl)(methyl)carbamate (105 mg, 0.5 mmol) to afford the title compound as a orange solid

(165 mg, 94 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.67-7.08 (11H, m), 5.22 (2H, s), 4.49 (2H, t, J 6.7), 4.14 (2H, t, J 7.3), 3.37 (2H, t, J 7.6), 3.16 (2H, t, J 7.1), 2.88 (3H, s), 2.01 (2H, quin, J 8.01), 1.28 (9H, s). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 155.7, 152.4, 149.0, 147.7, 141.5, 136.9, 133.9, 130.1, 129.1, 128.8, 128.7, 128.4, 127.1, 106.2, 78.47, 48.9, 46.3, 41.5, 37.4, 32.8, 28.5, 22.6.

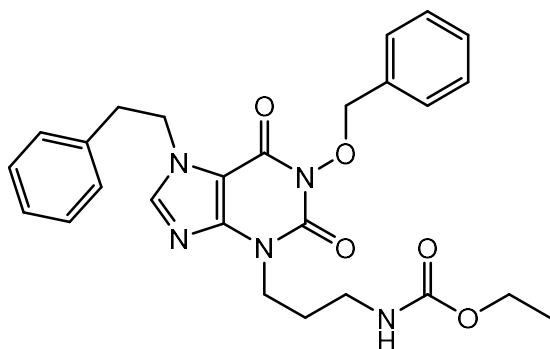
ethyl (3-bromopropyl)carbamate



A solution of 3-Bromopropylamine hydrobromide (600 mg, 2.7 mmol), ethyl chloroformate (257 μl , 2.7 mmol) and

triethylamine (1.126 ml, 8.1 mmol) in anhydrous DCM (15 ml) was stirred at 0 °C for 4 hours. The solution was washed with water (15 ml), dried (MgSO_4), filtered and the solvent was removed *in vacuo* to afford the title compound as a pink oil (467 mg, 83 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 4.87 (1H, s), 4.12 (2H, q, J 7.1), 3.46 (2H, t, J 6.5), 3.35 (2H, dt, J 6.5 and 6.1), 2.08 (2H, quin, J 6.5), 1.26 (3H, t, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 156.9, 61.0, 38.7, 33.0, 31.4, 14.6.

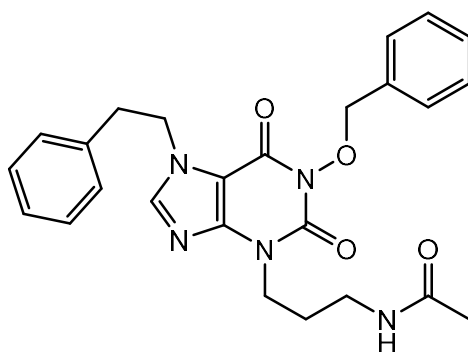
ethyl (3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)carbamate (15p)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(phenethyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.22 mmol) and ethyl (3-bromopropyl)carbamate (69 mg, 0.34 mmol) to afford the title compound as a yellow oil (92

mg, 91 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.67-7.08 (11H, m), 5.24 (2H, s), 4.58 (2H, t, J 6.8), 4.21 (2H, t, J 6.0), 4.15 (2H, q, J 6.8), 3.81 (2H, t, J 6.0), 3.12 (2H, t, J 6.0), 1.97 (2H, quin, J 6.1), 1.29 (3H, t, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 156.7, 152.3, 149.6, 148.0, 141.5, 137.9, 136.8, 130.2, 129.0, 128.9, 128.7, 128.5, 128.2, 125.3, 106.2, 78.7, 60.8, 49.1, 40.8, 37.3, 32.1, 29.7, 21.5, 14.9.

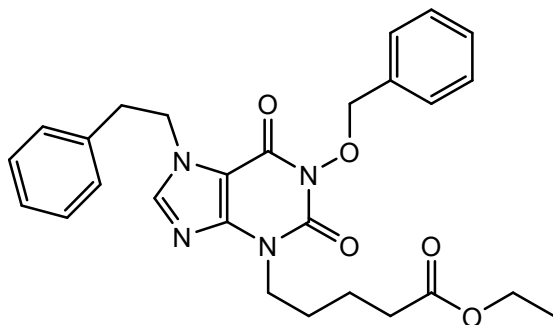
***N*-(3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)acetamide (15q)**



A mixture of 3-(3-aminopropyl)-1-(benzyloxy)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (60 mg, 0.14 mmol), acetic anhydride (132 μl , 0.14 mmol), CHCl_3 (2 ml) and saturated aqueous sodium bicarbonate solution (1 ml) was stirred at rt for 4 hours. The organic phase was separated and

solvent removed *in vacuo* to afford the title compound as a yellow oil (55 mg, 85 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.58-7.04 (11H, m), 6.85 (1H, t, J 5.9), 5.16 (2H, s), 4.47 (2H, t, J 7.3), 4.12 (2H, t, J 6.8), 3.16-3.09 (4H, m), 2.00 (3H, s), 1.91 (2H, quin, J 6.3). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 171.3, 151.2, 149.8, 147.4, 141.8, 136.8, 133.6, 130.1, 129.2, 128.8, 128.7, 128.4, 127.1, 106.3, 78.4, 49.0, 40.9, 37.1, 35.8, 27.5, 23.0.

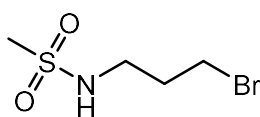
ethyl 5-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)pentanoate (15r)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(phenethyl)-3,7-dihydro-1H-purine-2,6-dione (140 mg, 0.39 mmol) and ethyl 5-bromovalerate (92 μ l, 0.58 mmol) to afford the title compound as a yellow oil

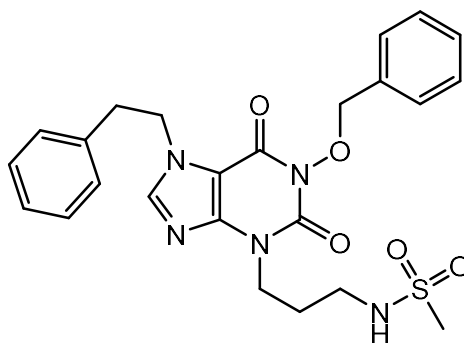
(182 mg, 95 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.76-7.08 (11H, m), 5.23 (2H, s), 4.50 (2H, t, J 7.0), 4.15 (2H, t, J 7.0), 3.16 (2H, t, J 7.0), 2.38 (4H, m), 1.84 (2H, t, quin, J 7.3), 1.72 (2H, t, J 7.3), 1.27 (3H, q, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 173.2, 152.5, 149.3, 147.8, 141.5, 137.0, 133.9, 130.1, 129.0, 128.9, 128.7, 128.4, 128.2, 106.2, 78.6, 60.3, 48.8, 43.3, 37.2, 33.9, 27.5, 22.0, 14.3.

***N*-(3-bromopropyl)methanesulfonamide**



To a stirred solution of 3-bromopropylamine hydrobromide (600 mg, 2.7 mmol) and triethylamine (495 μ l, 4.1 mmol) in THF (15 ml) at 5 $^{\circ}\text{C}$ was added methanesulphonyl chloride (273 μ l, 3.5 mmol) and the solution stirred for 3 hours. The resulting mixture was filtered and the solvent was removed *in vacuo* to afford the title compound as an orange oil (390 mg, 67 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.96 (1H, s), 3.54 (2H, t, J 6.5), 3.35 (2H, dt, J 6.5 and 6.4), 3.02 (3H, s), 2.16 (2H, quin, J 6.5). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 41.4, 40.5, 32.7, 30.1.

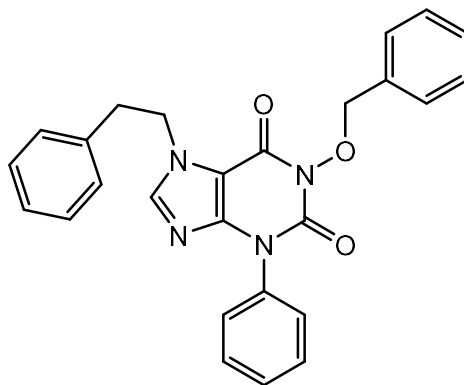
***N*-(3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)propyl)methanesulfonamide (15t)**



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(phenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (80 mg, 0.22 mmol) and *N*-(3-bromopropyl)methanesulfonamide (70 mg, 0.33 mmol) to afford the title compound as a yellow oil (48 mg, 42 %). ¹H NMR δ_H (400.13 MHz; CDCl₃):

7.65-7.07 (11H, m), 5.90 (1H, t, *J* 6.7), 5.21 (2H, s), 4.51 (2H, t, *J* 6.9), 4.22 (2H, t, *J* 6.2), 3.16 (2H, t, *J* 6.9), 3.07 (2H, q, *J* 6.2), 2.93 (3H, s), 2.03 (2H, quin, *J* 6.9). ¹³C NMR δ_C (101.6 MHz; CHCl₃): 152.2, 149.6, 147.8, 141.5, 136.8, 133.7, 130.1, 129.2, 128.9, 128.7, 128.5, 127.2, 106.2, 78.6, 49.0, 40.7, 40.5, 39.4, 37.1, 28.9.

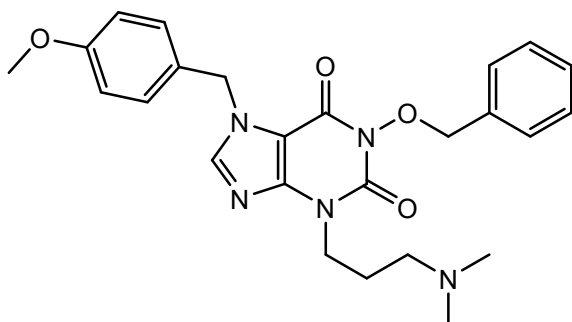
1-(Benzyloxy)-7-phenethyl-3-phenyl-3,7-dihydro-1*H*-purine-2,6-dione (15u)



A solution of 1-(benzyloxy)-7-phenethyl-3,7-dihydro-1*H*-purine-2,6-dione (150 mg, 0.41 mmol), phenyl boronic acid (92 mg, 0.82 mmol), copper (II) acetate (113 mg, 0.62 mmol), and pyridine (66 μl, 0.82 mmol) in DCM (10 ml) was stirred open to air, at rt for 7 days. DCM (10 ml) was added and the mixture filtered through

celite, washed with saturated aqueous EDTA solution (10 ml) and the solvent was removed *in vacuo* to afford a yellow oil. Purification by column chromatography eluting with EtOAc:hexane 1:1 to afford the title compound as a colourless glass (77 mg, 43 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.69-7.14 (16H, m), 5.29 (2H, s), 4.55 (2H, t, *J* 6.4), 3.20 (2H, t, *J* 6.8). ¹³C NMR δ_C (101.6 MHz; CHCl₃): 152.5, 149.2, 148.1, 141.5, 136.9, 134.4, 133.9, 130.2, 129.5, 129.2, 129.2, 128.9, 128.8, 128.5, 128.1, 127.2, 106.4, 78.8, 49.0, 37.4. HRMS (ESI⁺) [M+H]⁺ expected 439.1765 found 439.1762 [M+Na]⁺ expected 461.1584 found 461.1581

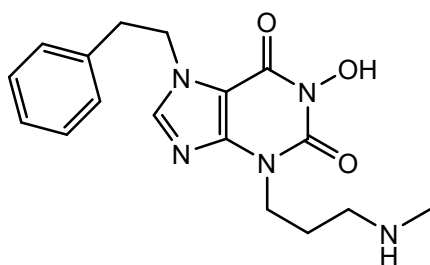
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-methoxybenzyl)-3,7-dihydro-1H-purine-2,6-dione (15v)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(4-methoxybenzyl)-3,7-dihydro-1H-purine-2,6-dione (6.8 g, 18.1 mmol) and 3-dimethylamino-1-propyl

chloride hydrochloride (3.31 g, 21.7 mmol) to afford the title compound as a yellow oil (7.48 g, 90 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.61-7.31 (5H, m), 7.55 (1H, s), 7.29 (2H, d, J 8.7), 6.85 (2H, d, J 8.7), 5.37 (2H, s), 5.15 (2H, s), 4.13 (2H, t, J 7.3), 4.09 (2H, q, J 7.3), 3.74 (3H, s), 2.35 (2H, t, J 7.3), 2.19 (6H, s), 1.90 (2H, quin, J 7.3), 1.22 (3H, t, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 159.8, 152.5, 149.1, 147.7, 141.1, 133.9, 130.0, 129.9, 129.0, 128.4, 127.1, 114.4, 106.4, 78.4, 60.3, 56.7, 55.3, 49.9, 45.3, 42.0, 26.1.

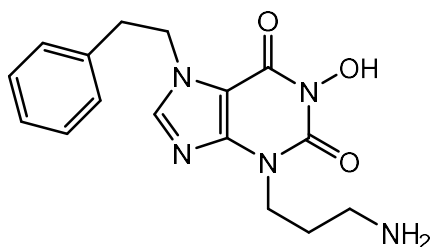
1-hydroxy-3-(3-(methylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16a)



Prepared according to standard procedure 7 using tert-butyl (3-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)(methyl)carbamate (75 mg, 0.17 mmol) to afford the title compound as a white solid (17 mg, 29 %). ^1H NMR δ_{H}

(400.13 MHz; DMSO): 10.58 (1H, s), 8.30 (1H, brs), 7.90 (1H, s), 7.33-7.16 (5H, m), 4.49 (2H, t, J 7.6), 4.07 (2H, t, J 6.4), 3.12 (2H, t, J 6.4), 2.93 (2H, t, J 7.6), 2.00 (2H, quin, J 7.6). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 153.0, 150.4, 147.0, 142.5, 137.8, 128.5, 128.3, 126.6, 106.1, 48.3, 46.2, 40.0, 36.5, 32.2, 24.7. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{17}\text{H}_{22}\text{N}_5\text{O}_3$, 344.1717: found 344.1724. $[\text{M}+\text{Na}]$ expected for $\text{C}_{17}\text{H}_{21}\text{N}_5\text{O}_3\text{Na}$, 366.1537: found 366.1534.

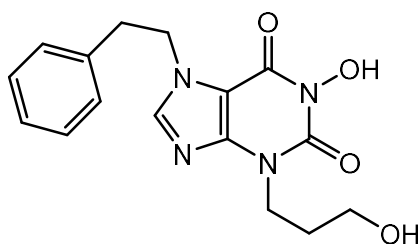
3-(3-aminopropyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16b)



Prepared according to standard procedure 7 using *tert*-butyl (3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)carbamate (231 mg, 0.44 mmol) to afford the title compound as a pink solid (176 mg, 95 %). ^1H NMR δ_{H}

(400.13 MHz; DMSO): 10.58 (1H, s), 7.91 (1H, s), 7.71 (2H, brs), 7.34-7.16 (5H, m), 4.49 (2H, t, J 7.3), 4.07 (2H, t, J 6.8), 3.12 (2H, t, J 7.3), 2.87-2.81 (2H, m), 1.97 (2H, quin, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 153.0, 150.4, 147.0, 142.5, 137.3, 128.5, 128.3, 126.5, 106.1, 40.1, 39.1, 36.7, 36.6, 26.0. HRMS (ESI $^{+}$) $[\text{M}+\text{H}]$ expected for $\text{C}_{16}\text{H}_{20}\text{N}_5\text{O}_3$, 330.1561: found 330.1572. $[\text{M}+\text{Na}]$ expected for $\text{C}_{16}\text{H}_{19}\text{N}_5\text{O}_3\text{Na}$, 352.1380: found 352.1393.

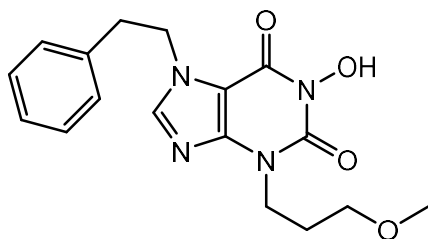
1-Hydroxy-3-(3-hydroxypropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16c)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-hydroxypropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (140 mg, 0.33 mmol) to afford the title compound as an orange oil (112 mg, 90 %). ^1H NMR δ_{H} (400.13 MHz; DMSO):

7.72 (1H, s, H-8), 7.30-7.13 (5H, m), 4.44-4.39 (2H, m), 4.14 (2H, t, J 6.5), 3.49 (2H, t, J 6.5), 3.08 (2H, t, J 6.5), 2.02-1.92 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 154.8, 152.5, 145.6, 140.5, 137.2, 128.6, 128.6, 126.7, 106.7, 58.2, 48.6, 40.5, 36.9, 31.7. HRMS (ESI $^{+}$) $[\text{M}+\text{Na}]$ expected for $\text{C}_{16}\text{H}_{18}\text{N}_4\text{O}_4\text{Na}$, 353.1220: found 353.1235.

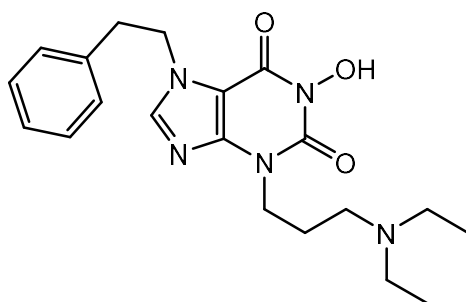
1-Hydroxy-3-(3-methoxypropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16d)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-methoxypropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (123 mg, 0.28 mmol) to afford the title compound as orange oil (59 mg, 61 %). ^1H NMR δ_{H}

(400.13 MHz; DMSO): 7.77 (1H, s, H-8), 7.31-7.15 (5H, m), 4.43 (2H, t, J 7.7), 4.02 (2H, t, J 7.0), 3.37-3.32 (2H, m), 3.19 (3H, s), 3.08 (2H, t, J 7.7), 1.86 (2H, quin, J 7.7). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 1.53, 150.7, 146.4, 140.7, 137.2, 128.7, 128.7, 126.7, 106.5, 69.9, 58.5, 48.8, 41.4, 37.1, 28.9. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{17}\text{H}_{21}\text{N}_4\text{O}_4$, 345.1557: found 345.1561. [M+Na] expected for $\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_4\text{Na}$, 367.1372: found 367.1392.

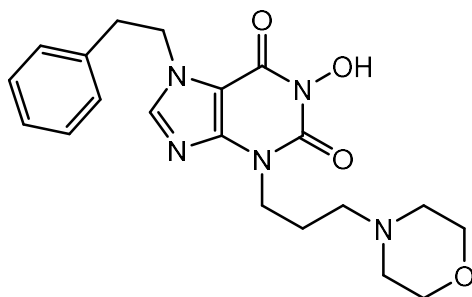
3-(3-(Diethylamino)propyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16e)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(diethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (117 mg, 0.25 mmol) to afford the title compound as a brown oil (81 mg, 88 %). ^1H NMR δ_{H} (400.13 MHz;

DMSO): 10.58 (1H, s), 7.89 (1H, s), 7.33-7.17 (5H, m), 4.49 (2H, t, J 7.2), 4.06 (2H, t, J 7.1), 3.12 (2H, t, J 7.1), 2.54-2.50 (6H, m), 2.02-1.94 (2H, m), 1.10 (6H, t, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.6, 149.4, 146.5, 141.3, 137.0, 128.8, 128.8, 127.1, 106.4, 48.9, 46.4, 41.2, 37.2, 29.8, 22.8, 14.2. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{20}\text{H}_{28}\text{N}_5\text{O}_3$, 386.2187: found 386.2194. [M+Na] expected for $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_3\text{Na}$, 408.2006: found 408.2010.

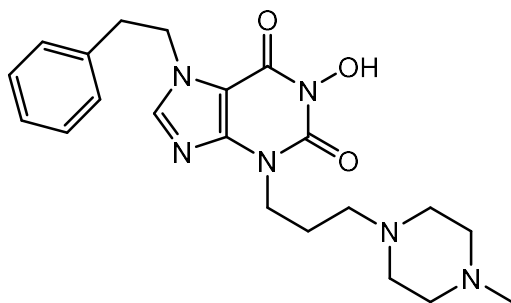
1-hydroxy-3-(3-morpholinopropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16f)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-morpholinopropyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (104 mg, 0.21 mmol) to afford the title compound as a colourless glass (30 mg, 36 %). ^1H NMR δ_{H} (400.13 MHz;

MeOD): 7.69 (1H, s), 7.31-7.15 (5H, m), 4.57 (2H, t, J 7.0), 4.24 (2H, t, J 7.0), 3.89-3.84 (4H, m), 3.21-3.11 (6H, m), 2.21 (2H, quin, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 153.1, 150.3, 147.0, 142.4, 137.3, 128.9, 128.5, 128.3, 126.6, 106.2, 64.2, 54.6, 52.0, 48.1, 40.5, 36.6, 22.7. HRMS (ESI $^+$) [M+H] $^+$ expected for $\text{C}_{20}\text{H}_{26}\text{N}_5\text{O}_4$, 400.1979: found 400.1980. [M+Na] $^+$ expected for $\text{C}_{20}\text{H}_{25}\text{N}_5\text{O}_4\text{Na}$, 422.1799: found 422.1792.

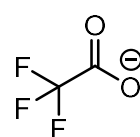
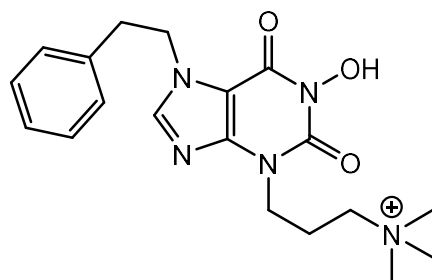
1-hydroxy-3-(3-(4-methylpiperazin-1-yl)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16g)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(4-methylpiperazin-1-yl)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (75 mg, 0.15 mmol) to afford the title compound as a yellow oil (60 mg, 97 %). ^1H NMR

δ_{H} (400.13 MHz; DMSO): 10.56 (1H, s), 7.89 (1H, s), 7.34-7.15 (5H, m), 4.49 (2H, t, J 7.4), 4.05 (2H, t, J 7.4), 3.12 (2H, t, J 6.2), 2.77-2.71 (2H, m), 2.55 (8H, s), 2.17 (3H, s), 1.96-1.86 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.8, 150.0, 143.4, 142.5, 137.2, 128.5, 128.3, 126.5, 106.4, 54.0, 52.0, 49.2, 42.2, 39.1, 36.6, 22.8. HRMS (ESI $^+$) [M+H] $^+$ expected for $\text{C}_{21}\text{H}_{29}\text{N}_6\text{O}_3$, 413.2296: found 413.2297. [M+Na] $^+$ expected for $\text{C}_{21}\text{H}_{28}\text{N}_6\text{O}_3\text{Na}$, 435.2115: found 435.2100.

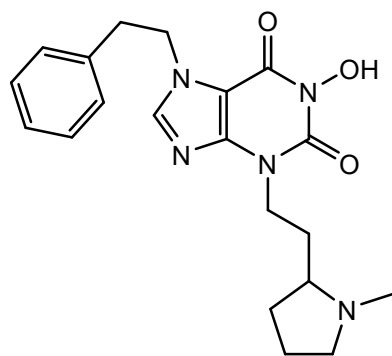
3-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)-N,N,N-trimethylpropan-1-aminium 2,2,2-trifluoroacetate (16h)



A solution of 3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)-N,N,N-trimethylpropan-1-aminium iodide (54 mg, 0.09 mmol) in

TFA (5 ml) was stirred at 120 °C under microwave radiation for 20 minutes. Solvent removed *in vacuo* to afford the title compound as a yellow oil (32 mg, 98 %). ¹H NMR δ_H (400.13 MHz; MeOD): 7.70 (1H, s), 7.31-7.17 (5H, m), 4.75 (2H, t, *J* 6.8), 4.23 (2H, t, *J* 6.1), 3.53-3.47 (2H, m), 3.19 (2H, t, *J* 6.8), 3.16 (9H, s), 2.35-2.27 (2H, m). ¹³C NMR δ_C (101.6 MHz; MeOD): 153.0, 150.3, 146.9, 142.5, 137.3, 128.5, 128.3, 126.5, 106.3, 63.7, 52.3, 48.5, 40.3, 36.6, 21.9. HRMS (ESI⁺) [*M*+]⁺ expected for C₁₉H₂₆N₅O₃, 372.2030: found 372.2034

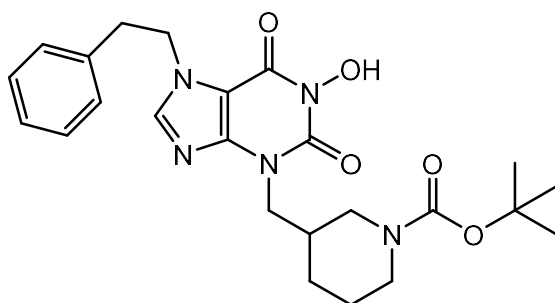
(RS) 1-Hydroxy-3-(2-(1-methylpyrrolidin-2-yl)ethyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (16i)



Prepared according to standard procedure 3 using (RS) 1-(benzyloxy)-3-(2-(1-methylpyrrolidin-2-yl)ethyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (67 mg, 0.14 mmol) to afford the title compound as an orange solid (47 mg, 88 %). ¹H NMR δ_H (400.13 MHz; DMSO): 10.53 (1H, s), 7.88 (1H, s), 7.32-7.15 (5H, m), 4.48 (2H, t, *J* 7.1),

4.05 (H₂, t, *J* 8.2), 3.12 (2H, t *J* 7.1), 2.41-2.28 (3H, m), 2.04 (3H, s), 1.77-1.67 (9H, m), 1.58-1.49 (2H, m). ¹³C NMR δ_C (101.6 MHz; MeOD): 153.0, 150.3, 146.5, 140.9, 137.1, 128.8, 128.8, 127.1, 106.4, 65.2, 56.3, 48.9, 41.1, 39.8, 37.2, 30.1, 29.8, 22.8. HRMS (ESI⁺) [*M*+H]⁺ expected for C₂₀H₂₆N₅O₃, 384.2030: found 384.2021.

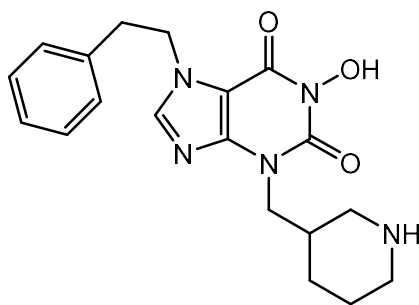
(RS) *tert*-butyl 3-((1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)methyl)piperidine-1-carboxylate (16j)



Prepared according to standard procedure 3 using (RS) *tert*-butyl 3-((1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)methyl)piperidine-1-carboxylate (64 mg, 0.14 mmol) to afford the title compound as a

yellow oil (12 mg, 18 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.56 (1H, s), 7.84 (1H, s), 7.34-7.10 (5H, m), 4.48 (2H, t, J 7.7), 3.88 (2H, d, J 6.9), 3.80 (2H, dt, J 13.3 and 4.0), 3.32-3.17 (3H, m), 3.11 (2H, t, J 6.8), 1.71-1.54 (4H, m), 1.40 (9H, s). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 154.8, 150.9, 148.6, 147.2, 141.3, 136.8, 128.9, 128.7, 127.2, 106.0, 79.7, 50.8, 49.0, 37.3, 35.0, 30.8, 28.4, 28.0, 26.9, 23.9. HRMS (ESI $^{+}$) $[\text{M}+\text{H}]$ expected for $\text{C}_{24}\text{H}_{32}\text{N}_5\text{O}_5$, 470.2398: found 470.2406. $[\text{M}+\text{Na}]$ expected for $\text{C}_{24}\text{H}_{31}\text{N}_5\text{O}_5\text{Na}$, 492.2217: found 492.2221.

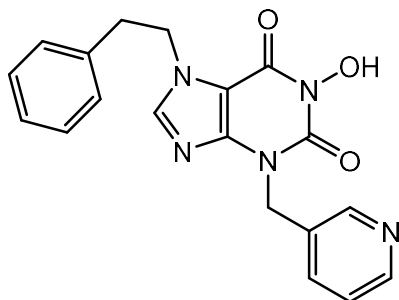
(RS) 1-hydroxy-7-phenethyl-3-(piperidin-3-ylmethyl)-3,7-dihydro-1H-purine-2,6-dione (16k)



Prepared according to standard procedure 7 using (RS) *tert*-butyl 3-((1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)methyl)piperidine-1-carboxylate (80 mg, 0.22 mmol) to afford the title compound as a pink solid (5 mg, 8 %). ^1H NMR δ_{H} (400.13 MHz; DMF): 7.75-7.38

(6H, m), 4.47 (2H, t, J 7.4), 4.24 (2H, d, J 7.4), 3.70-3.57 (3H, m), 3.08 (2H, t, J 6.8), 3.10 (2H, quin, J 1.7), 2.93 (2H, quin, J 2.0), 2.15-1.96 (4H, m). ^{13}C NMR δ_{C} (101.6 MHz; DMF): 153.0, 150.3, 147.6, 142.5, 138.2, 129.1, 128.7, 126.9, 106.2, 63.4, 48.3, 47.0, 45.9, 40.6, 27.1, 26.4, 25.4, 22.0. HRMS (ESI $^{+}$) $[\text{M}+\text{H}]$ expected for $\text{C}_{19}\text{H}_{24}\text{N}_5\text{O}_3$, 370.1874: found 370.1872. $[\text{M}+\text{Na}]$ expected for $\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}_3\text{Na}$, 392.1693: found 392.1690.

1-hydroxy-7-phenethyl-3-(pyridin-3-ylmethyl)-3,7-dihydro-1*H*-purine-2,6-dione (16l)

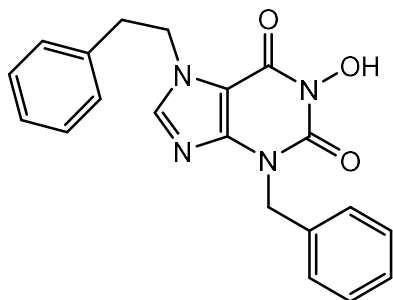


Prepared according to standard procedure 3 using benzyloxy)-7-phenethyl-3-(pyridin-3-ylmethyl)-3,7-dihydro-1*H*-purine-2,6-dione (99 mg, 0.22 mmol) to afford the title compound as an orange solid (35 mg, 44 %).

^1H NMR δ_{H} (400.13 MHz; DMSO): 10.64 (1H, s), 8.69-8.56 (2H, m), 7.90 (1H, s),

7.55-7.48 (2H, m), 7.32-7.15 (5H, m), 5.24 (2H, s), 4.49 (2H, t, J 7.4), 3.12 (2H, t, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 152.9, 150.2, 146.8, 144.0, 143.4, 143.3, 142.5, 143.2, 142.6, 137.2, 128.6, 128.3, 126.6, 106.2, 99.9, 43.5, 36.6. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{19}\text{H}_{18}\text{N}_5\text{O}_3$, 364.1404: found 364.1402. $[\text{M}+\text{Na}]$ expected for $\text{C}_{19}\text{H}_{19}\text{N}_5\text{O}_3\text{Na}$, 386.1224: found 386.1214.

1-Hydroxy-3,7-diphenethyl-3,7-dihydro-1*H*-purine-2,6-dione (16m)

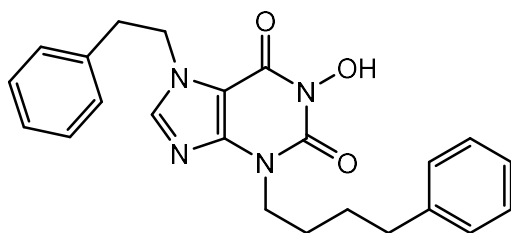


Prepared according to standard procedure 3 using 1-(benzyloxy)-3,7-diphenethyl-3,7-dihydro-1*H*-purine-2,6-dione (92 mg, 0.2 mmol) to afford the title compound as a cream solid (67 mg, 89 %).

^1H NMR δ_{H} (400.13 MHz; DMSO): 10.58 (1H, s), 7.67 (1H, s), 7.39-7.13 (10H, m), 4.45 (2H, t, J

7.7), 4.28 (2H, t, J 6.3), 3.61 (2H, t, J 7.7), 2.74 (2H, t, J 7.7). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 155.2, 150.1, 145.1, 141.1, 139.0, 137.5, 128.8, 128.7, 128.1, 128.0, 127.1, 126.4, 106.6, 50.1, 44.8, 38.8, 34.7. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{21}\text{H}_{21}\text{N}_4\text{O}_3$, 377.1608: found 377.1611. $[\text{M}+\text{Na}]$ expected for $\text{C}_{21}\text{H}_{20}\text{N}_4\text{O}_3\text{Na}$, 399.1428: found 399.1431.

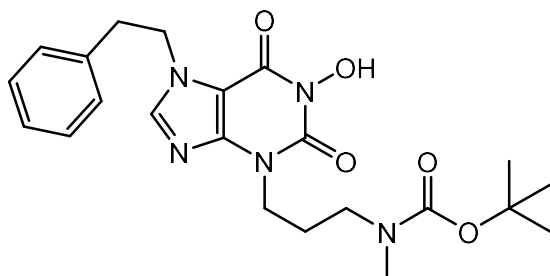
1-Hydroxy-7-phenethyl-3-(4-phenylbutyl)-3,7-dihydro-1H-purine-2,6-dione (16n)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-phenethyl-3-(4-phenylbutyl)-3,7-dihydro-1H-purine-2,6-dione (107 mg, 0.21 mmol) to afford the title

compound as an orange solid (21 mg, 25 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.53 (1H, s), 7.87 (1H, s), 7.52-7.19 (10H, m), 4.47 (2H, t, J 7.0), 4.02 (2H, t, J 7.6), 3.41 (2H, t, J 6.6), 3.11 (2H, t, J 7.0), 1.58 (2H, quin, J 6.6), 1.44 (2H, quin, J 8.0). ^{13}C NMR δ_{C} (101.6 MHz; DMSO): 152.9, 149.9, 147.2, 143.0, 142.5, 138.0, 129.1, 128.9, 128.8, 128.7, 127.1, 126.1, 105.9, 48.0, 43.2, 36.8, 35.5, 28.7, 27.6. HRMS (ESI $^{+}$) $[\text{M}+\text{H}]$ expected for $\text{C}_{23}\text{H}_{25}\text{N}_4\text{O}_3$, 405.1921: found 405.1925. $[\text{M}+\text{Na}]$ expected $\text{C}_{23}\text{H}_{24}\text{N}_4\text{O}_3\text{Na}$ 427.1741: found 427.1740.

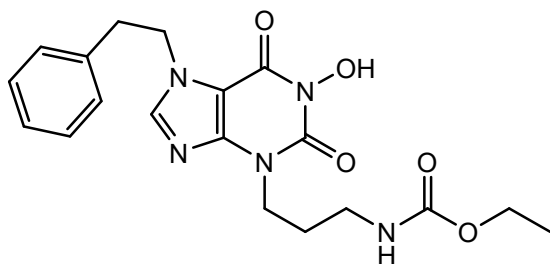
tert-butyl (3-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)(methyl)carbamate (16o)



Prepared according to standard procedure 3 using tert-butyl (3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)(methyl)carbamate

(165 mg, 0.31 mmol) to afford the title compound as an orange solid (130 mg, 95 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.75 (1H, s), 7.30-7.12 (5H, m), 4.43 (2H, t, J 6.3), 3.96 (2H, t, J 6.3), 3.62 (2H, t, J 6.3), 3.18 (3H, s), 3.08 (2H, t, J 6.9), 1.92 (2H, quin, J 7.0), 1.41 (9H, s). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 155.7, 140.3, 137.9, 137.2, 130.4, 129.0, 128.7, 128.7, 128.2, 106.6, 79.3, 53.5, 46.4, 37.1, 34.0, 32.0, 28.4, 22.8. HRMS (ESI $^{+}$) $[\text{M}+\text{H}]$ expected for $\text{C}_{22}\text{H}_{30}\text{N}_5\text{O}_5$, 444.2241: found 444.2246. $[\text{M}+\text{Na}]$ expected for $\text{C}_{22}\text{H}_{29}\text{N}_5\text{O}_5\text{Na}$, 466.2061: found 466.2062. (ESI $^{-}$) $[\text{M}-\text{H}]$ expected for $\text{C}_{22}\text{H}_{28}\text{N}_5\text{O}_5$, 442.2096: found 442.2085.

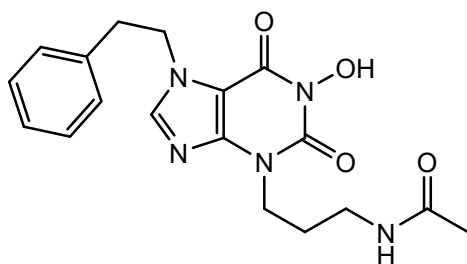
ethyl (3-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)propyl)carbamate (16p)



Prepared according to standard procedure 3 using ethyl (3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)propyl)carbamate (92 mg, 0.2 mmol) to afford the title

compound as an orange solid (70 mg, 94 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.66 (1H, s), 7.30-7.14 (5H, m), 7.04 (1H, t, J 6.6), 4.46 (2H, t, J 5.6), 4.02-3.92 (4H, m), 3.11 (2H, t, J 7.2), 2.97 (2H, t, J 6.7), 1.77 (2H, quin, J 6.7), 1.16 (3H, t, J 7.3). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.75 (1H, s), 7.30-7.12 (5H, m), 4.43 (2H, t, J 6.3), 3.96 (2H, t, J 6.3), 3.62 (2H, t, J 6.3), 3.18 (3H, s), 3.08 (2H, t, J 6.9), 1.92 (2H, quin, J 7.0), 1.41 (9H, s). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 156.7, 140.4, 137.9, 137.4, 130.4, 129.0, 128.7, 128.6, 126.9, 106.9, 77.2, 53.5, 48.4, 37.1, 34.0, 32.0, 29.7, 14.1. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}_5$, 402.1772: found 402.1778. $[\text{M}+\text{Na}]$ expected for $\text{C}_{19}\text{H}_{22}\text{N}_5\text{O}_5\text{Na}$, 424.1591: found 424.1589.

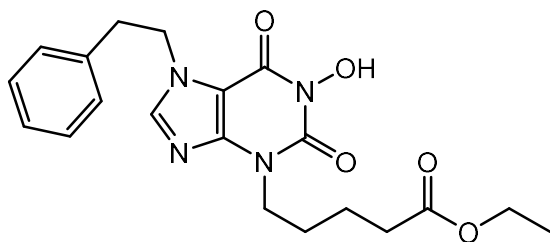
***N*-(3-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)propyl)acetamide (16q)**



Prepared according to standard procedure 3 using *N*-(3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3*H*-purin-3-yl)propyl)acetamide (55 mg, 0.12 mmol) to afford the title compound as a

yellow oil (36 mg, 81 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.85 (1H, s), 7.33-7.14 (6H, m), 4.46 (2H, t, J 7.2), 4.00 (2H, q, J 7.7), 3.13-3.02 (4H, m), 1.83-1.78 (5H, m). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 170.8, 153.0, 150.3, 148.4, 146.6, 136.7, 128.8, 128.6, 127.1, 106.3, 48.9, 40.1, 37.4, 35.9, 27.2, 23.2. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{18}\text{H}_{22}\text{N}_5\text{O}_4$, 372.1666: found 372.1662. $[\text{M}+\text{Na}]$ expected for $\text{C}_{18}\text{H}_{21}\text{N}_5\text{O}_4\text{Na}$, 394.1486: found 394.1480.

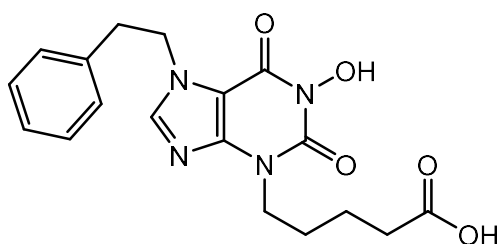
ethyl 5-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)pentanoate (16r)



Prepared according to standard procedure 3 using ethyl 5-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)pentanoate (182 mg, 0.39 mmol) to afford the title

compound as an orange solid (117 mg, 75 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.23-6.69 (6H, m), 4.18-4.05 (5H, m), 3.67 (2H, t, J 6.3), 2.99 (2H, t, J 7.4), 2.36 (2H, t, J 6.7), 1.77-1.56 (4H, m), 1.22 (3H, t, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 173.3, 156.7, 154.0, 146.1, 140.6, 137.2, 128.7, 128.7, 126.9, 106.5, 60.3, 48.7, 43.1, 37.1, 33.8, 28.7, 21.6, 14.2 HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{20}\text{H}_{25}\text{N}_4\text{O}_5$, 401.1891: found 401.1828.

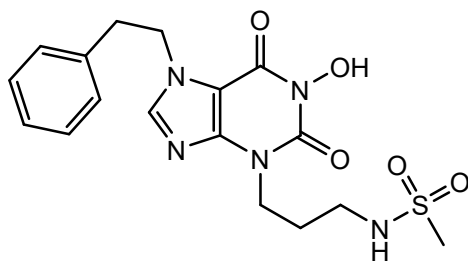
5-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)pentanoic acid (16s)



To a stirred solution of ethyl (3-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)carbamate (30 mg, 0.08 mmol) and aqueous sodium hydroxide (1 ml, 2 M) in EtOH (2

ml) was stirred for 24 hours. The solution was acidified with HCl (2 ml, 2 M), the solvent was removed *in vacuo* and the resulting residue dissolved in acetone (2 ml) and filtered. The solvent was removed *in vacuo* to afford the title compound as a brown oil (13 mg, 47 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.87 (1H, s), 7.33-7.14 (5H, m), 4.48 (2H, t, J 7.6), 3.99 (2H, t, J 7.6), 3.11 (2H, t, J 7.0), 2.25 (2H, t, J 7.0), 1.69 (2H, quin, J 8.2), 1.49 (2H, quin, J 8.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 174.6, 152.8, 149.5, 147.3, 142.9, 137.8, 129.1, 128.9, 127.9, 106.9, 47.9, 42.7, 36.9, 33.6, 27.5, 25.4. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{18}\text{H}_{21}\text{N}_4\text{O}_5$, 373.1506: found 373.1515. $[\text{M}+\text{Na}]$ expected for $\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_5\text{Na}$, 395.1326: found 395.1326. (ESI^-) $[\text{M}-\text{H}]$ expected for $\text{C}_{18}\text{H}_{19}\text{N}_4\text{O}_5$, 371.1361: found 371.1366.

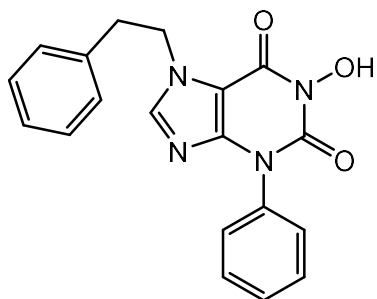
N-(3-(1-hydroxy-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)methanesulfonamide (16t)



Prepared according to standard procedure 3 using N-(3-(1-(benzyloxy)-2,6-dioxo-7-phenethyl-1,2,6,7-tetrahydro-3H-purin-3-yl)propyl)methanesulfonamide (48 mg, 0.1 mmol) to afford the title compound

as a yellow oil (39 mg, 99 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.78 (1H, s), 7.32-7.14 (5H, m), 7.01 (1H, s), 4.43 (2H, t, J 7.2), 4.01 (2H, t, J 7.2), 3.08 (2H, t, J 7.2), 2.98 (2H, t, J 5.9), 2.89 (2H, t, J 5.9), 1.85 (2H, quin, J 6.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 153.5, 150.7, 146.5, 142.0, 137.4, 128.6, 128.2, 126.5, 106.2, 47.9, 40.9, 40.0, 38.6, 36.6, 28.3. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{17}\text{H}_{22}\text{N}_5\text{O}_5\text{S}$, 408.1336: found 408.1342. [M+Na] expected for $\text{C}_{17}\text{H}_{21}\text{N}_5\text{O}_5\text{SNa}$, 430.1156: found 430.1163. (ESI $^{-}$) [M-H] expected for $\text{C}_{17}\text{H}_{20}\text{N}_5\text{O}_5\text{S}$, 406.1191: found 406.1189.

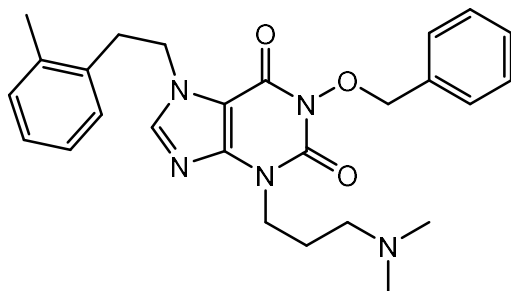
1-Hydroxy-7-phenethyl-3-phenyl-3,7-dihydro-1H-purine-2,6-dione (16u)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-phenethyl-3-phenyl-3,7-dihydro-1H-purine-2,6-dione (77 mg, 0.18 mmol) to afford the title compound as an orange solid (57 mg, 89 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.57 (1H, s), 7.71 (1H, s), 7.51-7.19 (10H, m), 4.41 (2H, t, J 7.1),

3.08 (2H, t, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; CHCl_3): 153.5, 150.5, 146.7, 140.4, 137.2, 134.8, 129.2, 128.8, 128.7, 128.7, 128.6, 128.1, 126.9, 106.6, 48.7, 37.1. HRMS (ESI $^{+}$) [M+Na] expected for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{O}_3\text{Na}$, 371.1115: found 371.1111.

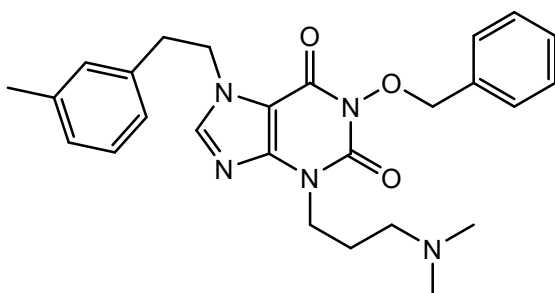
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22a)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(o-tolyl)ethan-1-ol (93 μ l, 0.7 mmol) to afford title compound as a

colourless oil (23 mg, 22 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.75 (1H, s), 7.61 (2H, dd, J 3.6 and 5.6), 7.43-7.39 (3H, m), 7.17 (1H, t, J 7.3), 7.13 (1H, t, J 7.3), 7.07 (1H, t, J 7.3), 7.00-6.96 (1H, m), 5.15 (2H, s), 4.54 (2H, t, J 7.3), 4.23 (2H, t, J 7.1), 3.19 (2H, t, J 7.3), 3.06 (2H, t, J 7.1), 2.47 (3H, s), 2.37 (6H, s), 2.16 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 161.6, 152.3, 149.8, 147.3, 142.7, 136.3, 135.2, 134.0, 130.0, 129.8, 129.4, 128.0, 126.7, 125.8, 106.3, 78.1, 42.4, 40.1, 34.2, 32.3, 24.7, 17.9.

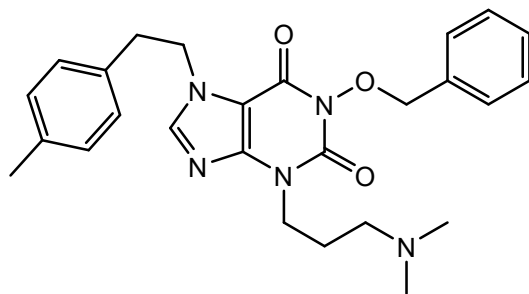
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22b)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (90 mg, 0.26 mmol) and 2-(3-fluorophenyl)ethan-1-ol (98 μ l, 0.78 mmol) to afford title

compound as a colourless oil (40 mg, 34 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.65 (1H, s), 7.61-6.89 (9H, m), 5.16 (2H, s), 4.53 (2H, t, J 7.1), 4.16 (2H, t, J 7.1), 3.10 (2H, t, J 7.1), 2.82 (2H, t, J 7.1), 2.58 (6H, s), 2.30 (3H, s), 2.07 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.4, 149.7, 147.5, 142.7, 138.0, 137.1, 134.2, 129.9, 129.2, 128.8, 128.1, 127.3, 125.5, 118.2, 115.1, 106.2, 78.0, 55.6, 48.5, 43.1, 40.8, 36.5, 24.4, 20.1.

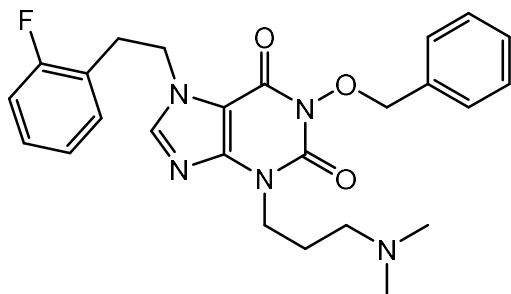
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22c)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(4-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (71 mg, 0.19 mmol) and 3-dimethylamino-1-propyl chloride hydrochloride (45 mg, 0.29 mmol) to afford the title

compound as a yellow oil (12 mg, 14 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.68-6.96 (10H, m), 5.21 (2H, s), 4.48 (2H, t, J 7.0), 4.21 (2H, t, J 7.1), 3.12 (2H, t, J 7.0), 2.60 (2H, t, J 7.1), 2.41 (6H, s), 2.33 (3H, s), 2.08 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 152.4, 149.3, 147.7, 141.5, 136.7, 133.9, 133.7, 130.1, 129.5, 129.1, 128.6, 128.5, 106.2, 78.5, 56.5, 49.1, 44.7, 41.8, 36.8, 25.3, 21.1.

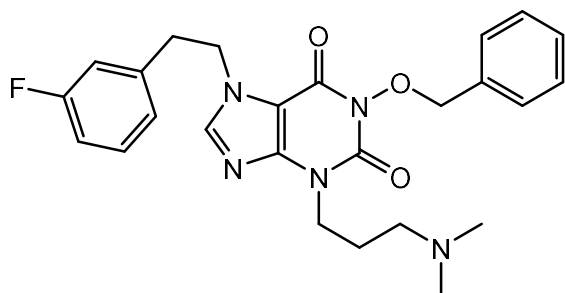
3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (22d)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (100 mg, 0.26 mmol) and 3-dimethylamino-1-propyl chloride hydrochloride (61 mg, 0.39 mmol) to afford the title

compound as a yellow oil (58 mg, 48 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.64-6.96 (6H, m), 5.19 (2H, s), 4.50 (2H, t, J 6.8), 4.14 (2H, t, J 7.1), 3.21 (2H, t, J 6.8), 2.54 (2H, t, J 7.1), 2.54 (6H, s), 1.93 (2H, quin, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 162.4, 160.0, 152.4, 149.2, 147.8, 141.4, 133.9, 131.3, 130.1, 129.1, 128.4, 123.8, 115.4, 106.3.

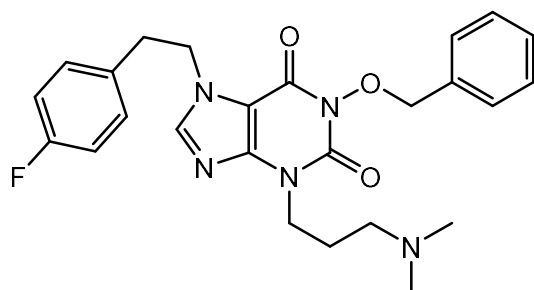
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-fluorophenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (22e)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1*H*-purine-2,6-dione (90 mg, 0.26 mmol) and 2-(3-fluorophenyl)ethan-1-ol (98 μ l,

0.78 mmol) to afford title compound as a white solid (29 mg, 25 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.62 (1H, s), 7.76-6.89 (5H, m), 5.17 (2H, s), 4.55 (2H, t, J 7.3), 4.17 (2H, t, J 6.9), 3.17 (2H, t, J 7.3), 2.84 (2H, t, J 6.9), 2.60 (6H, s), 2.08 (2H, quin, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 162.1, 152.3, 149.7, 147.5, 142.7, 140.0, 134.1, 130.9, 129.9, 128.8, 128.0, 124.5, 115.2, 113.2, 106.3, 78.0, 55.5, 48.7, 43.0, 40.8, 36.2, 24.3.

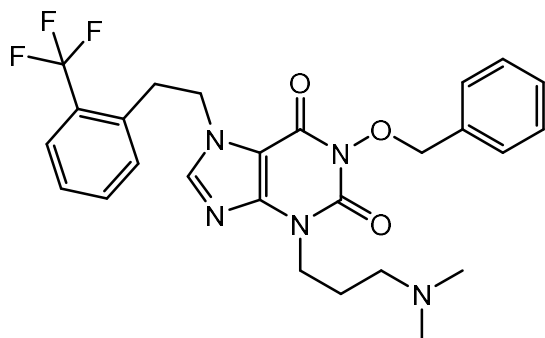
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-fluorophenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (22f)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(4-fluorophenethyl)-3,7-dihydro-1*H*-purine-2,6-dione (100 mg, 0.21 mmol) and 3-dimethylamino-1-propyl chloride hydrochloride (61 mg, 0.39 mmol) to afford the title

compound as a yellow oil (101 mg, 83 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.76-6.97 (10H, m), 5.22 (2H, s), 4.47 (2H, t, J 7.1), 4.19 (2H, t, J 7.1), 3.14 (2H, t, J 7.1), 2.39 (2H, t, J 7.1), 2.25 (6H, s), 1.96 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 160.8, 152.4, 149.2, 148.0, 141.3, 133.9, 132.8, 130.2, 130.1, 129.1, 128.4, 115.8, 106.2, 78.6, 56.6, 48.7, 45.4, 42.2, 36.5, 25.9.

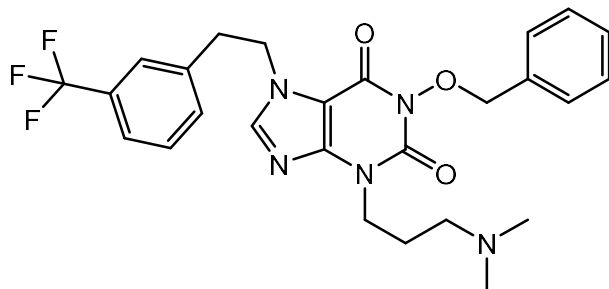
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (22g)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(2-(trifluoromethyl)phenyl)ethan-1-ol (111 μ l, 0.7 mmol) to afford title compound as a colourless oil

(52 mg, 44 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.72 (1H, s), 7.71 (1H, s), 7.62-7.58 (2H, m), 7.56-7.52 (1H, m), 7.41-7.38 (4H, m), 7.33-7.30 (1H, m), 5.14 (2H, s), 4.59 (2H, t, J 7.1), 4.17 (2H, t, J 7.0), 3.34-3.33 (2H, m), 2.76 (2H, t, J 7.4), 2.53 (6H, s), 2.06 (2H, quin, J 7.4). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.6, 152.4, 149.6, 147.5, 142.6, 135.7, 134.1, 132.1, 131.6, 129.8, 128.8, 128.1, 127.2, 125.9, 123.4, 106.3, 78.0, 55.6, 43.2, 41.3, 41.0, 33.6, 24.6.

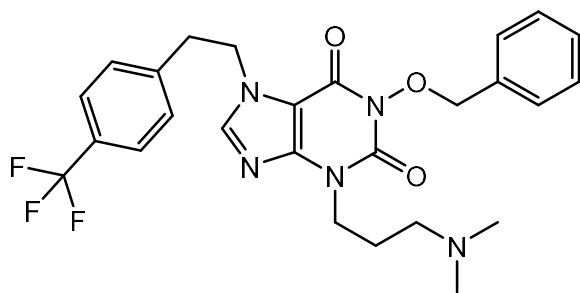
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (22h)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 3-(3-(trifluoromethyl)phenyl)ethan-1-ol (105 μ l, 0.7 mmol) to afford title

compound as a colourless oil (32 mg, 27 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.72 (1H, s), 7.73 (1H, s), 7.61-7.58 (2H, m), 7.54 (1H, t, J 8.0), 7.47-7.44 (2H, m), 7.42-7.38 (4H, m), 5.14 (2H, s), 4.58 (2H, t, J 7.0), 4.16 (2H, t, J 7.0), 3.25 (2H, t, J 7.0), 2.76 (2H, t, J 7.0), 2.52 (6H, s), 2.05 (2H, quin, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.5, 152.3, 149.5, 147.5, 142.6, 138.7, 134.0, 132.6, 129.9, 129.1, 128.9, 129.9, 128.0, 125.3, 123.4, 106.3, 78.1, 55.7, 43.2, 40.5, 36.4, 24.5.

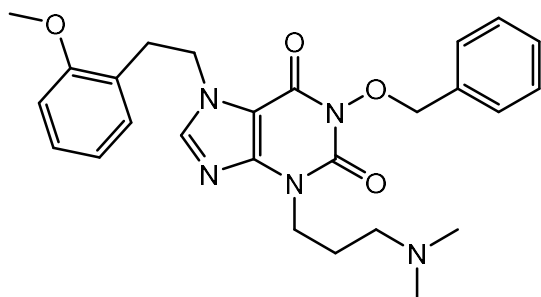
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (22i)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(4-(trifluoromethyl)phenyl)ethan-

1-ol (106 μ l, 0.7 mmol) to afford title compound as a colourless oil (39 mg, 33 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.72 (1H, s), 7.62-7.57 (4H, m), 7.41-7.34 (5H, m), 5.14 (2H, s), 4.58 (2H, t, J 7.0), 4.15 (2H, t, J 7.1), 3.26 (2H, t, J 7.0), 2.71 (2H, t, J 7.1), 2.49 (6H, s), 2.04 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.6, 152.3, 149.6, 142.6, 142.0, 134.0, 129.9, 129.8, 129.3, 128.8, 128.0, 125.1, 106.2, 78.0, 55.7, 43.4, 41.1, 36.4, 24.7.

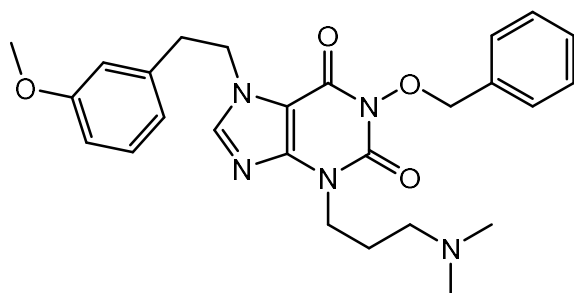
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22j)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (100 mg, 0.29 mmol) and 2-(2-methoxyphenyl)ethan-1-ol (123 μ l, 0.87 mmol) to afford title

compound as a colourless oil (31 mg, 22 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.76-6.79 (10H, m), 5.20 (2H, s), 4.51 (2H, t, J 6.6), 4.20 (2H, t, J 6.6), 3.82 (3H, s), 3.17 (2H, t, J 6.6), 2.68 (2H, t, J 6.6), 2.47 (6H, s), 2.09 (2H, quin, J 6.6). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 157.4, 152.2, 149.4, 147.4, 141.6, 133.8, 130.7, 130.1, 129.1, 128.6, 128.5, 125.1, 120.7, 110.3, 106.4, 78.5, 56.2, 55.2, 47.1, 44.4, 41.5, 32.1, 25.0.

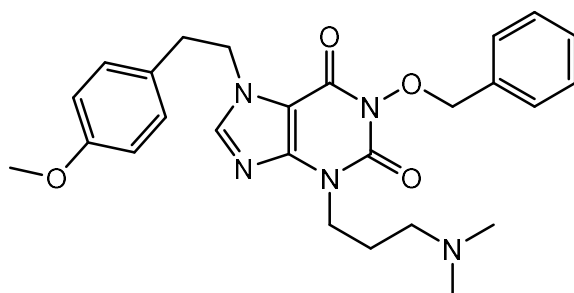
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22k)



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (100 mg, 0.26 mmol) and 3-dimethylamino-1-propyl

chloride hydrochloride (61 mg, 0.39 mmol) to afford the title compound as a yellow oil (64 mg, 51 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.69-6.62 (10H, m), 5.21 (2H, s), 4.49 (2H, t, J 7.1), 4.16 (2H, t, J 7.7), 3.78 (3H, s), 3.21 (2H, t, J 7.1), 2.57 (2H, t, J 7.7), 2.05 (6H, s), 1.95 (2H, quin, J 7.7). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 159.9, 152.4, 149.2, 147.9, 141.5, 138.5, 133.9, 130.1, 129.8, 129.1, 128.4, 121.0, 114.5, 112.4, 106.2, 78.5, 60.4, 55.2, 50.0, 48.8, 46.70, 42.4, 37.2, 25.4.

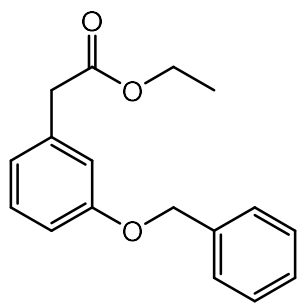
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-methoxybenzyl)-3,7-dihydro-1H-purine-2,6-dione (22l)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (100 mg, 0.29 mmol) and 2-(4-methoxyphenyl)ethan-1-ol (132

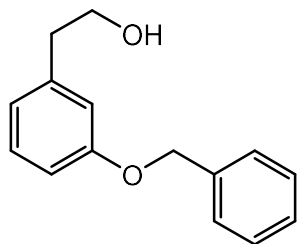
mg, 0.87 mmol) to afford title compound as a colourless oil (20 mg, 14 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.66-7.40 (5H, m), 7.25 (1H, s), 7.04 (2H, d, J 8.6), 6.86 (2H, d, J 8.6), 5.22 (2H, s), 4.48 (2H, t, J 6.9), 4.26 (2H, t, J 6.8), 3.81 (3H, s), 3.12 (2H, t, J 6.9), 2.84 (6H, s), 2.35 (2H, t, J 6.8), 2.02-1.93 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 158.8, 152.1, 149.6, 147.2, 141.7, 133.8, 130.1, 129.8, 129.2, 128.7, 128.5, 114.3, 106.2, 78.8, 55.5, 55.3, 49.2, 43.1, 40.7, 36.3, 24.0.

ethyl 2-(3-(benzyloxy)phenyl)acetate



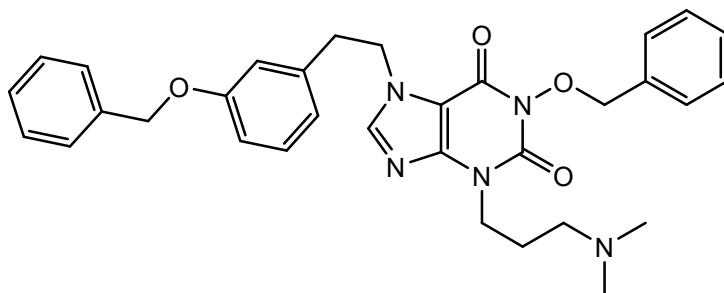
To a solution of ethyl 2-(3-hydroxyphenyl)acetate (296 mg, 1.64 mmol) in anhydrous THF (10 ml) at 0 °C was added potassium *tert*-butoxide (173 mg, 1.8 mmol) followed by benzyl bromide (195 μ l, 1.64 mmol) and the solution allowed to warm to rt and stirred for 24 hours. Solvent removed *in vacuo*, Residue dissolved in DCM (20 ml), washed with water, dried (MgSO₄), filtered and solvent removed *in vacuo* giving the title compound as a yellow oil (319 mg, 53 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.47-7.32 (6H, m), 7.27-7.24 (1H, m), 6.97-6.90 (2H, m), 5.08 (2H, s), 4.18 (2H, q, *J* 7.2), 3.61 (2H, s), 1.28 (3H, t, *J* 7.2). ¹³C NMR δ_C (101.6 MHz; CDCl₃): 171.2, 158.9, 137.0, 136.6, 129.5, 129.0, 127.9, 127.5, 121.9, 115.8, 113.7, 70.0, 60.9, 41.5, 14.2.

2-(3-(benzyloxy)phenyl)ethan-1-ol



Prepared according to standard procedure 12 using ethyl 2-(3-(benzyloxy)phenyl)acetate (319 mg, 1.28 mmol) to afford the title compound as a red oil (235 mg, 75 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.48-7.43 (2H, m), 7.43-7.39 (2H, m), 7.36 (1H, dt, *J* 7.0 and 1.7), 7.28-7.24 (2H, m), 5.09 (2H, s), 3.88 (2H, t, *J* 6.6), 2.87 (2H, t, *J* 6.6). ¹³C NMR δ_C (101.6 MHz; CDCl₃): 159.0, 141.0, 137.0, 129.6, 128.7, 128.0, 127.5, 121.6, 115.7, 112.7, 69.9, 63.5, 39.2.

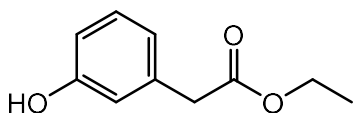
1-(benzyloxy)-7-(3-(benzyloxy)phenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22m)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-

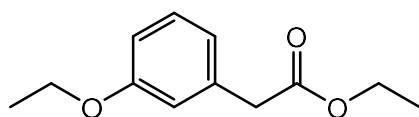
(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (90 mg, 0.26 mmol) and 2-(3-(benzyloxy)phenyl)ethan-1-ol (200 mg, 0.78 mmol) to afford title compound as a colourless oil (69 mg, 48 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.66-7.63 (2H, m), 7.46-7.33 (8H, m), 7.21 (2H, t, J 7.8), 6.87 (1H, dd, J 8.3 and 2.1), 6.76 (1H, t, J 2.1), 6.69 (1H, d, J 7.5), 5.21 (2H, s), 5.05 (2H, s), 4.48 (2H, t, J 7.0), 4.20 (2H, t, J 7.1), 3.13 (2H, t, J 7.0), 2.69 (2H, t, J 7.1), 2.46 (6H, s), 2.08 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 162.7, 159.2, 152.3, 149.4, 147.6, 141.6, 138.5, 136.8, 133.9, 130.1, 129.2, 128.8, 128.4, 128.1, 127.5, 121.4, 115.6, 113.3, 106.3, 78.6, 70.0, 56.1, 48.7, 41.5, 37.2, 36.5, 25.0.

ethyl 2-(3-hydroxyphenyl)acetate



To a stirred solution of 3-hydroxyphenylacetic acid (1 g, 6.57 mmol) in anhydrous ethanol (20 ml) was added dropwise thionyl chloride (119 μl , 1.38 mmol) and the solution stirred at rt for 6 hours. Solvent removed *in vacuo*, giving the title compound as a yellow liquid (1182 mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.20 (1H, t, J 7.7), 6.87-6.74 (3H, m), 4.19 (2H, q, J 7.1), 3.59 (2H, s), 1.29 (3H, t, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 172.0, 155.9, 135.6, 129.8, 121.6, 116.3, 114.3, 61.2, 41.4, 14.1.

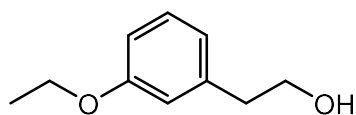
ethyl 2-(3-ethoxyphenyl)acetate



To a stirred solution of ethyl 2-(3-hydroxyphenyl)acetate (296 mg, 1.64 mmol) in anhydrous THF (10 ml) at 0 °C

was added potassium *tert*-butoxide (369 mg, 3.3 mmol) followed by ethyl iodide (262 μ l, 3.3 mmol) and the solution allowed to warm to rt and stirred for 24 hours. Solvent removed *in vacuo*, giving the title compound as a brown oil (309 mg, 91 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.24 (1H, t, J 7.9), 6.90-6.80 (3H, m), 4.18 (2H, q, J 7.2), 4.05 (2H, q, J 7.0), 3.60 (2H, s), 1.43 (3H, t, J 7.0), 1.28 (3H, t, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 171.5, 159.2, 135.4, 129.5, 121.5, 115.5, 113.2, 63.5, 60.8, 41.5, 14.9, 14.1.

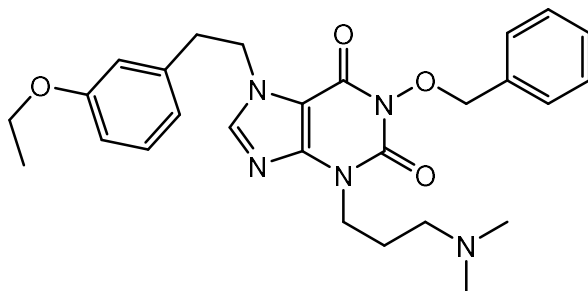
2-(3-ethoxyphenyl)ethan-1-ol



Prepared according to standard procedure 12 using ethyl 2-(3-ethoxyphenyl)acetate (208 mg, 1.49 mmol) to afford the title compound as a

yellow oil (200 mg, 69 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.27-6.78 (4H, m), 4.06 (2H, q, J 7.0), 3.88 (2H, t, J 6.4), 2.87 (2H, t, J 6.4), 1.44 (3H, t, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 159.1, 139.9, 129.6, 121.3, 115.3, 112.3, 63.7, 63.3, 39.2, 14.8.

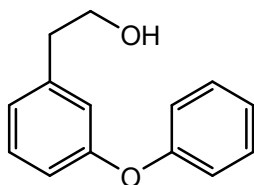
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-ethoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22n)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (90 mg, 0.26 mmol) and 2-(3-ethoxyphenyl)ethan-1-ol (130

mg, 0.78 mmol) to afford title compound as a colourless oil (39 mg, 30 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.69-6.69 (10H, m), 5.13 (2H, s), 4.53 (2H, t, J 7.2), 4.14 (2H, t, J 7.2), 3.98 (2H, q, J 7.0), 3.09 (2H, t, J 7.2), 2.83 (2H, t, J 7.2), 2.59 (6H, s), 2.06 (2H, quin, J 7.2), 1.35 (3H, t, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 159.2, 152.3, 149.5, 147.3, 142.7, 138.7, 134.1, 129.9, 129.2, 128.8, 128.1, 120.7, 114.8, 112.5, 106.3, 78.0, 63.0, 48.1, 43.0, 40.7, 36.7, 24.2, 13.9.

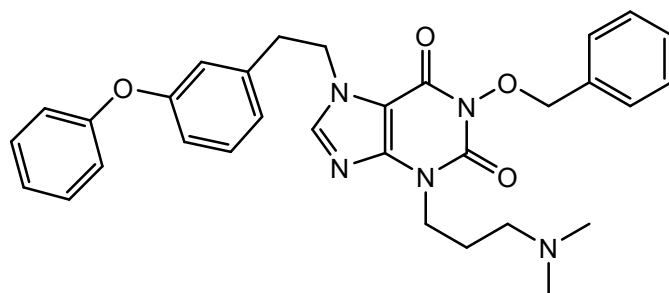
2-(3-phenoxyphenyl)ethan-1-ol



Prepared according to standard procedure 12 using 2-(3-phenoxyphenyl)acetic acid (300 mg, 1.32) to afford the title compound as an yellow oil (281 mg, 100 %). ^1H

NMR δ_{H} (400.13 MHz; CDCl_3): 7.36 (2H, t, J 7.8), 2.29 (1H, t, J 7.8), 7.13 (1H, t, J 7.5), 7.04 (2H, d, J 8.0), 7.00 (1H, d, J 7.5), 6.90 (2H, m), 3.86 (2H, t, J 6.8), 2.87 (2H, t, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 157.5, 157.1, 140.6, 129.8, 123.8, 123.3, 119.4, 118.4, 116.8, 63.4, 39.1.

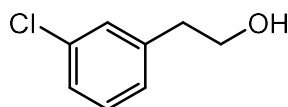
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-phenoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22o)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-

(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(3-phenoxyphenyl)ethan-1-ol (167 mg, 0.78 mmol) to afford title compound as a yellow oil (68 mg, 48 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.64 (1H, s), 6.55 (2H, dd, J 3.6 and 5.8), 7.35 (3H, m), 7.32 (2H, t, J 8.1), 7.24 (1H, t, J 7.9), 7.09 (1H, t, J 7.4), 6.90 (3H, m), 6.81 (1H, dd, J 2.0 and 8.1), 6.70 (1H, t, J 2.0), 5.09 (2H, s), 4.49 (2H, t, J 6.9), 4.12 (2H, t, J 7.1), 3.09 (2H, t, J 6.9), 2.68 (2H, t, J 7.1), 2.46 (6H, s), 2.00 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 157.4, 157.1, 152.2, 149.5, 147.4, 142.7, 139.4, 134.1, 129.9, 129.7, 129.3, 128.8, 128.1, 123.6, 123.1, 128.9, 128.5, 117.0, 106.2, 78.0, 55.7, 48.0, 43.4, 41.1, 36.4, 24.7.

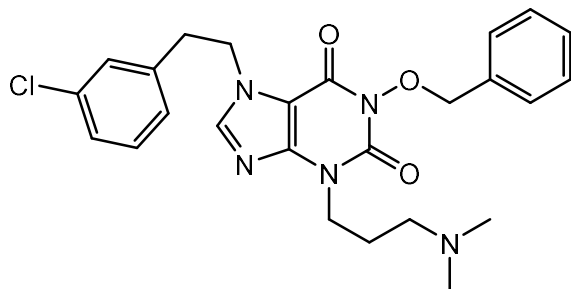
2-(3-chlorophenyl)ethan-1-ol



Prepared according to standard procedure 12 using 2-(3-chlorophenyl)acetic acid (250 mg, 1.47) to afford the title compound as an colourless oil (202 mg,

88%). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.27-7.22 (3H, m), 7.13 (1H, dd, J 6.9 and 1.6), 3.87 (2H, t, J 6.5), 2.86 (2H, t, J 6.5). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 140.7, 134.3, 129.8, 129.4, 127.2, 126.7, 63.3, 38.8.

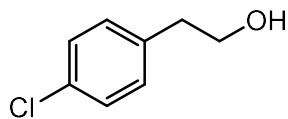
1-(benzyloxy)-7-(3-chlorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22p)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(3-chlorophenyl)ethan-1-ol (122

mg, 0.78 mmol) to afford title compound as a white solid (51 mg, 57 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.71 (1H, s), 7.58 (2H, dd, J 3.9 and 5.4), 7.40-7.38 (3H, m), 7.26-7.21 (2H, m), 7.15 (1H, t, J 1.8), 7.09 (1H, dt, J 1.8 and 7.1), 5.13 (2H, s), 4.52 (2H, t, J 7.3), 4.15 (2H, t, J 7.1), 3.13 (2H, t, J 7.3), 2.81 (2H, t, J 7.1), 2.57 (6H, s), 2.06 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.3, 149.6, 147.4, 142.6, 139.7, 134.1, 133.9, 129.9, 129.8, 128.8, 128.6, 128.1, 127.1, 126.7, 106.2, 78.0, 55.6, 46.2, 43.1, 40.8, 36.2, 24.4.

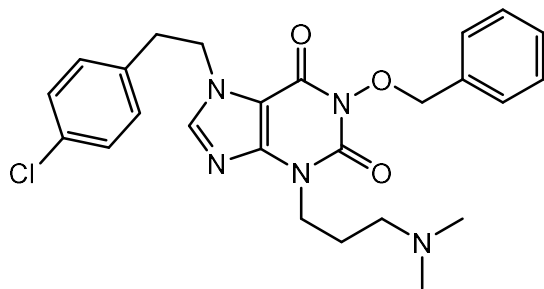
2-(4-chlorophenyl)ethan-1-ol



Prepared according to standard procedure 12 using 2-(4-chlorophenyl)acetic acid (250 mg, 1.47) to afford the title compound as an colourless oil (206 mg, 90

%). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.32-7.28 (2H, m), 7.19 (2H, d, J 8.5), 3.86 (2H, t, J 6.4), 2.86 (2H, t, J 6.4). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 137.1, 132.3, 130.3, 128.6, 63.4, 38.5.

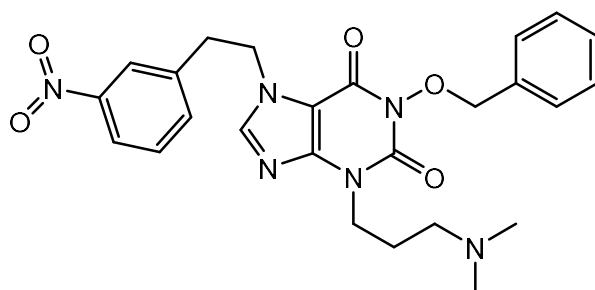
1-(benzyloxy)-7-(4-chlorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22q)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(4-chlorophenyl)ethan-1-ol (122 mg, 0.78 mmol) to afford title

compound as a white solid (55 mg, 61 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.70 (1H, s), 7.58 (2H, dd, J 3.9 and 5.4), 7.40-7.38 (3H, m), 7.26 (2H, d, J 8.5), 7.12 (2H, d, J 8.5), 5.13 (2H, s), 4.52 (2H, t, J 7.1), 4.15 (2H, t, J 6.9), 3.13 (2H, t, J 7.1), 2.78 (2H, t, J 6.9), 2.55 (6H, s), 2.06 (2H, quin, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.2, 149.6, 147.3, 142.6, 136.1, 134.0, 132.2, 130.3, 129.8, 128.9, 128.3, 128.1, 106.2, 78.0, 55.6, 47.6, 43.2, 40.9, 35.9, 24.4.

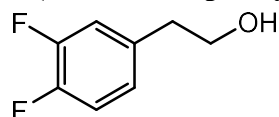
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-nitrophenethyl)-3,7-dihydro-1H-purine-2,6-dione (22s)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(3-nitrophenyl)ethan-1-ol (82 μl ,

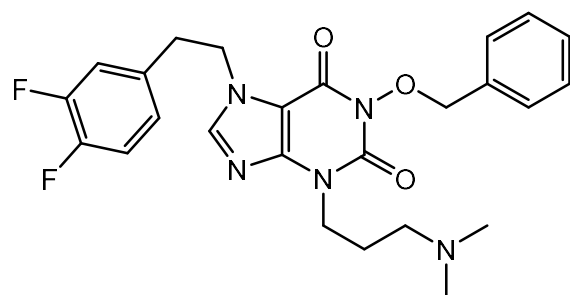
0.08 mmol) to afford title compound as a white solid (60 mg, 47 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 8.11 (1H, dq, J 1.0 and 8.1), 7.92 (1H, t, J 2.0), 7.73 (1H, s), 7.65-7.43 (4H, m), 7.42-7.37 (3H, m), 5.13 (2H, s), 4.59 (2H, t, J 6.9), 4.16 (2H, t, J 6.9), 3.29 (2H, t, J 6.9), 2.85 (2H, t, J 6.9), 2.60 (6H, s), 2.08 (2H, quin, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.3, 148.3, 147.5, 142.8, 139.6, 135.2, 134.1, 129.9, 129.8, 129.5, 128.9, 128.0, 123.3, 121.6, 106.3, 78.0, 55.6, 48.5, 43.0, 40.8, 36.1, 24.3.

2-(3,4-difluorophenyl)ethan-1-ol



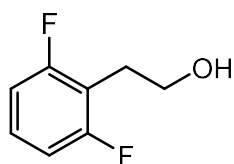
Prepared according to standard procedure 12 using 2-(3,4-difluorophenyl)acetic acid (250 mg, 1.50) to afford the title compound as a yellow oil (208 mg, 88 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.09-6.99 (2H, m), 6.93-6.89 (1H, m), 3.76 (2H, t, J 6.7), 2.77 (2H, t, J 6.7). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 150.7 (dd, J 12.9 and 109.8), 148.2 (dd, J 12.9 and 109.8), 135.9 (dd, J 4.2 and 5.7), 124.8 (dd, J 3.4 and 4.0), 117.6 (d, J 16.5), 117.0 (d, J 16.5), 62.9, 38.0.

1-(benzyloxy)-7-(3,4-difluorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22t)



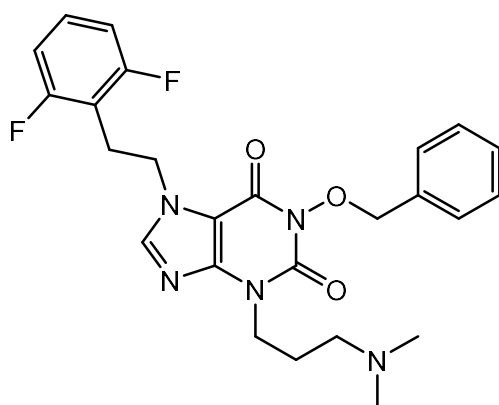
Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(3,4-difluorophenyl)ethan-1-ol (123 mg, 0.78 mmol) to afford title compound as a white solid (52 mg, 41 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.71 (1H, s), 7.58 (2H, dd, J 3.7 and 5.8), 7.40-7.37 (3H, m), 7.16 (1H, dt, J 8.5 and 10.6), 7.09 (1H, dq, J 2.1 and 7.9), 5.14 (2H, s), 4.51 (2H, t, J 6.9), 4.14 (2H, t, J 6.9), 3.13 (2H, t, J 6.9), 2.65 (2H, t, J 6.9), 2.45 (6H, s), 2.02 (2H, quin, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.7, 152.4, 150.8 (dd, J 13.1 and 81.3), 149.5, 148.4, (dd, J 12.4 and 80.6), 147.5, 142.6, 143.9 (dd, J 3.6 and 5.7), 134.1, 129.9, 128.8, 128.4, 125.1 (3.6 and 6.2), 117.5 (d, J 17.5), 117.0 (d, J 17.0), 106.2, 78.0, 55.8, 43.5, 41.1, 35.7, 24.8.

2-(2,6-difluorophenyl)ethan-1-ol



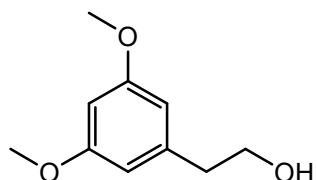
Prepared according to standard procedure 12 using 2-(2,6-difluorophenyl)acetic acid (250 mg, 1.41) to afford the title compound as an colourless oil (156 mg, 69 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.19 (1H, tt, J 6.6 and 8.2), 6.89 (2H, t, J 7.7), 3.86 (2H, t, J 6.9), 2.98 (2H, t, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 161.6 (dd, J 9.4 and 247.0), 128.0 (t, J 10.5), 114.0 (t, J 21.7), 111.1 (dd, J 7.2 and 19.3), 61.8, 30.3.

1-(benzyloxy)-7-(2,6-difluorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22u)



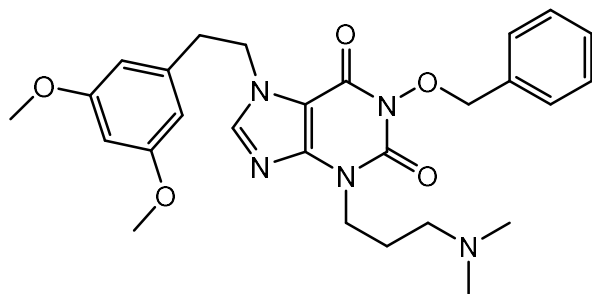
Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(2,6-difluorophenyl)ethan-1-ol (123 mg, 0.78 mmol) to afford title compound as a white solid (54 mg, 48 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.70 (1H, s), 7.59-7.56 (2H, m), 7.40-7.37 (3H, m), 7.27 (1H, tt, J 6.6 and 8.2), 6.90 (2H, t, J 7.7), 5.07 (2H, s), 4.59 (2H, t, J 6.4), 4.14 (2H, t, J 6.9), 3.28 (2H, t, J 6.4), 2.72 (2H, t, J 6.9), 2.51 (6H, s), 2.03 (2H, quin, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 160.2 (dd, J 63.5 and 194.5), 152.2, 149.4, 147.3, 142.8, 134.0, 129.8, 129.0 (d, J 22.4), 128.8, 128.1, 117.2 (d, J 180.9), 110.8 (d, J 24.7), 106.5, 78.0, 55.6, 45.9, 43.2, 40.9, 36.4, 24.5.

2-(3,5-dimethoxyphenyl)ethan-1-ol



Prepared according to standard procedure 12 using 2-(3,5-dimethoxyphenyl)acetic acid (250 mg, 1.27 mmol) to afford the title compound as a yellow oil (223 mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 6.38 (2H, d, J 2.2), 6.31 (1H, t, J 2.2), 3.78 (2H, t, J 6.9), 3.74 (6H, s), 2.77 (2H, t, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 160.8, 141.3, 106.9, 98.3, 63.2, 55.1, 39.6.

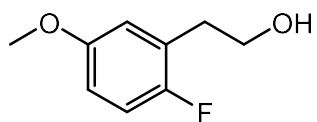
1-(benzyloxy)-7-(3,5-dimethoxyphenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22v)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(3,5-dimethoxyphenyl)ethan-

1-ol (127 mg, 0.70 mmol) to afford title compound as a white solid (41 mg, 35 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.69 (1H, s), 7.61-7.58 (2H, m), 7.41-7.38 (3H, m), 6.34 (1H, t, J 2.0), 6.29 (2H, t, J 2.0), 5.14 (2H, s), 4.54 (2H, t, J 7.0), 4.16 (2H, t, J 7.0), 3.73 (6H, s), 3.06 (2H, t, J 7.0), 2.82 (2H, t, J 7.0), 2.59 (6H, s), 2.07 (2H, quin, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.8, 161.1, 152.4, 149.6, 147.4, 142.7, 139.5, 134.8, 129.9, 128.8, 128.1, 106.5, 106.3, 98.3, 78.0, 55.4, 54.3, 43.0, 40.7, 36.9, 24.2.

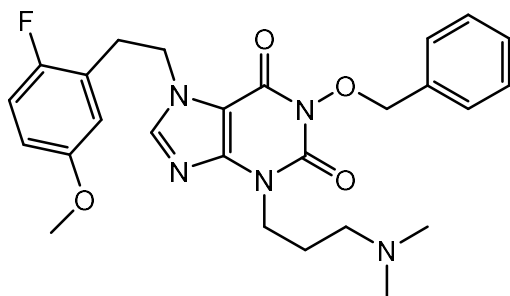
2-(2-fluoro-5-methoxyphenyl)ethan-1-ol



Prepared according to standard procedure 12 using 2-(2,6-difluorophenyl)acetic acid (250 mg, 1.35) to afford the title compound as an colourless oil (230

mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 6.97 (1H, t, J 9.2), 6.78 (1H, dd, J 3.1 and 6.0), 6.73 (1H, t, J 3.5 and 8.8), 3.88 (2H, t, J 6.8), 2.90 (2H, t, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 155.8 (d, J 237.1), 155.6 (d, J 2.2), 126.2 (d, J 17.8), 116.4 (d, J 4.6), 115.7 (d, J 24.4), 112.7 (d, J 8.2), 62.5, 32.8.

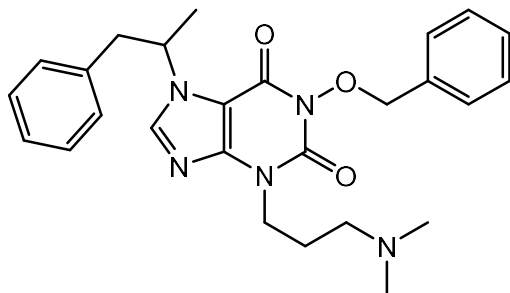
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-fluoro-5-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (22w)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(2-fluoro-5-methoxyphenyl)ethan-1-ol (132 mg, 0.78 mmol) to afford title compound

as a white solid (60 mg, 53 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.69 (1H, s), 7.58 (2H, dd, J 3.6 and 5.6), 7.41-7.38 (3H, m), 6.95 (1H, t, J 9.2), 6.77 (1H, dt, J 3.5 and 9.0), 6.65 (1H, dd, J 3.5 and 5.8), 5.11 (2H, s), 4.56 (2H, t, J 6.8), 4.14 (2H, t, J 6.9), 3.71 (3H, s), 3.17 (2H, t, J 6.8), 2.72 (2H, t, J 6.9), 2.50 (6H, s), 2.03 (2H, quin, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.6, 156.0, 155.6 (d, J 234.0), 152.2, 249.5, 147.4, 142.7, 134.1, 129.9, 128.8, 128.0, 124.6 (d, J 17.4), 115.9 (d, J 4.5), 115.4 (d, J 24.0), 113.2 (d, J 8.3), 106.4, 78.0, 55.7, 54.8, 46.8, 43.3, 40.9, 30.1, 24.6.

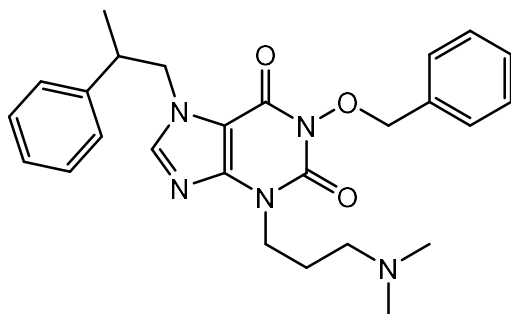
(RS) 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(1-phenylpropan-2-yl)-3,7-dihydro-1H-purine-2,6-dione (22x)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 1-phenylpropan-2-ol (106 mg, 0.78 mmol) to afford title compound as a

white solid (48 mg, 40 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.94 (1H, m), 7.62-7.58 (2H, m), 7.42-7.36 (3H, m), 7.27-7.16 (3H, m), 7.10 (2H, dd, J 6.8 and 2.0), 5.14 (2H, s), 7.12-7.08 (1H, m), 4.06 (2H, m), 3.30 (1H, dd, J 1.9 and 7.7), 3.14 (1H, dd, J 2.5 and 6.6), 3.01 (2H, t, J 7.1), 2.88 (2H, t, J 6.9), 2.64 (6H, s), 2.13 (2H, quin, J 6.9), 1.65 (1.5H, d, J 2.6), 1.64 (1.5H, d, J 2.6). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.6, 152.3, 149.3, 147.6, 141.3, 137.1, 134.0, 129.9, 128.8, 128.1, 128.0, 126.5, 106.2, 77.9, 56.2, 55.3, 46.2, 42.9, 42.3, 24.1, 18.8.

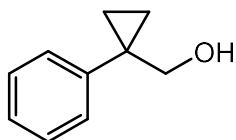
(RS) 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-phenylpropyl)-3,7-dihydro-1H-purine-2,6-dione (22y)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-phenylpropan-1-ol (106 mg, 0.78 mmol) to afford title compound as a

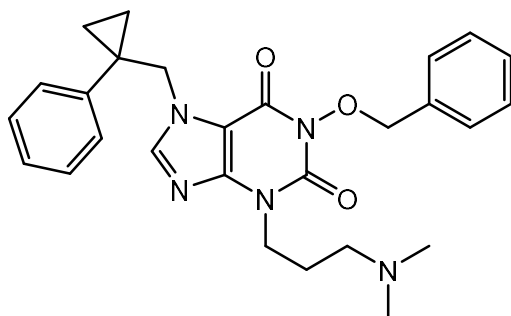
white solid (50 mg, 42 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.62-7.58 (2H, m), 7.57 (1H, s), 7.42-7.38 (3H, m), 7.30-7.25 (2H, m), 7.21-7.16 (3H, m), 5.15 (2H, s), 4.48 (1H, dd, J 7.2 and 13.2), 4.38 (1H, dd, J 8.7, 13.4), 4.11 (2H, t, J 7.0), 3.34-3.32 (1H, m), 2.67 (2H, t, J 7.0), 2.47 (6H, s), 2.00 (2H, quin, J 7.0), 1.33 (1.5H, s), 1.31 (1.5H, s). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.7, 152.4, 149.5, 147.4, 142.8, 134.1, 129.9, 128.8, 128.3, 128.0, 126.9, 126.8, 106.2, 78.0, 55.7, 53.5, 43.4, 40.9, 40.8, 24.6, 17.2.

(1-phenylcyclopropyl)methanol



Prepared according to standard procedure 12 using 1-phenylcyclopropane-1-carboxylic acid (333 mg, 2.06 mmol) to afford the title compound as a yellow oil (215 mg, 62 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.40-7.37 (2H, m), 7.33 (2H, t, J 7.6), 7.24 (2H, tt, J 1.4 and 7.2), 3.66 (2H, s), 2.08 (1H, br s), 0.88 (4H, dd, J 1.9 and 8.6). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 142.9, 129.0, 128.4, 126.5, 70.4, 25.6, 11.3.

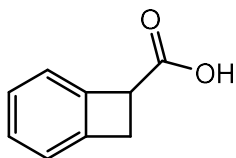
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-((1-phenylcyclopropyl)methyl)-3,7-dihydro-1H-purine-2,6-dione (22z)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (70 mg, 0.21 mmol) and (1-phenylcyclopropyl)methanol (97 mg, 0.70 mmol) to afford title

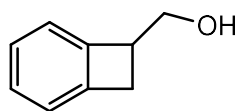
compound as a white solid (66 mg, 52 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.57 (2H, dd, J 2.2 and 7.3), 7.42-7.39 (3H, m), 7.26-7.20 (2H, m), 7.16-7.13 (2H, m), 5.50 (2H, s), 5.06 (2H, s), 4.11 (2H, t, J 7.1), 2.60 (2H, t, J 7.1), 2.42 (6H, s), 1.98 (2H, quin, J 7.1), 1.22 (2H, dd, J 1.7 and 6.0), 0.93 (2H, dd, J 1.7 and 6.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.3, 149.4, 147.0, 142.6, 141.4, 134.0, 129.9, 129.4, 128.8, 128.1, 128.0, 127.0, 106.3, 77.9, 55.9, 54.8, 53.4, 43.5, 41.1, 27.2, 24.8, 10.6.

bicyclo[4.2.0]octa-1,3,5-triene-7-carboxylic acid



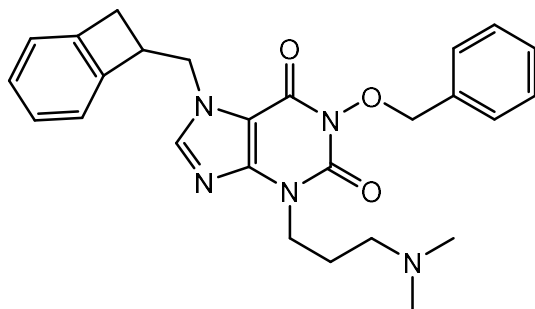
A solution of bicyclo[4.2.0]octa-1,3,5-triene-7-carbonitrile (316 μl , 2.6 mmol) and potassium hydroxide (1.0 g, 17.8 mmol) in EtOH (4 ml) was stirred at rt for 24 hours, water (2 ml) was added and the mixture stirred at reflux for 3 hours. Water (20 ml) was added followed by HCl (1 M, 10 ml) and the mixture extracted into EtOAc (2 x 20 ml), dried (MgSO_4), filtered and Solvent removed *in vacuo* to afford the title compound as orange crystals (389 mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.30-7.26 (2H, m), 7.22-7.20 (1H, m), 7.15-7.12 (1H, m), 4.37 (1H, t, J 4.2), 3.52 (2H, d, J 4.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 178.2, 144.1, 142.2, 128.4, 127.5, 123.0, 122.6, 45.7, 34.0.

bicyclo[4.2.0]octa-1,3,5-trien-7-ylmethanol



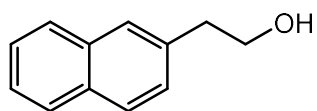
Prepared according to standard procedure 12 using bicyclo[4.2.0]octa-1,3,5-triene-7-carboxylic acid (381 mg, 2.57 mmol) to afford the title compound as a colourless oil (171 mg, 56 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.32-7.24 (2H, m), 7.17 (2H, dt, J 2.6 and 8.8), 3.92 (2H, d, J 6.8), 3.78-3.72 (1H, m), 3.37 (1H, dd, J 5.3 and 14.2), 2.98 (1H, dd, J 2.1 and 14.1), 2.75 (1H, br s). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 146.4, 144.4, 127.7, 126.9, 123.1, 122.3, 65.3, 45.4, 33.3.

(RS) 1-(benzyloxy)-7-(bicyclo[4.2.0]octa-1(6),2,4-trien-7-ylmethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22aa)



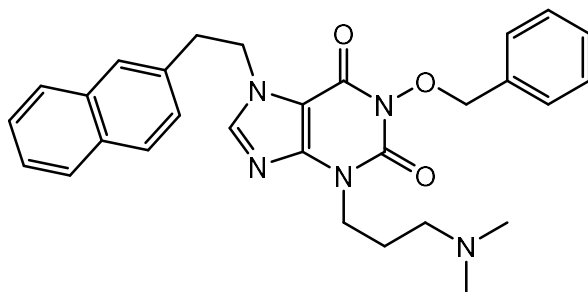
Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (70 mg, 0.21 mmol) and bicyclo[4.2.0]octa-1,3,5-trien-7-ylmethanol (94 mg, 0.70 mmol) to afford title compound as a white solid (39 mg, 33 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.95 (1H, s), 7.61-7.57 (2H, m), 7.40-7.36 (3H, m), 7.23 (1H, t, J 7.3), 7.16 (1H, t, J 7.3), 7.10 (1H, t, J 7.3), 6.84 (1H, t, J 7.3), 5.14 (2H, s), 4.68 (1H, dd, J 7.5 and 13.5), 4.59 (1H, dd, J 7.5 and 13.5), 4.18 (2H, t, J 7.0), 4.04 (1H, dq, J 1.9 and 7.5), 2.99 (2H, dd, J 1.7 and 14.5), 2.64 (2H, t, J 7.0), 2.42 (6H, s), 2.04 (2H, quin, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.5, 149.5, 147.5, 147.9, 143.2, 142.8, 134.0, 129.9, 128.5, 128.0, 127.9, 127.8, 122.9, 121.8, 106.5, 78.0, 56.0, 53.4, 48.5, 43.6, 43.0, 33.1, 24.9.

2-(naphthalen-2-yl)ethan-1-ol



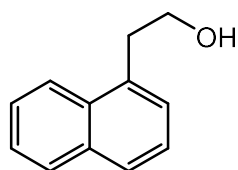
Prepared according to standard procedure 12 using 2-(naphthalen-2-yl)acetic acid (250 mg, 1.34 mmol) to afford the title compound as a white solid (234 mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.89-7.81 (3H, m), 7.70 (1H, s), 7.55-7.47 (2H, m), 7.38 (1H, d, J 8.80). 3.91 (2H, t, J 6.9), 3.03 (2H, t, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 136.3, 133.7, 132.3, 128.1, 127.7, 127.6, 127.6, 127.5, 126.1, 125.5, 63.4, 39.3.

1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-(naphthalen-2-yl)ethyl)-3,7-dihydro-1H-purine-2,6-dione (22ab)



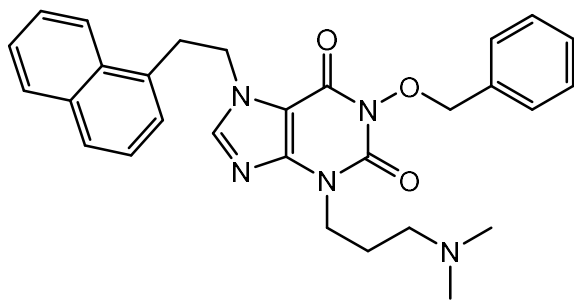
Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(naphthalen-2-yl)ethan-1-ol (134 mg, 0.78 mmol) to afford title compound as a white solid (33 mg, 24). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.81-7.76 (2H, m), 7.75-7.71 (1H, m), 7.64 (1H, s), 7.57 (2H, dd, J 1.8 and 7.4), 7.51 (1H, s), 7.44-7.37 (5H, m), 7.30 (1H, dd, J 1.3 and 6.9), 5.03 (2H, s), 4.62 (2H, t, J 7.0), 4.11 (2H, t, J 7.0), 3.29 (2H, t, J 7.0), 2.71 (2H, t, J 7.0), 2.48 (6H, s), 2.01 (2H, quin, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.5, 152.3, 149.6, 147.3, 142.7, 134.7, 134.1, 133.6, 132.5, 129.9, 128.8, 128.0, 127.9, 127.3, 127.2, 126.8, 125.8, 125.4, 106.3, 77.7, 55.6, 43.3, 43.2, 40.9, 36.9, 24.5.

2-(naphthalen-1-yl)ethan-1-ol



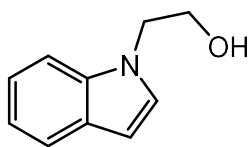
Prepared according to standard procedure 12 using 2-(naphthalen-1-yl)acetic acid (250 mg, 1.34) to afford the title compound as an orange oil (234 mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 8.15 (1H, d, J 8.8), 7.97-94 (1H, m), 7.84 (1H, d, J 8.4), 7.64-7.57 (2H, m), 7.51 (1H, t, J 7.9), 7.44 (1H, d, J 7.0), 3.99 (2H, t, J 7.0), 3.39 (2H, t, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 134.8, 134.1, 132.3, 129.0, 127.3, 127.2, 126.2, 125.8, 125.7, 123.8, 62.9, 36.3.

1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-(naphthalen-1-yl)ethyl)-3,7-dihydro-1H-purine-2,6-dione (22ac)



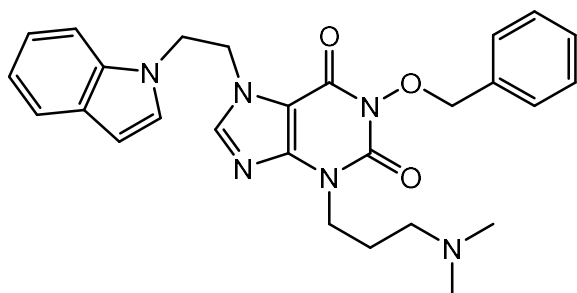
Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(naphthalen-1-yl)ethan-1-ol (134 mg, 0.78 mmol) to afford title compound as a white solid (38 mg, 29 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 8.17 (1H, d, J 8.4), 7.85 (1H, d, J 7.9), 7.75 (1H, d, J 8.3), 7.61-7.59 (2H, m), 7.55 (1H, s), 7.53-7.45 (2H, m), 7.41-7.33 (4H, m), 7.23 (1H, d, J 7.0), 5.12 (2H, s), 4.61 (2H, t, J 7.3), 4.10 (2H, t, J 7.2), 3.59 (2H, t, J 7.3), 2.69 (2H, t, J 7.2), 2.48 (6H, s), 1.99 (2H, quin, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.6, 152.4, 149.5, 147.4, 142.6, 134.1, 133.4, 131.8, 129.9, 128.9, 128.4, 128.1, 127.4, 127.0, 126.0, 125.4, 125.2, 123.2, 106.4, 78.0, 55.6, 47.7, 43.3, 40.9, 33.8, 24.6.

2-(1H-indol-1-yl)ethan-1-ol



To a solution of indole (500 mg, 4.27 mmol) and potassium hydroxide (263 mg, 4.70 mmol) in DMSO (10 ml) was added 2-chloroethan-1-ol (455 μ l, 6.41 mmol) and the solution stirred at rt for 24 hours. Water (50 ml) was added and the solution extracted into DCM (2 x 50 ml), dried (MgSO_4), filtered and Solvent removed *in vacuo*, residue purified by chromatography 4:1 Hexane:EtOAc to afford the title compound as a colourless oil (202 mg, 29 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.68 (1H, d, J 7.9), 7.39 (1H, d, J 8.2), 7.26 (1H, dt, J 0.9 and 7.1), 7.18-7.14 (2H, m), 6.55 (1H, d, J 3.2), 4.27-4.23 (2H, m), 3.92-3.88 (2H, m), 2.18 (1H, s). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 136.1, 128.7, 128.4, 121.7, 121.1, 119.6, 109.4, 101.5, 61.9, 48.7.

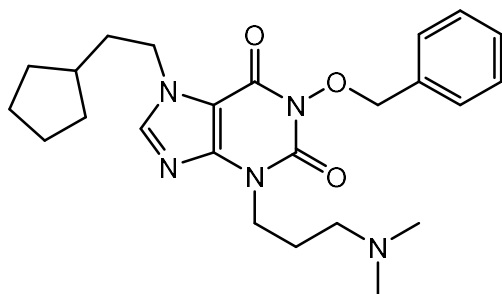
7-(2-(1H-indol-1-yl)ethyl)-1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22ad)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-(1H-indol-1-yl)ethan-1-ol (113 mg,

0.70 mmol) to afford title compound as a yellow solid (49 mg, 39 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.61-7.58 (2H, m), 7.49 (1H, d, J 7.7), 7.43-7.38 (3H, m), 7.22 (1H, s), 7.15 (1H, d, J 8.2), 7.07-6.97 (3H, m), 6.41 (1H, d, J 3.1), 5.10 (2H, s), 4.65 (2H, t, J 6.1), 4.59 (2H, t, J 6.1), 4.05 (2H, t, J 7.3), 2.71 (2H, t, J 7.3), 2.53 (6H, s), 1.96 (2H, quin, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.3, 149.4, 147.3, 142.9, 136.1, 134.1, 129.8, 128.9, 128.6, 128.1, 127.4, 121.2, 120.5, 119.2, 108.4, 106.3, 101.7, 78.1, 55.4, 53.5, 45.5, 43.1, 40.1, 24.3.

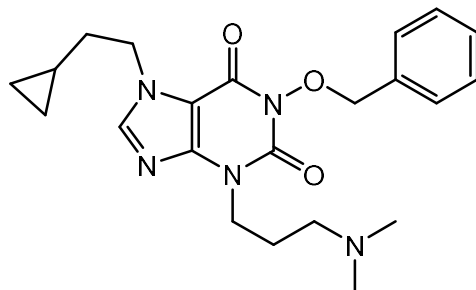
1-(benzyloxy)-7-(2-cyclopentylethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22ae)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 2-cyclopentylethan-1-ol (89 mg, 0.78 mmol) to afford title compound as a white solid (58

mg, 51 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 8.00 (1H, s), 7.59 (2H, dd, J 3.5 and 6.0), 7.39 (2H, m), 5.14 (2H, s), 4.34 (2H, t, J 7.5), 4.18 (2H, t, J 7.2), 2.72 (2H, t, J 7.5), 2.49 (6H, s), 2.06 (2H, quin, J 7.5), 1.92-1.75 (5H, m), 1.71-1.64 (2H, m), 1.61-1.54 (2H, m), 1.27-1.18 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.4, 149.6, 147.5, 142.5, 134.1, 129.9, 128.8, 128.0, 106.5, 78.0, 55.8, 46.4, 43.4, 41.0, 37.1, 37.0, 32.0, 24.7, 23.7.

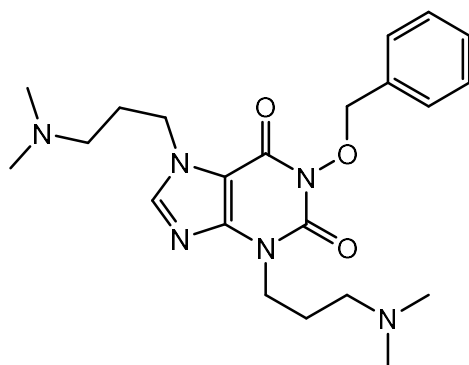
1-(benzyloxy)-7-(2-cyclopropylethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22af)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (70 mg, 0.21 mmol) and 2-cyclopropylethan-1-ol (75 μl , 0.70 mmol) to afford title compound as a yellow solid (43 mg, 40

%). ^1H NMR δ_{H} (400.13 MHz; MeOD): 8.01 (1H, s), 7.59-7.55 (2H, m), 7.40-7.37 (3H, m), 4.41 (2H, t, J 6.9), 4.19 (2H, t, J 7.1), 2.84 (2H, t, J 7.1), 2.59 (6H, s), 2.09 (2H, quin, J 7.1), 1.74 (2H, q, J 6.9), 0.70-0.61 (1H, m), 0.45-0.40 (2H, m), 0.03- -0.02 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.4, 149.6, 147.4, 142.9, 134.0, 129.3, 128.8, 127.9, 106.4, 78.0, 55.5, 53.5, 43.2, 40.9, 35.3, 24.4, 7.4, 3.0.

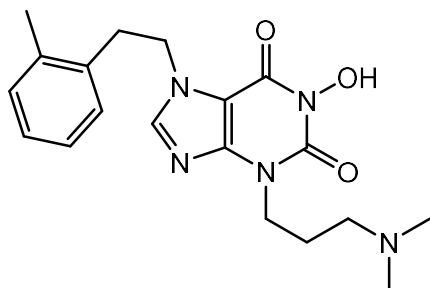
1-(benzyloxy)-3,7-bis(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (22ag)



Prepared according to standard procedure 9 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.23 mmol) and 3-(dimethylamino)propan-1-ol (83 μ l, 0.78 mmol) to afford title compound as a yellow solid (57 mg, 51 %). ^1H NMR δ_{H} (400.13 MHz; MeOD):

7.99 (1H, s), 7.59 (2H, dd, J 3.9 and 6.7), 7.41-7.37 (3H, m), 5.13 (2H, s), 4.36 (2H, t, J 7.2), 4.16 (2H, t, J 7.2), 2.55 (2H, t, J 7.2), 2.44 (2H, t, J 7.2), 2.35 (6H, s), 2.33 (6H, s), 2.10 (2H, quin, J 7.2), 2.01 (2H, quin, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.4, 149.5, 147.6, 142.8, 134.1, 129.8, 128.8, 128.0, 106.4, 77.9, 56.1, 55.8, 43.9, 43.8, 43.7, 41.5, 27.8, 25.1.

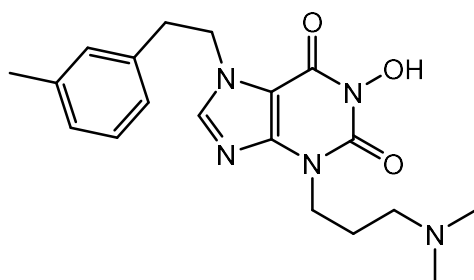
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(2-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23a)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23 mg, 0.05 mmol) to afford the title compound as a white solid (14 mg, 75 %). ^1H NMR δ_{H} (400.13 MHz; MeOD):

7.74 (1H, s), 7.16 (1H, dt, J 7.2 and 15.5), 7.07 (1H, t, J 7.2), 7.02-6.99 (1H, m), 4.65 (2H, t, J 6.8), 4.37 (2H, t, J 6.4), 3.28 (2H, t, J 6.8), 3.04 (2H, t, J 6.4), 2.34 (6H, s), 2.22 (2H, quin, J 6.4). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 152.4, 148.6, 146.9, 142.7, 136.4, 135.2, 130.2, 128.9, 126.7, 125.7, 106.5, 56.4, 46.7, 42.3, 40.0, 34.0, 25.4, 17.9. HRMS (ESI $^+$) $[\text{M}+\text{H}]$ expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_3$, 372.2030: found 372.2020.

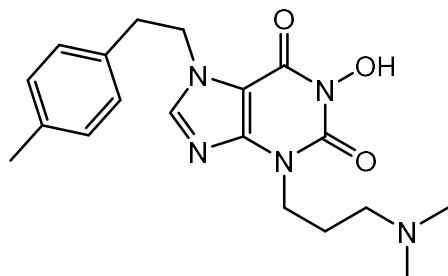
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(3-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23b)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (41 mg, 0.09 mmol) to afford the title compound as a cream

solid (24 mg, 73 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.71 (1H, s), 7.16 (1H, t, J 7.4), 7.07-6.92 (3H, m), 4.63 (2H, t, J 7.3), 4.33 (2H, t, J 7.3), 3.20 (2H, t, J 7.3), 2.88 (2H, t, J 7.3), 2.61 (6H, s), 2.30 (3H, s), 2.16 (2H, quin, J 7.3). ^1H NMR δ_{H} (400.13 MHz; MeOD): 160.5, 151.1, 150.0, 142.3, 138.0, 137.0, 129.1, 128.3, 127.1, 125.5, 106.9, 55.7, 48.1, 47.9, 43.1, 36.6, 24.5, 20.0. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_3$, 372.2030: found 372.2029. [M+Na] expected for $\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_3\text{Na}$, 394.1850: found 394.1847.

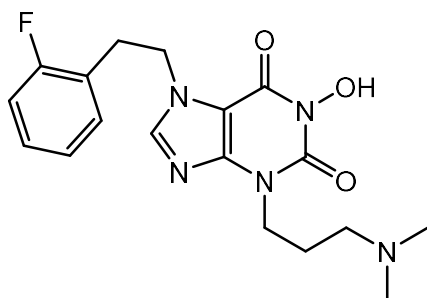
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(4-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23c)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-methylphenethyl)-3,7-dihydro-1H-purine-2,6-dione (12 mg, 0.03 mmol) to afford the title compound as a white solid (9 mg,

93 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.67 (1H, s), 7.09 (2H, d, J 8.0), 7.03 (2H, d, J 8.0), 4.54 (2H, t, J 7.2), 4.23 (2H, t, J 6.6), 3.22 (2H, t, J 7.2), 3.14 (2H, t, J 6.6), 2.90 (6H, s), 2.30 (3H, s), 2.22 (2H, quin, J 6.6). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 158.0, 153.0, 150.4, 142.5, 136.3, 134.2, 128.8, 128.4, 106.1, 54.9, 48.4, 47.9, 42.2, 40.2, 36.2, 23.4, 19.7. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_3$, 372.2030: found 372.2041. [M+Na] expected for $\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_3\text{Na}$, 394.1850: found 394.1846.

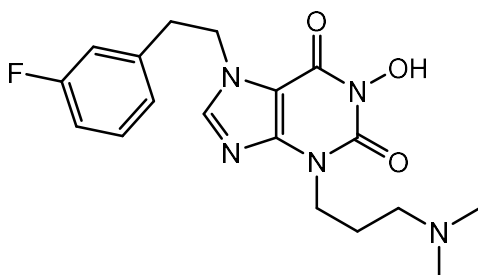
3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23d)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (58 mg, 0.12 mmol) to afford the title compound as a white solid (36 mg, 80 %). ^1H NMR δ_{H} (400.13 MHz; DMSO):

10.55 (1H, s), 7.87 (1H, s), 7.32-7.08 (4H, m), 4.49 (2H, t, J 6.8), 4.03 (2H, t, J 6.8), 3.17 (2H, t, J 6.8), 2.51 (6H, s), 2.42-2.36 (2H, m), 1.90 (2H, quin, 6.8). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 162.5, 160.0, 153.4, 150.8, 146.4, 142.1, 130.9, 128.7, 124.2, 114.9, 106.5, 55.2, 47.9, 46.9, 42.7, 40.5, 29.9, 23.9. ^{19}F NMR δ_{F} (376 MHz; DMSO): -118.8 (1F, s). HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{18}\text{H}_{23}\text{FN}_5\text{O}_3$, 376.1785: found 376.1778. [M+Na] expected for $\text{C}_{18}\text{H}_{22}\text{FN}_5\text{O}_3\text{Na}$, 398.1604: found 398.1595.

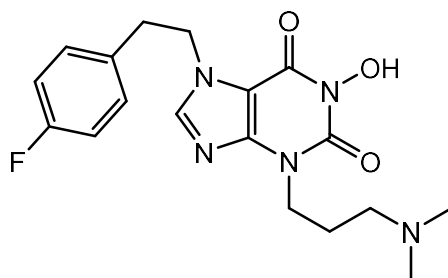
3-(3-(dimethylamino)propyl)-7-(3-fluorophenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23e)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (29 mg, 0.07 mmol) to afford the title compound as a white

solid (22 mg, 96 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.77 (1H, s), 7.35-6.90 (4H, m), 4.64 (2H, t, J 6.9), 4.34 (2H, t, J 6.1), 3.29-3.23 (4H, m), 2.92 (6H, s), 2.28 (2H, quin, J 6.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.7, 159.7, 144.4, 142.6, 142.4, 140.2, 130.0, 124.5, 115.3, 113.2, 104.2, 54.9, 48.4, 47.9, 42.1, 36.3, 23.5. ^{19}F NMR δ_{F} (376 MHz; DMSO): -73.7 (1F, s). HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{17}\text{H}_{21}\text{FN}_5\text{O}_3$, 376.1779: found 376.1778.

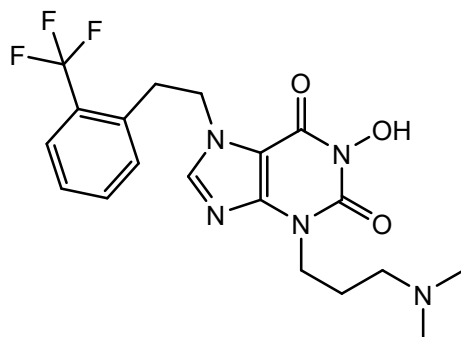
3-(3-(dimethylamino)propyl)-7-(4-fluorophenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23f)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (80 mg, 0.17 mmol) to afford the title compound as an orange solid (60

mg, 94 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.53 (1H, s), 7.87 (1H, s), 7.22-7.09 (4H, m), 4.47 (2H, t, J 7.3), 4.03 (2H, t, J 7.4), 3.11 (2H, t, J 7.3), 2.52 (6H, s), 2.34-2.46 (2H, m), 1.91-1.84 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 161.6, 159.2, 140.4, 136.1, 128.8, 127.1, 113.5, 104.7, 53.9, 46.5, 41.5, 39.3, 34.4, 22.6. ^{19}F NMR δ_{F} (376 MHz; DMSO): -116.3 (1F, s). HRMS (ESI^+) [$\text{M}+\text{H}$] expected for $\text{C}_{18}\text{H}_{23}\text{FN}_5\text{O}_3$, 376.1779: found 376.1774. [$\text{M}+\text{Na}$] expected for $\text{C}_{18}\text{H}_{22}\text{FN}_5\text{O}_3\text{Na}$, 398.1599: found 398.1603.

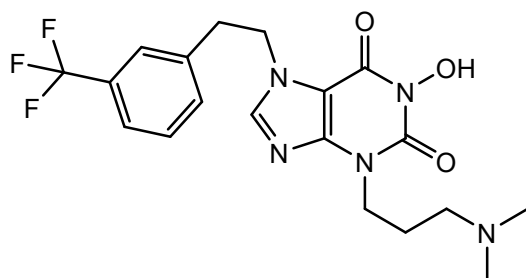
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(2-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (23g)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (52 mg, 0.10 mmol) to afford the title compound as a white solid (35 mg, 84 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.52

(1H, br s), 7.92 (1H, s), 7.74-7.70 (1H, m), 7.26 (1H, t, J 7.4), 7.46 (2H, dt, J 7.1 and 16.1), 4.54 (2H, t, J 7.1), 4.03 (2H, t, J 7.1), 2.91 (2H, t, J 7.1), 2.47-2.42 (2H, m), 2.26 (6H, s), 1.85 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.3, 142.2, 135.7, 132.0, 131.4, 127.2, 125.9, 118.3, 115.3, 106.6, 54.9, 46.4, 42.3, 33.4, 13.5. ^{19}F NMR δ_{F} (376 MHz; MeOD): -76.9 (3F, s). HRMS (ESI^+) [$\text{M}+\text{H}$] expected for $\text{C}_{19}\text{H}_{23}\text{F}_3\text{N}_5\text{O}_3$, 426.1748: found 426.1753. [$\text{M}+\text{Na}$] expected for $\text{C}_{19}\text{H}_{22}\text{F}_3\text{N}_5\text{O}_3\text{Na}$, 448.1567: found 448.1558.

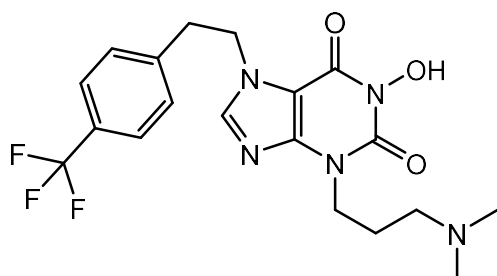
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(3-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (23h)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (32 mg, 0.06 mmol) to afford the title

compound as a white solid (21 mg, 80 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.52 (1H, br s), 7.89 (1H, s), 7.62-7.41 (4H, m), 4.51 (2H, t, J 6.8), 4.01 (2H, t, J 7.1), 3.22 (2H, t, J 6.8), 2.51 (6H, s), 2.25 (2H, t, J 7.1), 1.83 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.4, 149.4, 145.3, 142.3, 138.7, 134.0, 132.0, 129.2, 125.0, 123.5, 122.9, 118.1, 115.2, 106.5, 55.3, 48.3, 42.7, 40.2, 36.4, 24.1. ^{19}F NMR δ_{F} (376 MHz; MeOD): -76.9 (3F, s). HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{19}\text{H}_{23}\text{F}_3\text{N}_5\text{O}_3$, 426.1748: found 426.1752. [M+Na] expected for $\text{C}_{19}\text{H}_{22}\text{F}_3\text{N}_5\text{O}_3\text{Na}$, 448.1567: found 448.1550.

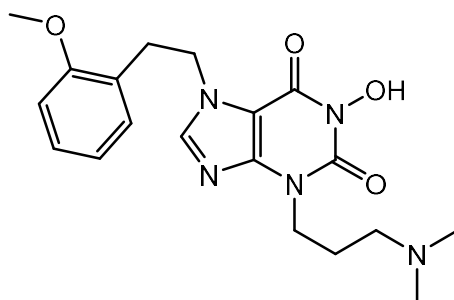
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(4-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (23i)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-(trifluoromethyl)phenethyl)-3,7-dihydro-1H-purine-2,6-dione (39 mg, 0.08 mmol) to afford the title

compound as a white solid (21 mg, 65 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.52 (1H, br s), 7.89 (1H, s), 7.77-7.64 (2H, m), 7.47-7.39 (2H, m), 4.51 (2H, t, J 6.8), 4.03 (2H, t, J 7.1), 3.22 (2H, t, J 6.8), 2.51 (6H, s), 2.33 (2H, t, J 7.1), 1.88 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.2, 149.1, 142.4, 142.0, 132.9, 129.3, 125.2 (q, J 3.6), 118.2, 115.3, 106.5, 54.9, 48.3, 42.3, 40.2, 36.3, 23.6. ^{19}F NMR δ_{F} (376 MHz; MeOD): -76.9 (3F, s). HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{19}\text{H}_{23}\text{F}_3\text{N}_5\text{O}_3$, 426.1748: found 426.1753. [M+Na] expected for $\text{C}_{19}\text{H}_{22}\text{F}_3\text{N}_5\text{O}_3\text{Na}$, 448.1567: found 448.1560.

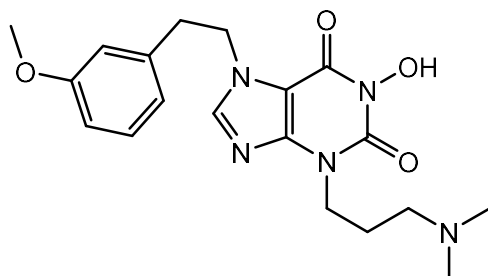
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(2-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23j)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (31 mg, 0.07 mmol) to afford the title compound as a white solid (17 mg, 69 %). ^1H NMR δ_{H} (400.13

MHz; DMSO): 10.53 (1H, s), 7.77 (1H, s), 7.25-6.81 (4H, m), 4.45 (2H, t, J 6.9), 4.04 (2H, t, J 6.6), 3.72 (3H, s), 3.09 (2H, t, J 6.9), 2.60-2.48 (8H, m), 2.02-1.93 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 157.7, 142.4, 130.2, 128.6, 127.5, 125.1, 120.1, 110.1, 106.4, 65.5, 54.4, 48.5, 46.9, 42.3, 31.4, 23.4. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_4$, 388.1979; found 388.1983.

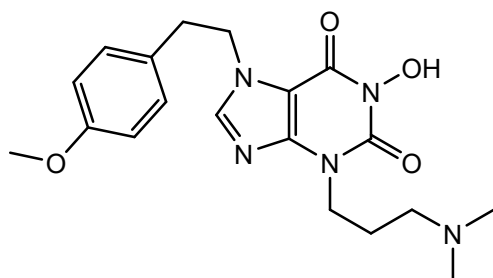
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23k)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (64 mg, 0.13 mmol) to afford the title compound as a white

solid (40 mg, 90 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.66 (1H, s), 7.17 (1H, t, J 8.1), 6.80-6.69 (3H, m), 4.52 (2H, t, J 7.2), 4.19 (2H, t, J 6.6), 3.75 (3H, s), 3.38 (6H, s), 3.14-3.07 (4H, m), 2.16 (2H, quin, J 6.6). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 160.0, 153.4, 150.7, 146.4, 142.1, 138.8, 129.3, 120.7, 114.1, 112.0, 106.2, 54.3, 49.1, 48.1, 46.6, 40.7, 36.6, 23.1. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_4$, 388.1985; found 388.1980. [M+Na] expected for $\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_4\text{Na}$, 410.1805; found 410.1793.

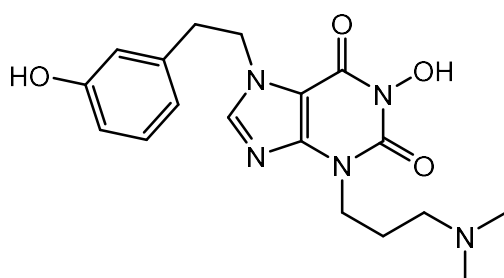
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(4-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23l)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (20 mg, 0.04 mmol) to afford the title compound as a

white solid (16 mg, 95 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.70 (1H, s), 7.07 (2H, d, J 8.0), 6.83 (2H, d, J 8.0), 4.61 (2H, t, J 6.2), 4.36 (2H, t, J 6.5), 3.77 (3H, s), 3.25 (2H, t, J 6.2), 3.18 (2H, t, J 6.5), 2.90 (6H, s), 2.29 (2H, quin, 6.5). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 158.7, 157.8, 152.4, 145.3, 142.4, 129.5, 129.1, 113.7, 106.6, 55.0, 54.2, 48.5, 47.9, 42.2, 35.8, 23.5. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_4$, 388.1979: found 388.1972.

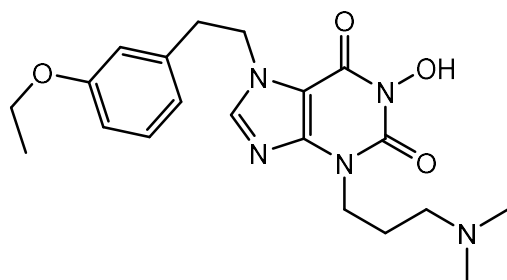
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(3-hydroxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23m)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(3-(benzyloxy)phenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (69 mg, 0.13 mmol) to afford the title compound

as a white solid (45 mg, 96 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 9.45 (1H, br s), 7.88 (1H, s), 7.07 (1H, t, J 7.8), 7.64-7.56 (3H, m), 4.43 (2H, t, J 7.1), 4.04 (2H, t, J 6.8), 3.01 (2H, t, J 7.1), 2.87 (2H, t, J 6.8), 2.51 (6H, s), 1.99 (2H, quin, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 157.3, 151.0, 146.6, 142.4, 138.8, 129.3, 119.6, 115.4, 113.4, 106.2, 54.7, 42.2, 40.1, 36.4, 23.4. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{18}\text{H}_{24}\text{N}_5\text{O}_4$, 374.1823: found 374.1833. [M+Na] expected for $\text{C}_{18}\text{H}_{23}\text{N}_5\text{O}_4\text{Na}$, 396.1642: found 396.1634. HRMS (ESI $^{-}$) [M-H] expected for $\text{C}_{18}\text{H}_{22}\text{N}_5\text{O}_4$, 372.1677: found 372.1673.

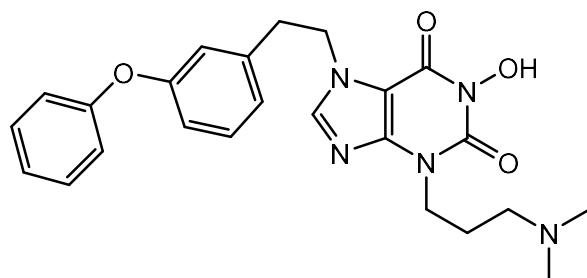
3-(3-(dimethylamino)propyl)-7-(3-ethoxyphenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23n)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-ethoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (39 mg, 0.08 mmol) to afford the title compound as a

white solid (31 mg, 99 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.74-6.67 (5H, m), 4.53 (2H, t, J 7.1), 4.30-4.22 (2H, m), 3.97 (2H, q, J 6.7), 3.20 (2H, t, J 7.1), 3.12 (2H, t, J 6.6), 2.88 (6H, s), 2.21 (2H, t, J 6.6), 1.34 (3H, t, J 6.7). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.8, 161.4, 159.3, 142.4, 138.9, 129.2, 120.8, 118.2, 115.0, 122.5, 106.4, 63.0, 54.7, 48.1, 47.9, 42.2, 36.6, 23.4, 13.9. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{20}\text{H}_{28}\text{N}_5\text{O}_4$, 402.2136: found 402.2147. [M+Na] expected for $\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_4\text{Na}$, 424.1955: found 424.1964.

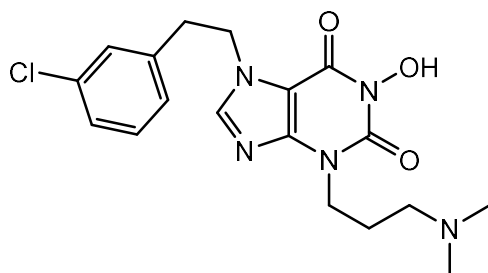
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(3-phenoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (23o)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-phenoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione

(68 mg, 0.15 mmol) to afford the title compound as a yellow solid (46 mg, 82 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 7.88 (1H, s), 7.38 (2H, t, J 8.0), 7.30 (1H, t, J 8.0), 7.13 (1H, t, J 7.6), 6.95 (3H, d, J 7.3), 6.86 (1H, dd, J 2.0 and 8.0), 6.82-6.80 (1H, m), 4.47 (2H, t, J 7.2), 4.02 (2H, t, J 7.1), 3.10 (2H, t, J 7.2), 2.44-2.36 (2H, m), 2.22 (6H, s), 1.82 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 157.4, 157.1, 151.1, 146.1, 142.0, 139.6, 129.8, 129.5, 123.5, 123.0, 119.0, 118.4, 116.9, 115.4, 106.3, 55.3, 48.4, 42.8, 40.7, 36.4, 24.0. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{24}\text{H}_{28}\text{N}_5\text{O}_4$, 450.2136: found 450.2152. [M+Na] expected for $\text{C}_{24}\text{H}_{27}\text{N}_5\text{O}_4\text{Na}$, 472.1955: found 472.1950.

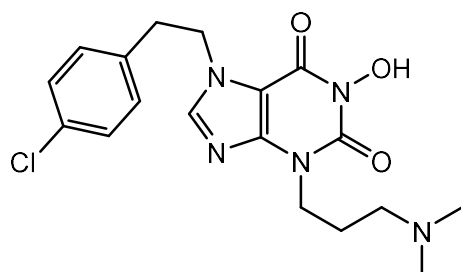
7-(3-chlorophenethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23p)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(3-chlorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (51 mg, 0.13 mmol), purified by preparative HPLC

eluting with MeCN:H₂O 3:7 to afford the title compound as a trifluoro acetate salt as a red oil (5 mg, 13 %). ¹H NMR δ_H (400.13 MHz; MeOD): 7.75 (1H, s), 7.30-7.24 (2H, m), 7.20 (1H, t, *J* 1.6), 7.13 (1H, dt, *J* 1.6 and 7.2), 4.58 (2H, t, *J* 7.1), 4.24 (2H, t, *J* 6.5), 3.23 (2H, t, *J* 7.1), 3.20 (2H, t, *J* 6.5), 2.92 (6H, s), 2.22 (2H, quin, *J* 6.5). ¹³C NMR δ_C (101.6 MHz; MeOD): 152.9, 150.3, 147.0, 142.4, 139.6, 134.0, 129.9, 128.6, 126.8, 126.7, 106.2, 54.9, 46.2, 42.2, 39.9, 36.1, 23.3. HRMS (ESI⁺) [M+Na] expected for C₁₈H₂₂ClN₅O₃Na, 414.1303: found 414.1297.

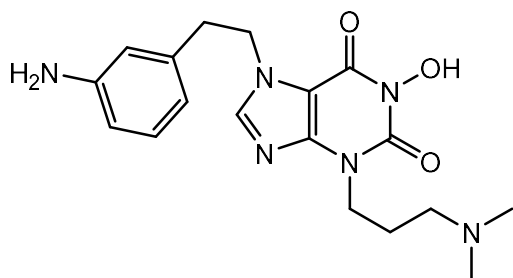
7-(4-chlorophenethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23q)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(4-chlorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (55 mg, 0.14 mmol), purified by preparative HPLC

eluting with MeCN:H₂O 3:7 to afford the title compound as a trifluoro acetate salt as a red oil (5 mg, 12 %). ¹H NMR δ_H (400.13 MHz; MeOD): 7.72 (1H, s), 7.28 (2H, dt, *J* 1.8 and 8.4), 7.16 (2H, dt, *J* 1.8 and 8.4), 4.57 (2H, t, *J* 7.0), 4.23 (2H, t, *J* 6.8), 3.22 (2H, t, *J* 7.0), 3.19 (2H, t, *J* 6.8), 2.91 (6H, s), 2.21 (2H, quin, *J* 6.8). ¹³C NMR δ_C (101.6 MHz; MeOD): 152.9, 150.4, 147.1, 142.5, 136.1, 132.5, 130.1, 128.3, 106.2, 55.0, 46.2, 42.1, 39.9, 35.8, 23.3. HRMS (ESI⁺) [M+H] expected for C₁₈H₂₃ClN₅O₃, 392.1484: found 392.1492. [M+Na] expected for C₁₈H₂₂ClN₅O₃Na, 414.1303: found 414.1308

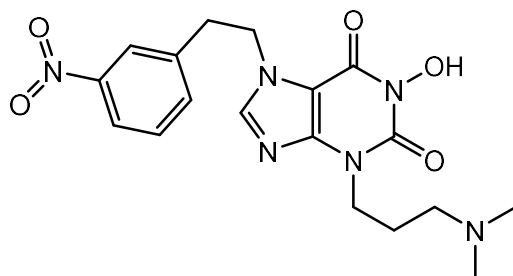
7-(3-aminophenethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23r)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-nitrophenethyl)-3,7-dihydro-1H-purine-2,6-dione (30 mg, 0.06 mmol) to afford the title compound

as a yellow solid (23 mg, 99 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.54 (1H, s), 7.90 (1H, s), 6.92 (2H, t, J 7.6), 6.43-6.32 (3H, m), 5.00 (2H, br s), 4.41 (2H, t, J 7.2), 4.04 (2H, t, J 7.1), 2.94 (2H, t, J 7.2), 2.51 (6H, s), 2.46-2.42 (2H, m), 1.92 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 147.7, 142.5, 138.0, 129.0, 118.3, 118.0, 115.4, 113.6, 106.2, 54.9, 42.3, 40.0, 36.5, 23.5. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{18}\text{H}_{25}\text{N}_6\text{O}_3$, 373.1983: found 373.1981. [M+Na] expected for $\text{C}_{18}\text{H}_{24}\text{N}_6\text{O}_3\text{Na}$, 395.1802: found 395.1785.

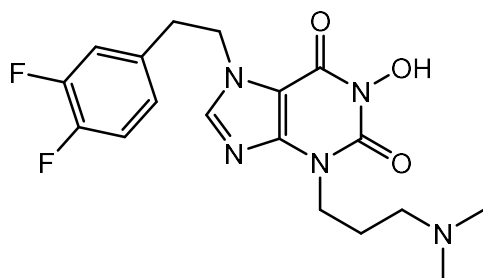
3-(3-(dimethylamino)propyl)-1-hydroxy-7-(3-nitrophenethyl)-3,7-dihydro-1H-purine-2,6-dione (23s)



A solution of 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(3-nitrophenethyl)-3,7-dihydro-1H-purine-2,6-dione (30 mg, 0.06 mmol) in TFA (5 ml) was stirred at 120 °C under microwave radiation

for 20 minutes. Solvent removed *in vacuo* to afford the title compound as the TFA salt as an orange oil (10 mg, 33 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.59 (1H, s), 8.11 (1H, dt, J 2.4 and 6.5), 8.06 (1H, s), 7.90 (1H, s), 7.60-7.58 (2H, m), 4.54 (2H, t, J 7.0), 4.06 (2H, t, J 6.8), 3.28 (2H, t, J 7.0), 2.55 (6H, s), 2.04 (2H, quin, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.9, 150.3, 148.3, 147.0, 142.6, 139.6, 135.2, 129.6, 123.3, 121.6, 106.3, 54.9, 46.2, 42.2, 40.1, 36.1, 23.6. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{18}\text{H}_{23}\text{N}_6\text{O}_5$, 403.1724: found 403.1736. [M+Na] expected for $\text{C}_{18}\text{H}_{22}\text{N}_6\text{O}_5\text{Na}$, 425.1544: found 425.1535.

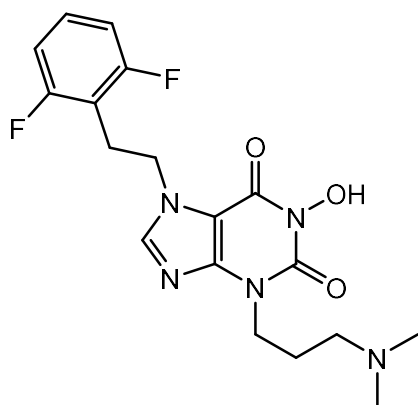
7-(3,4-difluorophenethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23t)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(3,4-difluorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (52 mg, 0.11 mmol) to afford the title compound as

a white solid (40 mg, 95 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.53 (1H, s), 7.86 (1H, s), 7.33 (1H, dt, J 8.6 and 10.5), 7.30-7.24 (1H, m), 6.97-6.94 (1H, m), 4.48 (2H, t, J 6.8), 4.03 (2H, t, J 7.1), 3.12 (2H, t, J 6.8), 2.51 (6H, s), 2.35-2.33 (2H, m), 1.88 (2, quin, J 6.8). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.4, 150.7 (dd, J 12.5 and 82.4), 148.3 (dd, J 13.2 and 82.4), 142.2, 134.9 (dd, J 3.8 and 5.6), 125.1 (dd, J 3.0 and 6.0), 118.4, 117.4 (d, J 17.4), 117.0 (d, J 17.4), 115.4, 106.2, 55.2, 48.5, 42.5, 40.6, 35.7, 23.9. ^{19}F NMR δ_{F} (376 MHz; MeOD): -76.9 (1F, s), -76.8 (1F, s). HRMS (ESI $^{+}$) [$\text{M}+\text{H}$] expected for $\text{C}_{18}\text{H}_{22}\text{F}_2\text{N}_5\text{O}_3$, 394.1685; found 394.1693. [$\text{M}+\text{Na}$] expected for $\text{C}_{18}\text{H}_{21}\text{F}_2\text{N}_5\text{O}_3\text{Na}$, 416.1405; found 416.1503.

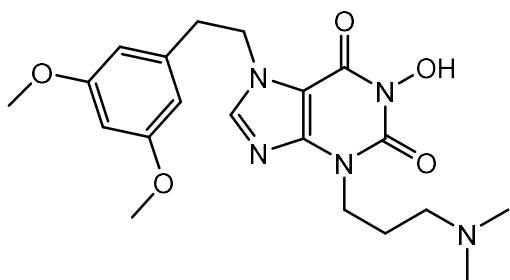
7-(2,6-difluorophenethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23u)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(2,6-difluorophenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (54 mg, 0.11 mmol) to afford the title compound as a white solid (38 mg, 88 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.51 (1H, s), 7.85 (1H, s), 7.38-7.29 (1H, m), 7.05-6.99 (2H, m), 4.48 (2H,

t, J 6.4), 4.01 (2H, t, J 7.3), 3.20 (2H, t, J 6.4), 2.51 (6H, s), 2.24-2.19 (2H, m), 1.82 (2H, quin, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.5 (dd, J 7.7 and 247.3), 147.2, 145.5, 142.5, 129.2 (d, J 10.8), 116.8 (d, J 292.8), 110.9 (d, J 26.5), 106.7, 55.1, 45.9, 42.6, 40.8, 33.9, 24.1. ^{19}F NMR δ_{F} (376 MHz; MeOD): -76.9 (2F, s). HRMS (ESI $^{+}$) [$\text{M}+\text{H}$] expected for $\text{C}_{18}\text{H}_{23}\text{F}_2\text{N}_5\text{O}_3$, 394.1685; found 394.1689. [$\text{M}+\text{Na}$] expected for $\text{C}_{18}\text{H}_{22}\text{F}_2\text{N}_5\text{O}_3\text{Na}$, 416.1505; found 416.1481.

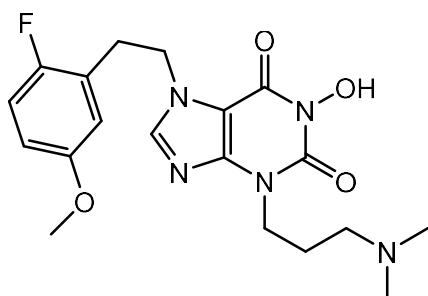
7-(3,5-dimethoxyphenethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23v)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(3,5-dimethoxyphenethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (41 mg, 0.08 mmol) to afford the title

compound as a white solid (31 mg, 74 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 7.87 (1H, s), 6.37-6.34 (1H, m), 6.33-6.32 (2H, m), 4.47 (2H, t, J 7.0), 4.03 (2H, t, J 7.2), 3.69 (6H, s), 3.35-3.33 (2H, m), 3.04 (2H, t, J 7.2), 2.51 (6H, s), 1.85 (2H, quin, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.4, 161.2, 145.6, 139.6, 118.4, 115.3, 106.7, 106.4, 98.0, 54.9, 54.3, 42.2, 40.5, 36.7, 23.5. HRMS (ESI $^{+}$) [M+H] $^{+}$ expected for $\text{C}_{20}\text{H}_{28}\text{N}_5\text{O}_5$, 418.2085: found 418.2087.

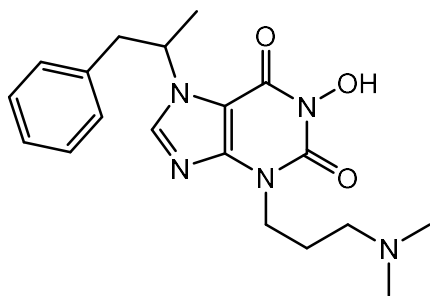
3-(3-(dimethylamino)propyl)-7-(2-fluoro-5-methoxyphenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23w)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-fluoro-5-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (60 mg, 0.12 mmol) to afford the title compound as a white solid (44 mg, 90 %). ^1H NMR δ_{H} (400.13 MHz;

DMSO): 10.53 (1H, s), 7.88 (1H, s), 7.06 (1H, t, J 8.9), 6.81 (1H, m), 6.75 (1H, m), 4.49 (2H, t, J 7.0), 4.04 (2H, t, J 7.0), 3.69 (3H, s), 3.13 (2H, t, J 7.0), 2.51 (6H, s), 2.38 (2H, m), 1.90 (2H, quin, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 155.9, 155.5 (d, J 237.4), 151.5, 147.0, 142.3 (dd, J 4.1 and 11.1), 124.7 (d, J 17.1), 121.2, 118.3, 116.8 (d, J 295.0), 115.9 (dd, J 6.7 and 12.3), 115.4 (d, J 23.9), 54.9, 54.8, 46.9, 42.3, 40.4, 30.1, 23.6. ^{19}F NMR δ_{F} (376 MHz; MeOD): -76.8 (1F, s). HRMS (ESI $^{+}$) [M+H] $^{+}$ expected for $\text{C}_{19}\text{H}_{25}\text{FN}_5\text{O}_4$, 406.1885: found 406.1889. [M+Na] $^{+}$ expected for $\text{C}_{19}\text{H}_{24}\text{FN}_5\text{O}_4\text{Na}$, 428.1705: found 428.1705.

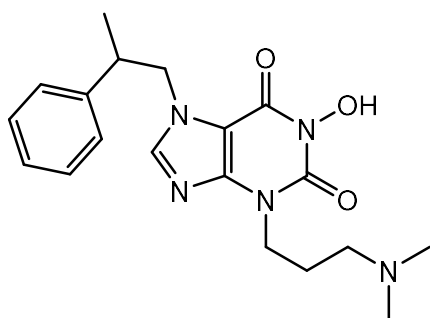
(RS) 3-(3-(dimethylamino)propyl)-1-hydroxy-7-(1-phenylpropan-2-yl)-3,7-dihydro-1H-purine-2,6-dione (23x)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(1-phenylpropan-2-yl)-3,7-dihydro-1H-purine-2,6-dione (48 mg, 0.10 mmol) to afford the title compound as an orange oil (13 mg, 35 %). ^1H NMR δ_{H} (400.13 MHz;

DMSO): 10.55 (1H, s), 8.11 (1H, s), 7.26 (2H, t, J 7.2), 7.20 (1H, t, J 7.2), 7.12 (1H, d, J 7.2), 5.04 (0.5H, q, J 7.1), 4.11 (0.5H, q, J 5.3), 4.04 (2H, t, J 6.7), 3.30 (1H, dd, J 8.0 and 13.7), 3.12 (1H, dd, 6.3 and 13.9), 2.92 (2H, t, J 6.7), 2.64 (6H, s), 1.99 (2H, quin, J 6.7), 1.54 (3H, d, J 6.7). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 160.6, 150.3, 147.2, 141.2, 137.2, 128.8, 128.1, 126.4, 106.1, 56.2, 54.8, 46.1, 42.1, 39.9, 23.3, 19.0. HRMS (ESI $^{+}$) [$\text{M}+\text{H}$] expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_3$, 372.2030; found 372.2029.

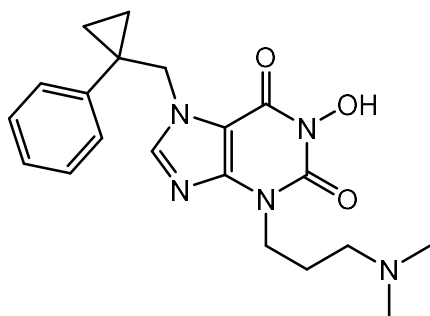
(RS) 3-(3-(dimethylamino)propyl)-1-hydroxy-7-(2-phenylpropyl)-3,7-dihydro-1H-purine-2,6-dione (23y)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-phenylpropyl)-3,7-dihydro-1H-purine-2,6-dione (50 mg, 0.10 mmol) to afford the title compound as a white solid (37 mg, 92 %). ^1H NMR δ_{H} (400.13 MHz; DMSO):

10.52 (1H, s), 7.84 (1H, s), 7.31 (2H, t, J 7.1), 7.26-7.21 (3H, m), 4.40 (2H, d, J 7.5), 4.01 (2H, t, J 7.2), 2.69 (0.5H, m), 2.59-2.54 (2H, m), 2.52 (6H, s), 2.22 (0.5, m), 1.88 (2H, quin, J 7.2), 1.20 (3H, d, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.4, 151.3, 142.5, 128.4, 127.0, 126.7, 118.3, 115.4, 106.3, 55.0, 53.4, 42.5, 40.8, 40.4, 23.8, 17.2. HRMS (ESI $^{+}$) [$\text{M}+\text{H}$] expected for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_3$, 372.2030; found 372.2032. [$\text{M}+\text{Na}$] expected for $\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_3\text{Na}$, 394.1850; found 394.1841.

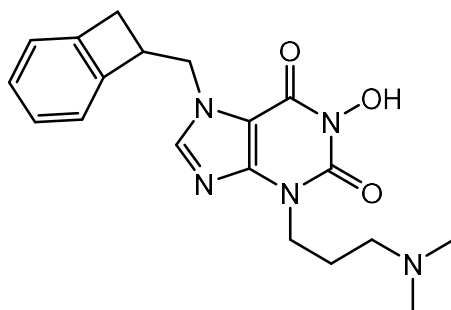
3-(3-(dimethylamino)propyl)-1-hydroxy-7-((1-phenylcyclopropyl)methyl)-3,7-dihydro-1H-purine-2,6-dione (23z)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-((1-phenylcyclopropyl)methyl)-3,7-dihydro-1H-purine-2,6-dione (66 mg, 0.14 mmol) to afford the title compound as a colourless oil (42 mg, 57 %). ^1H NMR δ_{H} (400.13

MHz; MeOD): 7.38 (1H, s), 7.27-7.17 (5H, m), 4.58 (2H, s), 4.20 (2H, t, J 6.2), 3.21 (2H, t, J 6.2), 2.90 (6H, s), 2.20 (2H, quin, J 6.2), 1.26 (2H, dd, J 1.0 and 5.1), 0.94 (2H, dd, J 1.0 and 5.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.9, 150.2, 146.5, 142.4, 141.3, 129.3, 128.2, 127.0, 106.2, 54.9, 42.2, 39.9, 38.9, 26.9, 23.3, 10.5. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{20}\text{H}_{26}\text{N}_5\text{O}_3$, 406.1850: found 406.1856.

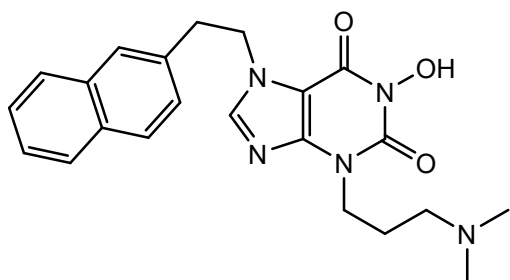
7-(bicyclo[4.2.0]octa-1(6),2,4-trien-7-ylmethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23aa)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(bicyclo[4.2.0]octa-1(6),2,4-trien-7-ylmethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (39 mg, 0.10 mmol) to afford the title compound as a yellow solid (25 mg, 70

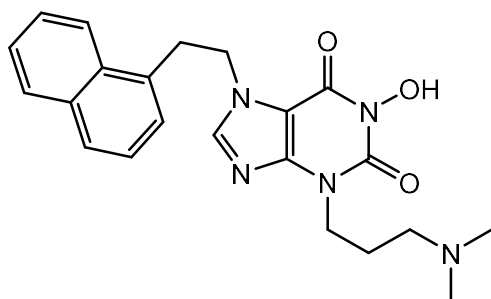
%). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.55 (1H, s), 8.15 (1H, s), 7.27-7.12 (3H, m), 6.88-6.84 (1H, m), 4.62 (1H, dd, J 7.9 and 13.7), 4.55 (1H, dd, J 7.9 and 13.7), 4.05 (2H, t, J 7.4), 4.01-3.91 (1H, m), 3.27 (2H, dd, J 4.9 and 14.3), 2.50 (6H, s), 2.37 (2H, t, J 7.4), 1.85 (2H, quin, J 7.4). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 151.5, 150.1, 144.9, 143.1, 142.6, 142.2, 127.9, 126.7, 122.8, 121.7, 106.6, 50.5, 49.8, 42.1, 39.5, 33.1, 29.2, 24.4. HRMS (ESI^+) $[\text{M}+\text{H}]$ expected for $\text{C}_{19}\text{H}_{24}\text{N}_5\text{O}_3$, 370.1874: found 370.1872.

3-(3-(dimethylamino)propyl)-1-hydroxy-7-(2-(naphthalen-2-yl)ethyl)-3,7-dihydro-1H-purine-2,6-dione (23ab)



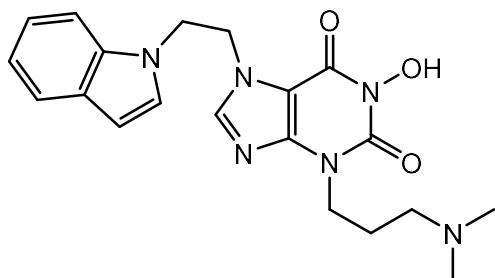
Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-(naphthalen-2-yl)ethyl)-3,7-dihydro-1H-purine-2,6-dione (33 mg, 0.07 mmol) to afford the title compound as a white solid (20 mg, 74 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 7.92-7.80 (5H, m), 7.64 (1H, s), 7.53-7.48 (3H, m), 7.39 (1H, d, J 8.2), 4.58 (2H, t, J 7.0), 4.01 (2H, t, J 7.2), 3.29 (2H, t, J 7.0), 2.52 (6H, s), 2.23-2.17 (2H, m), 1.82 (2H, quin, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.5, 142.3, 134.7, 133.5, 132.3, 128.0, 127.3, 127.1, 126.5, 125.8, 125.4, 122.9, 121.2, 118.4, 115.4, 106.4, 55.0, 48.8, 42.6, 40.6, 35.5, 23.8. HRMS (ESI $^{+}$) [M+H] $^{+}$ expected for $\text{C}_{22}\text{H}_{26}\text{N}_5\text{O}_3$, 408.2030: found 408.2034. [M+Na] $^{+}$ expected for $\text{C}_{22}\text{H}_{25}\text{N}_5\text{O}_3\text{Na}$, 430.1850: found 430.1848.

3-(3-(dimethylamino)propyl)-1-hydroxy-7-(2-(naphthalen-1-yl)ethyl)-3,7-dihydro-1H-purine-2,6-dione (23ac)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-(naphthalen-1-yl)ethyl)-3,7-dihydro-1H-purine-2,6-dione (38 mg, 0.08 mmol) to afford the title compound as a white solid (23 mg, 73 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.58 (1H, s), 8.36 (1H, d, J 8.1), 7.96 (1H, d, J 8.1), 7.95 (1H, s), 7.85 (1H, d, J 8.1), 7.62-7.53 (2H, m), 7.45 (1H, t, J 7.9), 4.56 (2H, t, J 7.2), 4.04 (2H, t, J 7.2), 3.59 (2H, t, J 7.2), 2.51 (6H, s), 2.26-2.21 (2H, m), 1.89 (2H, quin, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.5, 133.9, 133.4, 131.8, 128.5, 127.4, 126.9, 126.0, 125.4, 125.1, 118.3, 115.4, 106.6, 54.9, 42.4, 40.2, 33.8, 23.6. HRMS (ESI $^{+}$) [M+H] $^{+}$ expected for $\text{C}_{22}\text{H}_{26}\text{N}_5\text{O}_3$, 408.2030: found 408.2038. [M+Na] $^{+}$ expected for $\text{C}_{22}\text{H}_{25}\text{N}_5\text{O}_3\text{Na}$, 430.1850: found 430.1856.

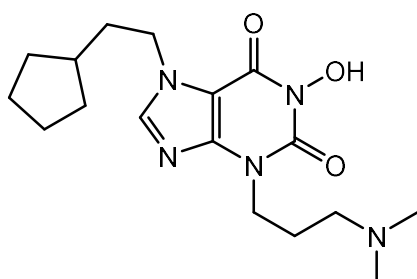
7-(2-(1H-indol-1-yl)ethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23ad)



Prepared according to standard procedure 3 using 7-(2-(1H-indol-1-yl)ethyl)-1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (49 mg, 0.13 mmol) to afford the title compound

as a yellow solid (44 mg, 85 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.61 (1H, s), 7.55 (1H, s), 7.53 (1H, d, J 7.7), 7.43 (1H, d, J 8.4), 7.16 (1H, d, J 3.1), 7.10 (1H, dt, J 0.9 and 8.1), 7.01 (1H, t, J 7.4), 6.42 (1H, d, J 3.0), 4.00 (4H, t, J 6.6), 3.32-3.30 (2H, m), 2.51 (6H, s), 2.46-2.38 (2H, m), 1.87 (2H, quin, J 6.6). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.1, 142.7, 136.2, 128.6, 127.4, 121.2, 120.5, 119.2, 118.3, 115.4, 108.4, 106.3, 101.8, 54.7, 54.3, 47.1, 45.3, 42.2, 23.3. HRMS (ESI $^{+}$) $[\text{M}+\text{H}]$ expected for $\text{C}_{20}\text{H}_{25}\text{N}_6\text{O}_3$, 397.1988: found 397.2132.

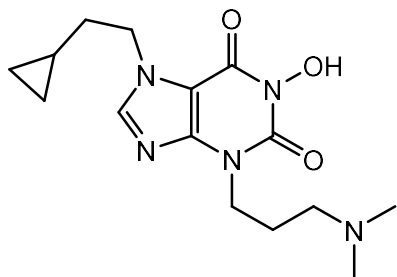
7-(2-cyclopentylethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23ae)



Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(2-cyclopentylethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (58 mg, 0.17 mmol) to afford the title compound as a white solid

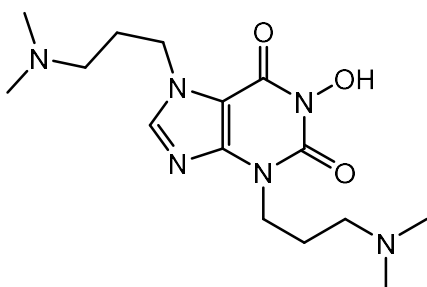
(44 mg, 99 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.48 (1H, s), 8.13 (1H, s), 4.25 (2H, t, J 7.3), 4.04 (2H, t, J 7.0), 2.63-2.56 (2H, m), 2.36 (6H, s), 1.90 (2H, quin, J 7.0), 1.80-1.64 (5H, m), 1.60-1.52 (2H, m), 1.50-1.43 (2H, m), 1.17-1.07 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.0, 142.0, 118.4, 115.4, 106.5, 55.1, 48.0, 46.2, 42.6, 37.0, 36.9, 32.0, 24.6, 23.7. HRMS (ESI $^{+}$) $[\text{M}+\text{H}]$ expected for $\text{C}_{17}\text{H}_{28}\text{N}_5\text{O}_3$, 350.2187: found 350.2200. $[\text{M}+\text{Na}]$ expected for $\text{C}_{17}\text{H}_{27}\text{N}_5\text{O}_3\text{Na}$, 372.2006: found 372.1998.

7-(2-cyclopropylethyl)-3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23af)



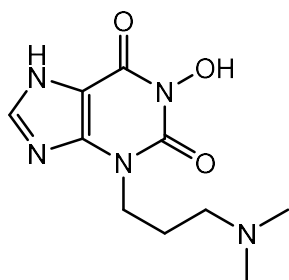
Prepared according to standard procedure 3 using 1-(benzyloxy)-7-(2-cyclopropylethyl)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (43 mg, 0.10 mmol) to afford the title compound as a yellow solid (25 mg, 75 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 8.03 (1H, s), 4.44 (2H, t, J 7.1), 4.26 (2H, t, J 7.1), 3.26 (2H, t, J 7.1), 2.93 (6H, s), 2.24 (2H, quin, J 7.1), 1.78 (2H, q, J 7.1), 0.73-0.63 (1H, m), 0.47-0.42 (2H, m), 0.06-0.00 (2H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 152.8, 150.3, 146.9, 142.7, 106.3, 55.0, 47.1, 42.2, 40.0, 35.4, 23.4, 7.3, 3.0. HRMS (ESI $^{+}$) [M+H] $^{+}$ expected for $\text{C}_{15}\text{H}_{24}\text{N}_5\text{O}_3$, 322.1824: found 322.1890. [M+Na] $^{+}$ expected for $\text{C}_{15}\text{H}_{23}\text{N}_5\text{O}_3\text{Na}$, 344.1693: found 344.1685.

3,7-bis(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23ag)



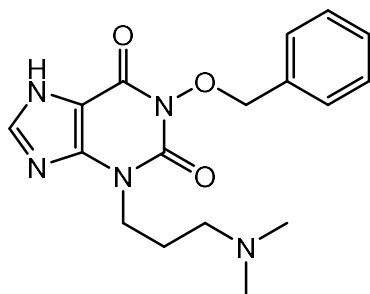
Prepared according to standard procedure 3 using 1-(benzyloxy)-3,7-bis(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (57 mg, 0.13 mmol) to afford the title compound as a yellow solid (43 mg, 88 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.44 (1H, s), 8.06 (1H, s), 4.25 (2H, t, J 7.1), 4.03 (2H, t, J 7.1), 2.33 (2H, t, J 7.1), 2.22 (2H, t, J 7.1), 2.18 (6H, s), 2.15 (6H, s), 1.93 (2H, quin, J 7.1), 1.83 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 153.9, 151.1, 145.8, 141.8, 106.5, 55.6, 55.3, 44.4, 43.5, 43.1, 41.0, 27.4, 24.7. HRMS (ESI $^{+}$) [M+H] $^{+}$ expected for $\text{C}_{15}\text{H}_{27}\text{N}_6\text{O}_3$, 339.2139: found 339.2142. [M+Na] $^{+}$ expected for $\text{C}_{15}\text{H}_{26}\text{N}_6\text{O}_3\text{Na}$, 361.1959: found 361.1968.

3-(3-(dimethylamino)propyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (23ah)



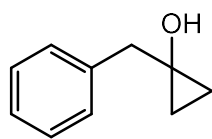
Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-methoxybenzyl)-3,7-dihydro-1H-purine-2,6-dione (20 mg, 0.04 mmol) to afford the title compound as a white solid (15 mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.91 (1H, s), 4.22 (2H, t, J 7.1), 3.07 (2H, t, J 7.1), 2.77 (6H, s), 2.18 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.5, 151.1, 145.9, 140.5, 107.65, 55.2, 42.5, 40.6, 24.0. HRMS (ESI⁺) [M+H]⁺ expected for $\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_3$, 253.1248: found 253.1241. [M+Na]⁺ expected for $\text{C}_{10}\text{H}_{15}\text{N}_5\text{O}_3\text{Na}$, 276.1067: found 276.1060. HRMS (ESI⁻) [M-H]⁻ expected for $\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_3$, 252.1102: found 252.1091.

1-(benzyloxy)-3-(3-(dimethylamino)propyl)-3,7-dihydro-1H-purine-2,6-dione (29)



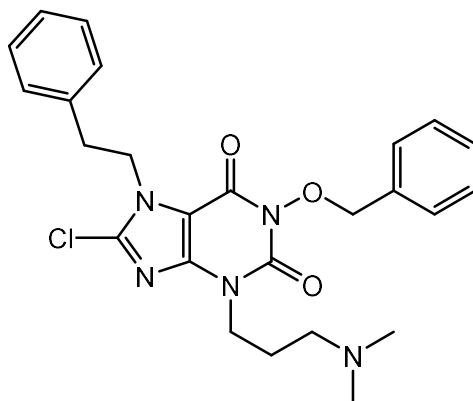
A stirred solution of 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(4-methoxybenzyl)-3,7-dihydro-1H-purine-2,6-dione (650 mg, 1.4 mmol) in TFA (50 ml) was heated at 70 °C under microwave radiation for 3.5 hours. Solvent was removed *in vacuo* giving a purple residue. Purification by column chromatography eluting with 3:1 MeOH:DCM to afford the title compound as a yellow solid (272 mg, 57 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.59 (1H, s), 7.57-7.18 (5H, m), 5.14 (2H, s), 4.21 (2H, t, J 7.2), 2.80 (2H, t, J 7.2), 2.56 (6H, s), 2.17 (2H, quin, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 152.8, 149.6, 146.8, 140.9, 137.9, 129.9, 129.1, 107.6, 78.6, 55.7, 45.2, 44.4, 25.4.

1-benzylcyclopropan-1-ol (31)



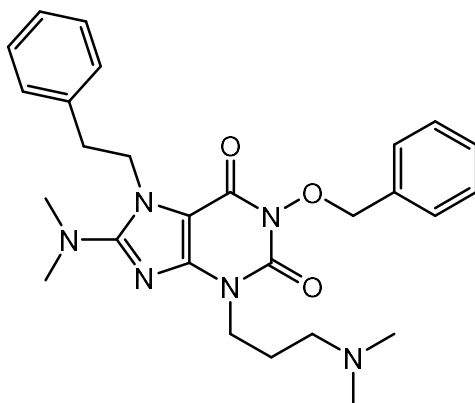
To a mixture of bromoethane (593 μ l, 8 mmol), magnesium (192 mg, 8 mmol), and anhydrous THF (5 ml) was added iodine (3 mg) under nitrogen atmosphere and the mixture stirred at 50 °C until it became a homogenous solution. This solution was added dropwise to a solution of methyl 2-phenylacetate (283 μ l, 2 mmol) and titanium isopropoxide (727 μ l, 2.4 mmol) under nitrogen atmosphere at -78 °C and stirred for 4 hours, allowing to warm to rt. The reaction was quenched with the dropwise addition of methanol (1 ml) and water (3 ml) and the solvent removed *in vacuo*. The residue was partitioned between aqueous HCl (10 mL, 2 M) and DCM (10 ml). The aqueous layer was extracted into DCM (3 x 10 ml). Combined organic fractions were dried (MgSO₄), filtered and the solvent was removed *in vacuo* to afford the title compound as a colourless oil (266 mg, 90 %). ¹H NMR δ_{H} (400.13 MHz; CDCl₃): 7.40-7.35 (2H, m), 7.34-7.32 (2H, m), 7.24 (1H, m), 2.65 (2H, s), 0.71 (2H, dd, *J* 1.9 and 8.9), 0.49 (2H, dd, *J* 1.9 and 8.9). ¹³C NMR δ_{C} (101.6 MHz; CDCl₃): 139.8, 128.8, 128.3, 126.2, 57.6, 51.3, 16.5.

1-(benzyloxy)-8-chloro-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (33)



Prepared according to standard procedure 10 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (160 mg, 0.36 mmol) to afford the title compound as an orange oil (130 mg, 75 %). ¹H NMR δ_{H} (400.13 MHz; CDCl₃): 7.63-7.18 (10H, m), 5.17 (2H, s), 4.52 (2H, t, *J* 7.8), 4.18 (2H, t, *J* 5.7), 3.17-3.7 (4H, m), 2.81 (6H, s), 2.23 (2H, quin, *J* 7.2). ¹³C NMR δ_{C} (101.6 MHz; CDCl₃): 151.3, 149.1, 145.6, 139.1, 136.4, 133.7, 130.0, 129.3, 129.0, 128.8, 128.5, 127.2, 107.3, 78.7, 55.2, 47.9, 42.9, 40.6, 36.4, 23.4.

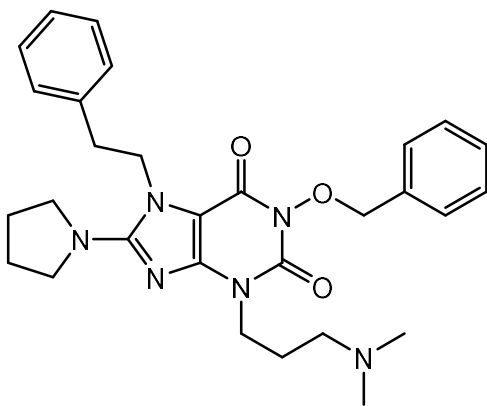
1-(benzyloxy)-8-(dimethylamino)-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1*H*-purine-2,6-dione (34b)



Prepared according to standard procedure 11 using 1-(benzyloxy)-8-chloro-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1*H*-purine-2,6-dione (50 mg, 0.08 mmol) and dimethylamine, stirring for 2 hours to afford the title compound as a yellow oil (51 mg, 100 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.67-7.15 (10H,

m), 5.18 (2H, s), 4.38 (2H, t, J 7.0), 4.13 (2H, t, J 7.1), 3.09 (2H, t, J 6.4), 2.89 (6H, s), 2.43 (2H, t, J 7.0), 2.30 (6H, s), 1.96 (2H, quin, J 7.1). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 158.1, 152.0, 149.5, 147.1, 137.6, 134.3, 130.1, 129.2, 128.9, 128.5, 128.4, 127.2, 103.6, 78.4, 56.7, 47.1, 45.4, 45.2, 41.8, 36.3, 26.2.

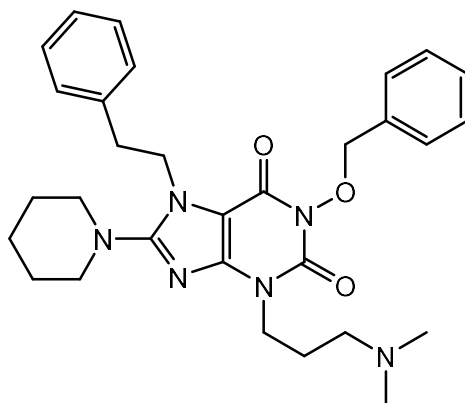
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-(pyrrolidin-1-yl)-3,7-dihydro-1*H*-purine-2,6-dione (34c)



Prepared according to standard procedure 11 using 1-(benzyloxy)-8-chloro-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1*H*-purine-2,6-dione (65 mg, 0.12 mmol) and pyrrolidine, stirring for 24 hours to afford the title compound as an orange oil (49 mg, 79 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.67-7.08

(10H, m), 5.16 (2H, s), 4.46 (2H, t, J 7.5), 4.12 (2H, t, J 7.6), 3.46 (4H, t, J 6.7), 3.02 (2H, t, J 7.3), 2.53 (2H, t, J 8.0), 2.37 (6H, s), 2.00 (2H, quin, J 8.0), 1.90 (4H, t, J 6.4). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 155.3, 151.5, 149.7, 147.9, 137.6, 134.3, 130.1, 129.0, 128.9, 128.5, 128.4, 126.8, 102.5, 78.4, 56.6, 49.8, 46.6, 44.9, 41.4, 36.8, 25.8, 25.6.

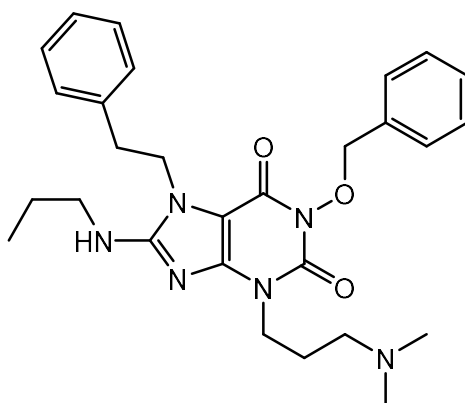
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-(piperidin-1-yl)-3,7-dihydro-1H-purine-2,6-dione (34d)



Prepared according to standard procedure 11 using 1-(benzyloxy)-8-chloro-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (60 mg, 0.12 mmol) and piperidine, stirring for 24 hours to afford the title compound as a yellow oil (64 mg, 96 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.68-7.61 (2H, m), 7.42-

7.34 (3H, m), 7.27-7.19 (3H, m), 7.13-7.08 (2H, m), 5.19 (2H, s), 4.29 (2H, t, J 7.3), 3.11 (2H, t, J 7.3), 3.07-2.98 (6H, m), 2.38 (2H, t, J 7.3), 2.25 (6H, s), 1.93 (2H, quin, J 7.3), 1.64-1.54 (6H, m). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 157.9, 152.1, 149.5, 146.9, 137.7, 134.2, 130.1, 129.0, 128.9, 128.5, 128.4, 126.7, 103.8, 70.4, 56.8, 51.6, 47.2, 45.4, 41.8, 36.4, 26.2, 25.5, 24.1.

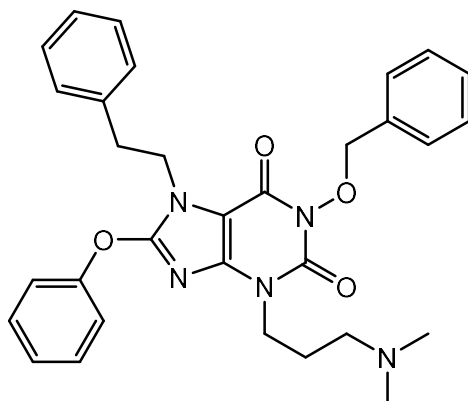
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-(propylamino)-3,7-dihydro-1H-purine-2,6-dione (34e)



Prepared according to standard procedure 11 using 1-(benzyloxy)-8-chloro-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (60 mg, 0.12 mmol) and propan-1-amine, stirring for 3 days to afford the title compound as a yellow oil (30 mg, 48 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.66-7.59 (2H, m), 7.40-7.33

(3H, m), 7.31-7.23 (3H, m), 7.16-7.12 (2H, m), 5.18 (2H, s), 4.21 (2H, t, J 6.5), 4.11 (2H, t, J 7.4), 3.19 (2H, q, J 7.4), 3.10-3.03 (5H, m), 2.40 (2H, t, J 7.4), 2.26 (6H, s), 1.95 (2H, quin, J 7.4), 1.33 (2H, sex, J 7.4), 0.91 (3H, t, J 7.4). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 153.9, 151.5, 149.6, 147.9, 138.5, 134.3, 130.0, 129.0, 129.0, 128.7, 128.4, 127.1, 102.0, 78.4, 56.8, 45.9, 45.2, 45.0, 41.8, 36.5, 26.0, 23.3, 11.3.

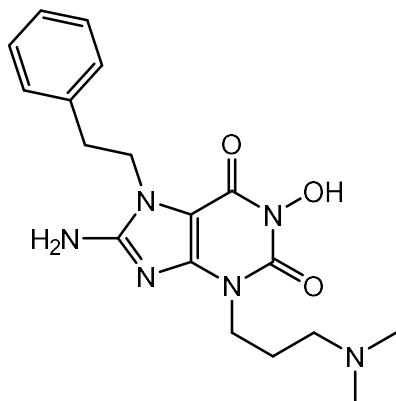
1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-phenoxy-3,7-dihydro-1H-purine-2,6-dione (34f)



A solution of 8-chloro-3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (50 mg, 0.08 mmol), Phenol (10 mg, 0.10 mmol) and caesium carbonate (14 mg, 0.10 mmol) in DMF (4 ml) was stirred for 2 days at rt. Solvent removed *in vacuo* and resulting brown residue purified by chromatography eluting

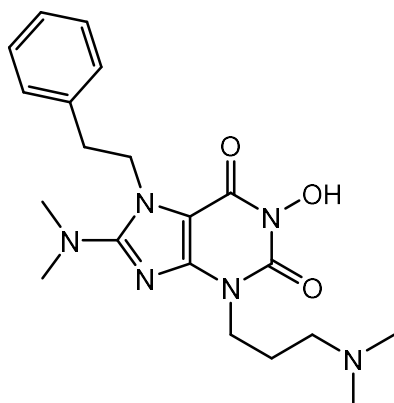
1:20 MeOH:DCM to afford the title compound as a colourless oil (53 mg, 77 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.65 (2H, dd, J 1.8 and 7.2), 7.42-7.36 (4H, m), 7.27-7.24 (3H, m), 7.20-7.15 (4H, m), 6.99 (2H, d, J 8.0), 5.20 (2H, s), 4.49 (2H, t, J 7.1), 4.07 (2H, t, J 6.9), 3.19 (2H, t, J 7.1), 2.74 (2H, t, J 6.9), 2.49 (6H, s), 2.09 (2H, quin, J 6.9). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 153.9, 152.9, 151.9, 149.5, 144.7, 137.4, 133.9, 130.1, 129.7, 129.0, 128.7, 128.5, 126.8, 125.9, 125.2, 129.8, 102.5, 78.6, 55.8, 45.5, 43.7, 41.3, 36.5, 24.4.

8-amino-3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (35a)



To a solution of 8-chloro-3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (70 mg, 1.40 mmol) in DMF (4 ml) was added sodium azide (10 mg, 1.5 mmol) and stirred at rt for 24 hours. Solvent removed *in vacuo*, residue dissolved in DCM (20 ml), washed with water, dried (MgSO₄), filtered and solvent removed *in vacuo*, residue dissolved in DCM (2 ml). Pd/C (5 mg) and MeOH (18 ml) were added. The resulting mixture was stirred under H₂ environment for 0.5 hours, the mixture was filtered through celite and solvent removed *in vacuo* to give a brown residue. Purified by preparative HPLC eluting with MeCN:H₂O 3:7 to afford the title compound as a trifluoro acetate salt as a red oil (4 mg, 8 %). ¹H NMR δ_{H} (400.13 MHz; DMSO): 10.23 (1H, s), 7.34-7.22 (5H, m), 4.20 (2H, t, *J* 7.3), 3.98-3.94 (2H, m), 2.94 (2H, t, *J* 7.3), 2.51 (6H, s), 2.02-1.95 (2H, m). ¹³C NMR δ_{C} (101.6 MHz; MeOD): 156.8, 152.5, 149.0, 142.7, 137.4, 128.7, 128.1, 126.4, 107.4, 56.1, 54.4, 41.9, 37.9, 29.3. HRMS (ESI⁺) [M+H] expected for C₁₈H₂₅N₆O₃, 373.1983; found 373.1992. [M+Na] expected for C₁₈H₂₄N₆O₃Na, 395.1802; found 395.1801.

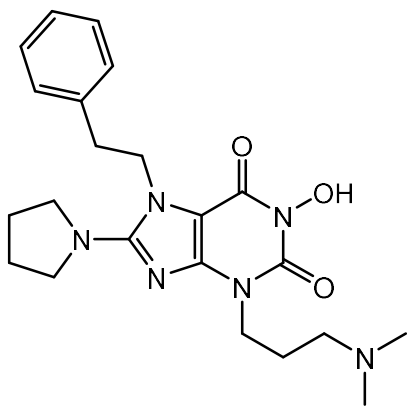
8-(dimethylamino)-3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (35b)



Prepared according to standard procedure 3 using 1-(benzyloxy)-8-(dimethylamino)-3-(3-(dimethylamino)propyl)-7-phenethyl-3,7-dihydro-1H-purine-2,6-dione (41 mg, 0.09 mmol) to afford the title compound as a white solid (23 mg, 63 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.26-7.11 (5H, m), 4.45 (2H, t, J 7.1), 4.18 (2H, t, J 6.7), 3.23 (2H, t, J 7.1), 3.08 (2H, t, J 7.1), 2.94 (6H, s), 2.94

(6H, s). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 158.6, 152.4, 150.5, 146.6, 137.6, 128.5, 128.1, 126.4, 103.5, 54.5, 46.9, 42.1, 40.4, 39.7, 35.6, 23.4. HRMS (ESI⁺) [M+H] expected for C₂₀H₂₉N₆O₃, 401.2296: found 401.2301. [M+Na] expected for C₂₀H₂₈N₆O₃Na, 423.2115: found 423.2108.

3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-8-(pyrrolidin-1-yl)-3,7-dihydro-1H-purine-2,6-dione (35c)

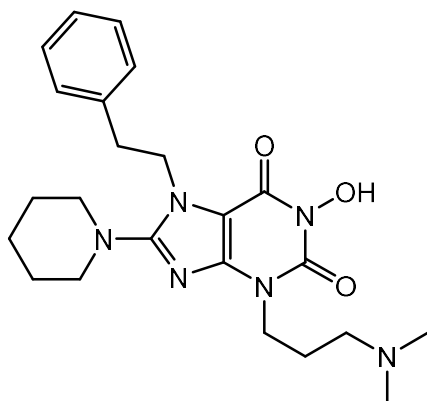


Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-(pyrrolidin-1-yl)-3,7-dihydro-1H-purine-2,6-dione (49 mg, 0.1 mmol) to afford the title compound as a white solid (23 mg, 45 %).

^1H NMR δ_{H} (400.13 MHz; DMSO): 9.54 (1H, s), 7.32-7.15 (5H, m), 4.41 (2H, t, J 7.0), 4.00 (2H, t, J 7.0), 3.46 (4H, t, J 6.2),

3.16-3.10 (2H, m), 2.97 (2H, t, J 7.0), 2.80 (6H, s), 2.03 (2H, quin, J 7.8), 1.85 (4H, t, J 6.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 154.9, 152.0, 150.0, 147.1, 138.2, 129.2, 128.8, 127.0, 102.2, 54.7, 49.8, 46.5, 42.7, 42.6, 36.6, 25.7, 23.6. HRMS (ESI⁺) [M+H] expected for C₂₂H₃₁N₆O₃, 427.2452: found 427.2456. [M+Na] expected for C₂₂H₃₁N₆O₃Na, 449.2279: found 449.2272.

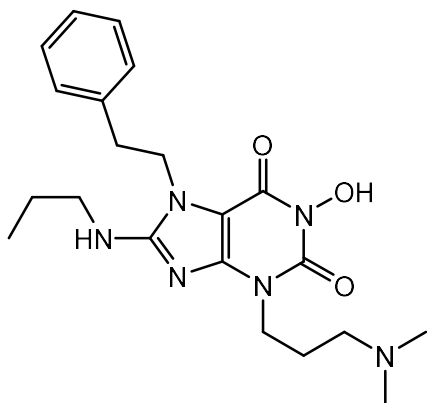
3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-8-(piperidin-1-yl)-3,7-dihydro-1H-purine-2,6-dione (35d)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-(piperidin-1-yl)-3,7-dihydro-1H-purine-2,6-dione (64 mg, 0.12 mmol) to afford the title compound as a yellow solid (5 mg, 10 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.26-7.17 (3H, m), 7.10-7.08 (2H, m), 4.36 (2H, m), 4.19 (2H, m), 3.23 (2H, m), 3.11

(2H, t, J 7.1), 3.04 (4H, m), 2.93 (6H, s), 2.20 (2H, m), 1.63 (6H, m). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 158.4, 1512.6, 150.5, 146.3, 137.7, 128.7, 128.1, 126.3, 103.7, 55.0, 51.2, 44.3, 42.2, 39.8, 35.8, 25.1, 23.6, 23.3. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{23}\text{H}_{33}\text{N}_6\text{O}_3$, 441.2609: found 441.2631.

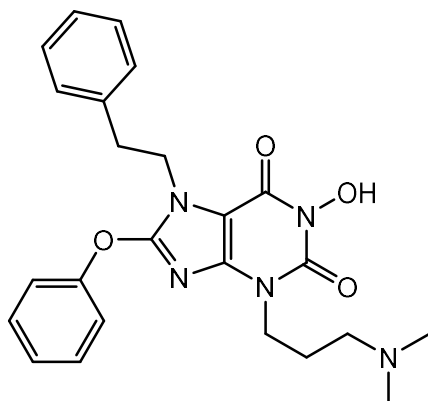
3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-8-(propylamino)-3,7-dihydro-1H-purine-2,6-dione (35e)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-(propylamino)-3,7-dihydro-1H-purine-2,6-dione (30 mg, 0.07 mmol) to afford the title compound as a yellow solid (10 mg, 20 %). ^1H NMR δ_{H} (400.13 MHz; MeOD): 7.29-7.19 (5H, m), 4.29 (2H, t, J 6.8), 4.20 (2H, t, J 6.9), 3.28 (2H, t, J 6.8), 3.24-3.22

(2H, m), 3.03 (2H, t, J 6.9), 2.93 (6H, s), 2.25-2.20 (2H, m), 1.57 (2H, sex, J 7.3), 0.93 (3H, t, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 154.6, 151.8, 150.5, 147.8, 137.7, 128.7, 128.1, 126.3, 101.4, 55.1, 44.4, 44.2, 42.2, 39.7, 35.3, 23.4, 22.6, 10.3. HRMS (ESI $^{+}$) [M+H] expected for $\text{C}_{21}\text{H}_{31}\text{N}_6\text{O}_3$, 415.2452: found 415.2471. [M+Na] expected for $\text{C}_{21}\text{H}_{30}\text{N}_6\text{O}_3\text{Na}$, 437.2272: found 437.2262.

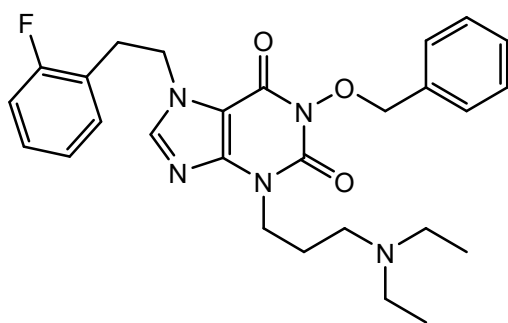
3-(3-(dimethylamino)propyl)-1-hydroxy-7-phenethyl-8-phenoxy-3,7-dihydro-1H-purine-2,6-dione (35f)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-phenethyl-8-phenoxy-3,7-dihydro-1H-purine-2,6-dione (53 mg, 0.10 mmol) to afford the title compound as a yellow solid (36 mg 81 %). ¹H NMR δ_H (400.13 MHz; MeOD): 7.37 (2H, t, *J* 7.8), 7.27-7.20 (4H, m), 7.14-7.09 (2H, m), 6.94 (2H, d, *J* 8.2), 4.45 (2H, t, *J*

6.4), 4.03 (2H, t, *J* 6.4), 3.14 (2H, t, *J* 6.4), 2.19 (2H, t, *J* 6.4), 2.65 (6H, s), 2.09-2.02 (2H, m). ¹³C NMR δ_C (101.6 MHz; MeOD): 154.0, 153.2, 137.7, 129.3, 128.7, 128.3, 126.5, 125.6, 119.6, 102.5, 55.2, 47.0, 42.7, 40.6, 36.0, 23.9. HRMS (ESI⁺) [M+H] expected for C₂₄H₂₈N₅O₄, 450.2136: found 450.2161. [M+Na] expected for C₂₄H₂₇N₅O₄Na, 472.1955: found 472.1938.

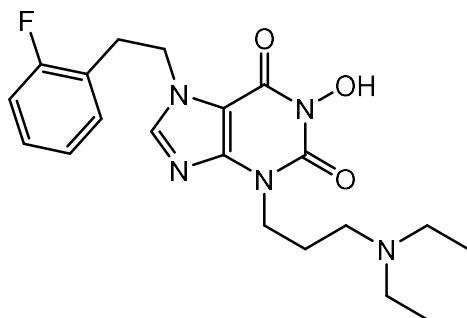
1-(benzyloxy)-3-(3-(diethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione



Prepared according to standard procedure 8, using ethyl 4-amino-1-(2-fluorophenethyl)-1H-imidazole-5-carboxylate (831 mg, 3.0 mmol) to afford the title compound as a white solid. (482 mg, 42 %). ¹H NMR δ_H (400.13 MHz; CDCl₃): 7.66-6.99

(11H, m), 5.19 (2H, s), 4.50 (2H, t, *J* 6.8), 4.14 (2H, t, *J* 7.3), 3.21 (2H, t, *J* 6.8), 2.45 (2H, t, *J* 7.3), 2.44 (4H, q, *J* 7.2), 1.93 (2H, quin, *J* 7.3), 1.01 (3H, t, *J* 7.2). ¹³C NMR δ_C (101.6 MHz; CDCl₃): 162.5, 159.9, 152.5, 149.1, 147.9, 141.5, 133.9, 131.2, 130.1, 129.1, 128.4, 124.5, 123.6, 115.4, 106.3, 78.5, 50.0, 47.2, 46.7, 42.5, 30.9, 29.6, 25.5, 11.6.

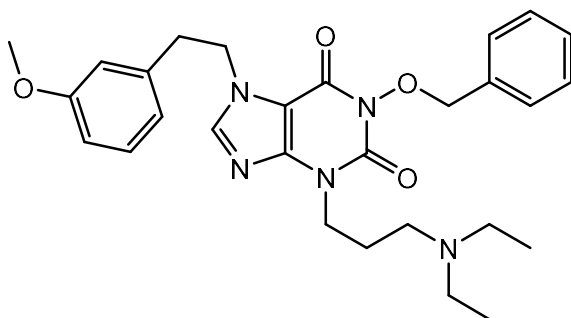
3-(3-(diethylamino)propyl)-7-(2-fluorophenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (36)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (58 mg, 0.12 mmol) to afford the title compound as a white solid (36 mg, 80 %). ^1H NMR δ_{H}

(400.13 MHz; MeOD): 7.69 (1H, s), 7.29-7.00 (4H, m), 4.57 (2H, t, J 6.7), 4.21 (2H, t, J 6.4), 3.25 (2H, t, J 6.7), 3.18 (6H, m), 2.19 (2H, quin, J 6.7), 1.29 (6H, t, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 162.6, 160.0, 153.2, 150.5, 146.5, 142.2, 130.9, 128.7, 124.2, 114.9, 106.3, 49.1, 48.5, 46.9, 40.6, 29.9, 22.9, 13.9. ^{19}F NMR δ_{F} (376 MHz; DMSO): -118.8 (1F, s). HRMS (ESI $^{+}$) [M+H] $^{+}$ expected for $\text{C}_{18}\text{H}_{23}\text{FN}_5\text{O}_3$, 376.1785; found 376.1778. [M+Na] $^{+}$ expected for $\text{C}_{18}\text{H}_{22}\text{FN}_5\text{O}_3\text{Na}$, 398.1604; found 398.1595.

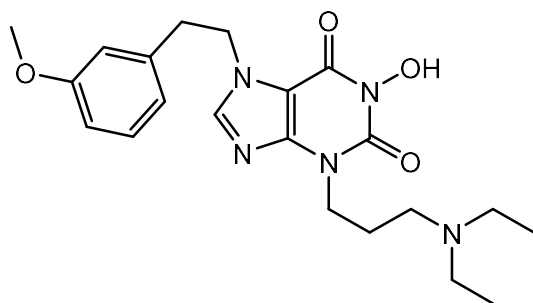
1-(benzyloxy)-3-(3-(diethylamino)propyl)-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione



Prepared according to standard procedure 1 using 1-(benzyloxy)-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (60 mg, 0.15 mmol) and 3-diethylamino-1-propyl chloride

hydrochloride (43 mg, 0.23 mmol) to afford the title compound as a yellow oil (70 mg, 93 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.76-6.63 (10H, m), 5.22 (2H, s), 4.49 (2H, t, J 7.1), 4.16 (2H, t, J 7.0), 4.13 (2H, q, J 7.3), 3.78 (3H, s), 3.12 (2H, t, J 7.1), 2.59-2.55 (2H, m), 1.95 (2H, quin, J 7.0), 1.03 (6H, t, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 159.9, 152.4, 149.3, 147.9, 141.6, 138.5, 134.0, 130.2, 129.9, 129.0, 128.4, 121.0, 114.5, 112.4, 106.2, 78.6, 60.4, 55.1, 49.9, 48.7, 42.4, 37.2, 25.2, 11.5.

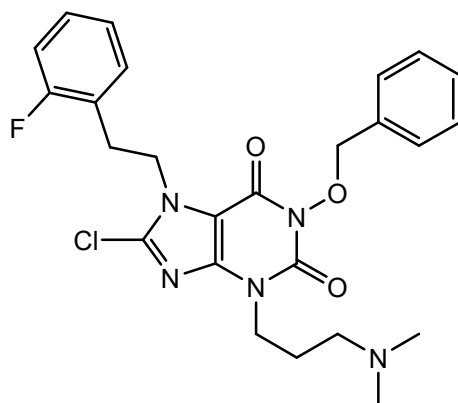
3-(3-(diethylamino)propyl)-1-hydroxy-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (37)



Prepared according to standard procedure 3 using 1-(benzyloxy)-3-(3-(diethylamino)propyl)-7-(3-methoxyphenethyl)-3,7-dihydro-1H-purine-2,6-dione (70 mg, 0.14 mmol) to afford the title compound as a white solid (45 mg, 95 %). ^1H

NMR δ_{H} (400.13 MHz; MeOD): 7.65 (1H, s), 7.17 (1H, t, J 7.2), 6.79-6.69 (3H, m), 4.52 (2H, t, J 7.2), 4.19 (2H, t, J 6.7), 3.75 (3H, s), 3.15-3.08 (8H, m), 2.16 (2H, quin, J 6.7), 1.26 (6H, t, J 7.3). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 160.0, 153.4, 150.7, 146.3, 142.3, 138.8, 129.2, 120.6, 114.1, 112.0, 106.3, 54.2, 49.1, 48.1, 46.6, 40.7, 36.6, 23.1, 13.7. HRMS (ESI $^{+}$) [$\text{M}+\text{H}$] expected for $\text{C}_{21}\text{H}_{30}\text{N}_5\text{O}_4$, 416.2292: found 416.2298. [$\text{M}+\text{Na}$] expected for $\text{C}_{21}\text{H}_{29}\text{N}_5\text{O}_4\text{Na}$, 436.2121: found 436.2111.

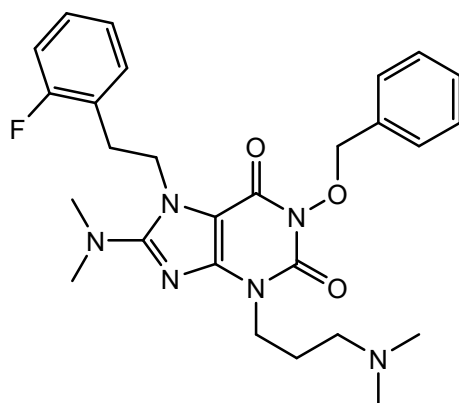
1-(benzyloxy)-8-chloro-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione



Prepared according to standard procedure 10 using 1-(benzyloxy)-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (130 mg, 0.36 mmol) to afford the title compound as an orange oil (115 mg, 64 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.62 (2H, d, J 6.7), 7.42-7.34 (3H, m), 7.27-7.20 (1H, m), 7.08-

6.99 (3H, m), 5.16 (2H, s), 4.54 (2H, t, J 7.0), 4.13 (2H, t, J 7.1), 3.17 (2H, t, J 7.0), 2.37 (2H, t, J 7.1), 2.24 (6H, s), 1.93 (2H, quin, J 7.1), ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 161.3 (d, J 245.0), 151.5, 149.0, 146.2, 139.2, 133.8, 131.3 (d, J 4.5), 130.1, 129.2 (d, J 8.4), 128.4, 128.3 (d, J 13.0), 124.4, 123.4 (d, J 16.0), 115.4 (d, J 21.7), 107.3, 78.5, 56.6, 46.4, 45.3, 42.2, 29.6, 25.8.

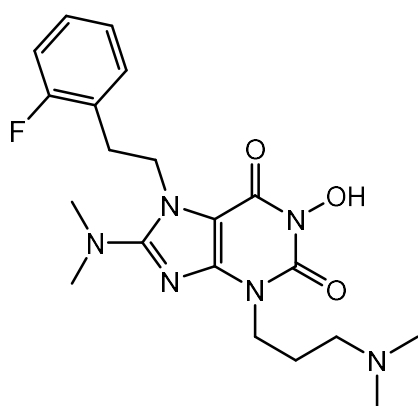
1-(benzyloxy)-8-(dimethylamino)-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione



Prepared according to standard procedure 11 using 1-(benzyloxy)-8-chloro-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (58 mg, 0.11 mmol) and pyrrolidine, stirring for 24 hours at rt to afford the title compound as a yellow oil (47 mg, 80 %). ^1H NMR δ_{H} (400.13 MHz; CDCl_3): 7.65 (1H, dd, J

1.7 and 7.5), 7.42-7.35 (4H, m), 7.23-7.17 (1H, m), 7.12 (1H, dt, J 1.3 and 7.5), 7.05-6.98 (2H, m), 5.18 (2H, s), 4.38 (2H, t, J 7.2), 4.12 (2H, t, J 7.2), 3.15 (2H, t, J 7.2), 2.88 (6H, s), 2.41 (2H, t, J 7.2), 2.28 (6H, s), 1.94 (2H, quin, J 7.2). ^{13}C NMR δ_{C} (101.6 MHz; CDCl_3): 161.3 (d, J 245.3), 158.1, 152.8, 149.6, 147.2, 134.2, 131.5 (d, J 5.1), 130.1, 128.9, 128.7 (d, J 8.6), 128.4, 124.4 (d, J 16.4), 124.2 (d, J 3.6), 115.0 (d, J 22.2), 103.5, 78.4, 56.7, 45.7, 45.3, 41.8, 41.7, 29.7, 26.0.

8-(dimethylamino)-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-1-hydroxy-3,7-dihydro-1H-purine-2,6-dione (38)



Prepared according to standard procedure 3 using 1-(benzyloxy)-8-(dimethylamino)-3-(3-(dimethylamino)propyl)-7-(2-fluorophenethyl)-3,7-dihydro-1H-purine-2,6-dione (47 mg, 0.11 mmol) to afford the title compound as a yellow solid (30 mg, 78 %). ^1H NMR δ_{H} (400.13 MHz; DMSO): 10.29 (1H, s), 7.37-7.23 (2H, m), 7.16-7.05 (2H, m), 4.36 (2H, t, J 7.1), 3.95 (2H, t, J

7.0), 3.05 (2H, t, J 7.1), 2.84 (6H, s), 2.51 (6H, s), 2.34-2.48 (2H, m), 1.83 (2H, quin, J 7.0). ^{13}C NMR δ_{C} (101.6 MHz; MeOD): 161.3 (d, J 244.2), 158.5, 151.2, 150.6, 146.4, 131.1 (d, J 4.8), 128.6 (d, J 8.3), 127.9 (d, J 16.7), 124.3 (d, J 16.0), 124.0 (d, J 3.7), 114.7 (d, J 22.2), 103.5, 55.6, 45.3, 43.1, 40.6, 40.5, 29.1, 24.5. ^{19}F NMR δ_{F} (376 MHz; MeOD): -76.9 (1F, s). HRMS (ESI $^{+}$) [$\text{M}+\text{H}$] expected for $\text{C}_{20}\text{H}_{28}\text{FN}_6\text{O}_3$, 419.2201: found 419.2212. [$\text{M}+\text{Na}$] expected for $\text{C}_{20}\text{H}_{27}\text{FN}_6\text{O}_3\text{Na}$, 441.2021: found 441.2001.

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