





Unlocking therapeutic potential: the role of adamantane in drug discovery

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ABSTRACT

The unique structural and physicochemical properties of adamantane and its derivatives have attracted considerable attention in the field of medicinal chemistry. Substituting phenyl rings for adamantane or its derivatives has provided a promising strategy to introduce lipophilicity and escape the ‘flat land’ of modern drug discovery. Additionally, the unique three-dimensional structure of adamantane facilitates the precise positioning of substituents allowing for a more effective exploration of drug targets. Evidently, we have seen an increased use of adamantane in pharmaceutically relevant molecules. The following Account highlights our group’s research in five drug discovery programs over the past 15 years showcasing the use of adamantane and its analogues in these studies.

Keywords: adamantane, cannabinoid receptors, CNS, medicinal chemistry, NMDA receptor, P2X7 receptor, sigma-2 receptor, structure–activity relationships, tau aggregation.

Introduction

Adamantane (tricyclo[3.3.1.1^{1,7}]decane; **1**, Fig. 1), the smallest of the diamonoids, was first elucidated in 1933 following its isolation from crude oil.^{1,2} Despite being synthesised chemically for the first time in 1941, it was not until Schleyer reported the synthesis in 1957 that adamantane and its derivatives became readily available.^{3,4} Subsequently this moiety began to be incorporated into drug discovery programs, with the first promising pharmaceutical breakthrough occurring with the identification of the antiviral activity of amantadine (**2**, Fig. 1) in 1963.^{5,6} In the 60 years following this seminal report, adamantyl-based compounds have seen numerous applications in medicinal chemistry and drug discovery, with seven adamantyl-based drugs (**3–8**, Fig. 1) currently in clinical use. These compounds are used to treat a range of diseases including viral infections, neurodegenerative disorders, acne vulgaris and type 2 diabetes mellitus.⁷

Incorporation of adamantane or its derivatives into drug-like molecules has been adopted by medicinal chemists for a variety of reasons. For instance, drug discovery programs targeting ion channels have exploited the three-dimensional space occupied by the adamantyl group. The rigid, highly symmetric (T_d point group) hydrocarbon scaffold can be used to control the orientation of functional groups to facilitate the optimisation of both potency and selectivity at a particular target.^{2,5,8} Additionally, inclusion of the sterically bulky adamantyl group can increase stability through the contribution of both London dispersion forces and electrostatic interactions.^{9,10} Adamantane has also shown utility as a suitable polycyclic replacement of phenyl rings to escape the ‘flat land’ of modern drug discovery.¹¹ Finally, the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of a drug candidate can be modulated by the incorporation of an adamantyl group. For example, the increased lipophilicity of an adamantyl-derivative can enhance central nervous system (CNS) drug exposure by improving

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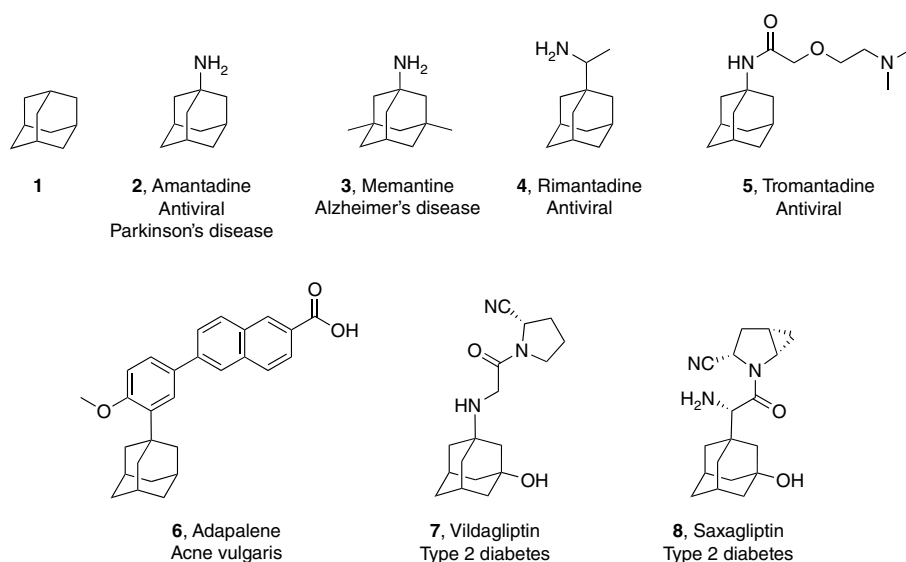


Fig. 1. Adamantane (**1**) and the structure and target indication of adamantyl-based drugs **2–8** that are currently available for clinical use.

blood–brain barrier (BBB) permeability and membrane solubility.^{5,7} Inclusion of hydrophobic substituents such as adamantane has been estimated to increase the calculated partition coefficients ($c \log P$) value of a given drug by ~ 3.1 log units and therefore may be clinically useful for highly water soluble compounds.⁷ In terms of metabolism, the rigid hydrocarbon scaffold of adamantane has been demonstrated to protect functional groups in its proximity from metabolic cleavage, thereby increasing drug stability and plasma half-life.⁷ Further insight into the benefits of using adamantyl-based small molecules in medicinal chemistry campaigns can be found in a number of detailed reviews.^{5,7,8,12}

Over the past 15 years, our research group has attempted to harness the beneficial properties of the adamantyl group and its derivatives in our CNS drug discovery projects. The following section highlights five of these projects.

Adamantane in CNS drug discovery

The sigma-2 receptor

The sigma (σ) receptors are a unique class of proteins located throughout the CNS and peripheral organs.¹³ Categorized into two subtypes, the sigma-1 and sigma-2 receptors (σ_1R and σ_2R respectively) acknowledge similar ligands, suggesting significant homology within the ligand-binding domain. Structurally and genetically, however, the relationship between the two remains unclear as the σ_2R has yet to be cloned. The σ_1R is a chaperone protein that resides in the endoplasmic reticulum, although it can still be regulated by small molecule antagonists and agonists. Within the CNS both the σ_1R and σ_2R play a notable role in synaptic signalling pathways by modulating ion channels and regulating calcium homeostasis.¹⁴ Evidently, both σ -receptors have been implicated in a multitude of neurological

disorders, prompting the development of selective and potent ligands.¹⁵

The incorporation of polycyclic moieties into medicinal chemistry campaigns has been an area of growing interest over the past several decades. Amantadine (**2**, Fig. 2), the first FDA-approved drug containing the adamantane scaffold, was clinically approved for the treatment of Parkinson's disease (PD) in 1968. Despite the mechanism of action being poorly understood there is considerable evidence that it acts as an antagonist of the *N*-methyl-D-aspartate (NMDA) glutamate receptor thereby increasing dopamine release.¹⁶ Following this, studies showed that amantadine was found to behave as a σ_1R agonist at therapeutically relevant concentrations.¹⁶ This initiated the inclusion of polycyclic frameworks, such as trishomocubane and adamantane, in the development of novel σ -receptor agonists.

Initially, the trishomocubane framework **9** was explored resulting in a novel series of compounds exhibiting selectivity and high affinity for the σ -binding site.¹⁷ Subtype selectivity was achieved by varying the distance between the polycyclic hemiaminal and the aryl group (Fig. 2). Benzyl derivatives **10** displayed a preference for the σ_1R , whereas phenethyl derivatives **11** had better activity at the σ_2R (Fig. 2).^{18–20} Affinity and selectivity were shown to both be improved when aryl halides were utilised, especially when substituted at the 3-position (**10** and **11**).^{18–20} The isomeric hemiaminal ethers **12** and **13** did not retain activity as expected despite their structural similarity to previously reported agonists (Fig. 2).^{21,22}

Considering the affinity of amantadine (**2**) at σ -receptors, and the promising results observed within the trishomocubane scaffold **9**, a structure–activity relationship (SAR) study of polycarbocyclic amines was explored. *N*-Arylalkyl-2-azaadamantan-1-ols **14** and **19** and their deoxygenated derivatives **15** and **20** were synthesised to gain information about

the steric and electronic tolerance of the hydrophobic region of the σ -receptor binding site. The hydroxy functional group was found to be detrimental to binding affinity with the achiral azaadamantane derivatives **15** and **20**, increasing affinity for both receptors by an order of magnitude (Fig. 3).²³ The azaadamantane analogues displayed excellent selectivity over 42 other CNS targets screened; however, only minimal selectivity between the σ -receptor subtypes was observed. A similar deoxygenation method was attempted for trishomocubane derivatives; however, it was deemed to be not synthetically viable.²³

The effects of the steric contribution of polycarbocyclic amines on σ -receptor binding and selectivity were expanded upon through a series of azabicyclo[2.2.1]heptanes, representing the simplest aza-bridged bicyclic subunit, and its analogous *N*-substituted pyrrolidines (Fig. 2).²⁴ Aryl and

cubyl moieties were connected to the polycarbocycle by alkyl chains of varying length. Reducing the steric bulk of the polycarbocycle in the azabicyclo[2.2.1]heptane derivatives **16** and **21** diminished binding affinity at both σ -receptors and was further illustrated by the significant decrease in σ_2R activity for the pyrrolidine derivatives **17** and **22**. Among the azabicyclo[2.2.1]heptane library, subtype discrimination was achieved with analogues incorporating benzylic substituents. This was further improved through electron-rich functional groups, such as a methoxy group (**16**, Fig. 2). The electronic argument was supported by the high σ_1R affinity of cyclohexyl-derived azabicyclo[2.2.1]heptane.²⁴

Substitution of the trishomocubane moiety for other polycycles provided valuable insight into the steric and electronic tolerance of the σ -receptor binding sites (Fig. 4).

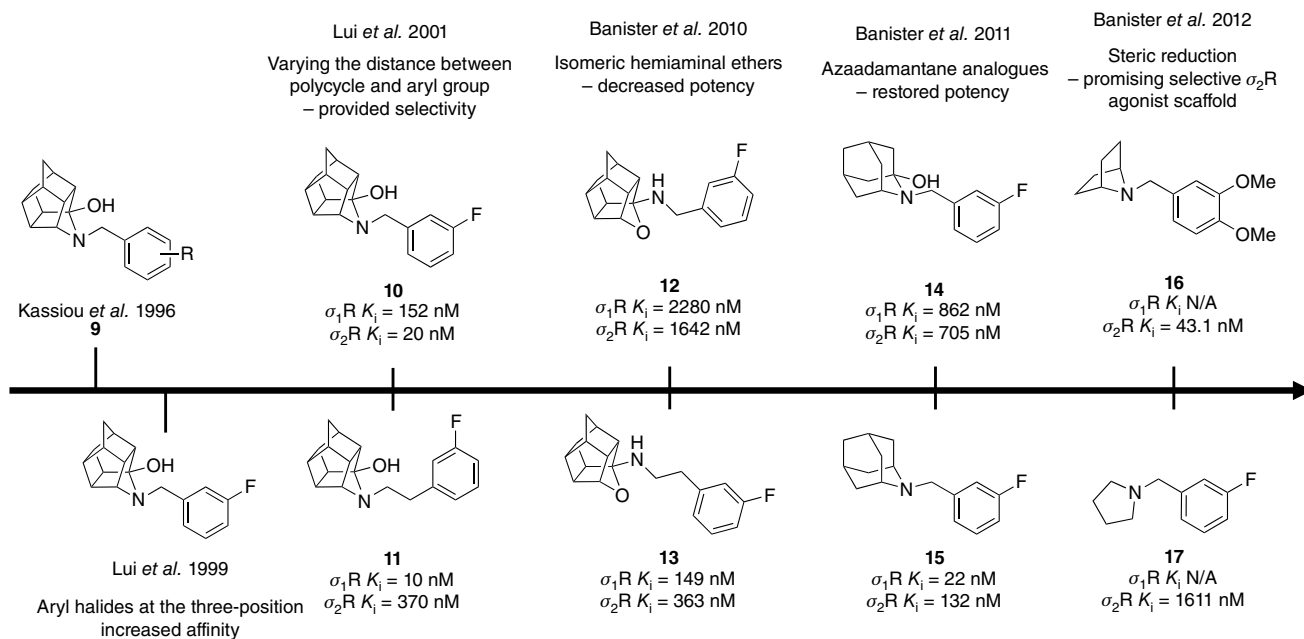


Fig. 2. Structure-activity relationship studies conducted on the trishomocubane scaffold led to the exploration of polycycles such as azaadamantane.^{17, 19–21, 23, 24} Data extracted from Liu et al.^{19, 20} and Banister et al.^{21, 23, 24} Competitive binding affinity measurements were performed using [³H](+)-pentazocine and [³H]DTG as radioligands and either guinea pig or rat as the source of σ_1 and σ_2 receptors.

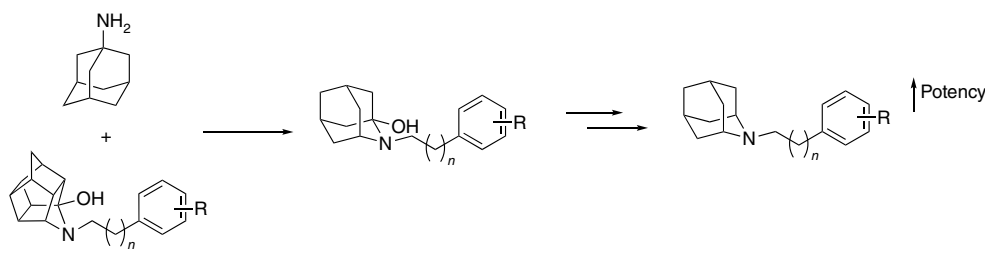


Fig. 3. Hybrid scaffold campaign between amantadine (**2**) and the trishomocubane σ -agonists. Deoxygenation of the *N*-aryllalkyl-2-azaadamantan-1-ols was found to increase affinity at both σ -receptors.

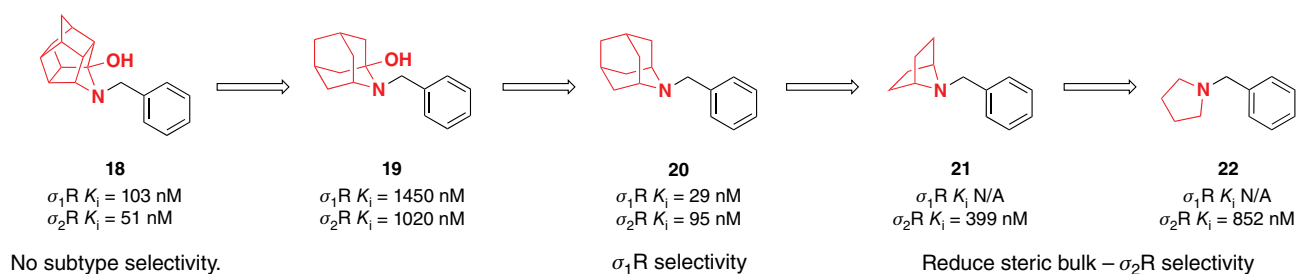


Fig. 4. Exploration of the effects of steric contribution on polycarbocyclic amines on σ -receptor binding. Azaadamantane and azabicyclo[2.2.1]heptane frameworks were highlighted as promising scaffolds for selective σ_1R and σ_2R agonists respectively. Data extracted from Liu *et al.*²⁰ and Banister *et al.*^{23,24} Competitive binding affinity measurements were performed using [³H](+)-pentazocine and [³H]DTG as radioligands and either guinea pig or rat as the source of σ_1 and σ_2 receptors.

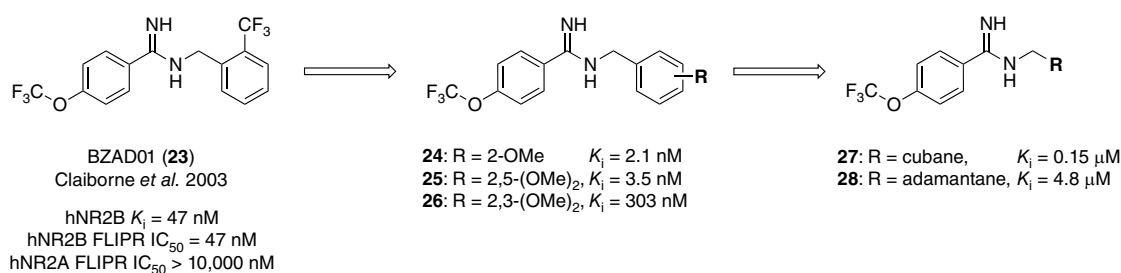


Fig. 5. The introduction of polycycles reduced activity at the hNR2B receptor by several orders of magnitude.^{26,31} Data extracted from Moir *et al.*²⁶ Competitive binding affinity measurements were performed using [³H]ifenprodil as the radioligand and rat as the source of GluN2B-containing NMDA receptors.

This information can then be utilised for the development of selective agonists and improve our general understanding of these two receptors. Reducing steric bulk through the introduction of an azabicyclo[2.2.1]heptane polycycle reduced binding affinity, however, highlighted a promising scaffold for the development of selective σ_2R agonists. Furthermore, the azaadamantane framework was highlighted as a novel lead for σ_1R selective agonists.

The N-methyl-D-aspartate receptor

NMDA receptors (NMDARs) are distributed widely throughout the CNS and play an essential role in memory function and information processing.^{25,26} Under physiological conditions, activation by their endogenous ligand L-glutamate in the presence of glycine results in the influx of calcium ions. Defects within the functioning of NMDAR signalling pathways have been implicated in a multitude of CNS pathologies including PD, neuropathic pain and stroke, thus highlighting this family of receptors as a therapeutic target for symptomatic treatment of such diseases.^{27,28} NMDAR antagonists have previously been reported, however, have failed pre-clinical trials due to non-selective subunit inhibition.²⁸

Structurally, NMDARs are hetero-oligomeric ligand-gated ion channels (LGIC) that exist as either tetrameric or pentameric complexes of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 (A–D) subunits.²⁹ Subunit

selectivity of potential therapeutics is crucial as broad-range inhibition of NMDARs can lead to serious adverse effects such as hallucinations and hypertension.²⁹ Inhibiting the GluN2B subunit selectively has been proposed to reduce the adverse effect profiles due to its expression being confined to regions of the forebrain, unlike GluN2A which is distributed throughout the brain.³⁰

In 2003 BZAD01 (**23**), a benzamidine reported by Claiborne *et al.* was shown to be an NR2B-subtype selective NMDAR antagonist ($hNR2B$ K_i = 47 nM, $hNR2B$ FLIPR half maximal inhibitory concentration (IC_{50}) = 47 nM, Fig. 5).^{27,31} When co-administered with Levodopa, BZAD01 demonstrated positive results in a rat model of PD. To investigate this scaffold further a library of *N*-benzyl benzamidines (**24–28**, Fig. 5) was prepared to determine the steric and electronic requirements of the GluN2B binding site. The synthesised library included a range of substituted benzene (**24–26**) and polycycle moieties, such as adamantane **28** (Fig. 5).²⁶ The library was evaluated for affinity at the ifenprodil binding site of GluN2B-containing NMDARs. The binding affinity (K_i) was determined by the compound's ability to outcompete the binding of [³H]ifenprodil (5 nM) at various concentrations.²⁶

Replacement of the benzyl ring with polycycles identified that steric bulk was not tolerated in this part of the molecule, with the adamantyl analogue **27** reducing the activity by several orders of magnitude (K_i = 4.8 μ M).²⁶ Our

findings also suggested a subtle electronic requirement for affinity at the GluN2B-containing NMDA receptors. This was evident by the replacement of the electron-withdrawing trifluoromethyl group of BZAD01 with an electron-donating methoxy moiety. Direct substitution saw an increase in binding affinity (**24**), and di-substitution was generally well tolerated around the benzene ring except at the 3-position (**26**).²⁶ The synthesised library provided valuable SAR data that increased our understanding of GluN2B pharmacology.

Within this case study, substitution of the phenyl ring for adamantane was detrimental to activity. Therefore it was proposed that the GluN2B binding pocket does not favour sterically bulky functional groups and aromatic interactions may confer activity.

Tau aggregation inhibitors

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases, accounting for 60–70% of all cases of dementia.³² In 2019, 55 million people worldwide were reported to be living with dementia, with this number projected to increase to 139 million by 2050.^{32,33} However, strategies that slow or prevent its clinical progression have largely remained elusive. Within AD and other neurodegenerative disorders, a common characteristic of the disease is the accumulation of aggregates comprised of post-translationally modified tau proteins in the cytoplasm of neurons.^{34,35} Many tau-centric approaches to treating AD have been proposed, including the direct inhibition of tau aggregation.³⁶ Small molecule tau aggregation inhibitors have been the subject of many drug candidate campaigns. A high throughput screening (HTS) campaign identified aminothienopyridazines (ATPZs) as potent inhibitors of tau aggregation with favourable physicochemical properties.^{35,37} However, the mechanism of action employed by ATPZs and similar compounds is unknown.³⁶

Preliminary SAR of the thienopyridazine substituents by Ballatore *et al.* modified the ATPZ scaffold for optimal activity and pharmacokinetic (PK) properties.³⁷ ATPZ **29** was thereby proposed as a suitable lead compound for further structural modifications and *in vivo* evaluation. A compound library was thus proposed of a series of ring-opened analogues of ATPZs (Fig. 6).³⁸ Disconnection of the nitrogen–nitrogen bond of the pyridazine ring was anticipated to produce analogues with a similar arrangement of functional groups in space and therefore have similar or improved binding to tau monomers or aggregates. These compounds would also allow for the evaluation of oxidation mechanisms in tau aggregation inhibition due to their differing redox properties to that of the lead ATPZs.^{37,38} Through simple modifications, this also provided us with the opportunity to improve the drug-like properties such as solubility. A range of oxime and oxime

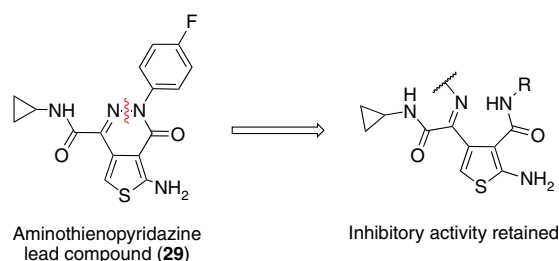


Fig. 6. Structural modifications to the lead ATPZ compound **29** were explored through the disconnection of the pyridazine nitrogen–nitrogen bond to result in the respective imine. Reprinted (adapted) with permission from Moir *et al.*³⁸ Copyright 2017 The Royal Society of Chemistry.

ether derivatives were also synthesised to account for the potential hydrolytic instability of the imine motif.³⁸

Our group reported a novel synthetic approach that enabled the synthesis of ring-opened ATPZ analogues and easy functionalisation of the C3 position of the thiophene with aliphatic amines. This methodology provided an efficient divergent synthesis to further explore the chemical space. The library was assessed for *in vitro* activity by both a fluorescence thioflavin T assay and an aggregate trap assay. Disconnection of the nitrogen–nitrogen bond of the pyridazine ring was shown to not affect activity with the ring-opened oxime allyl ether analogue **30** achieving 33% inhibition in the thioflavin T assay (**29**: 51% inhibition).³⁸ Aromatic amides at the 3-position of the thiophene were shown to not be necessary for inhibition of tau aggregation with the adamantane derivate **32** reporting 30% inhibition. The aggregate trap assay demonstrated that the oxime analogues **31** and **32** had a higher tendency to reduce higher-order tau aggregates compared to **29** and **30** (Fig. 7).³⁸

Oxidation of the cysteine residues in tau has been highlighted as a potential mechanism by which ATPZs exhibit their anti-aggregation effects.³⁵ Therefore, each compound's ability to oxidise tau was also assessed using Oregon green binding to non-oxidised cysteine. The oxime analogues **31** and **32** demonstrated oxidising activity, whereas **30** did not (Fig. 7). This suggested that the mechanism by which **31** and **32** inhibit tau aggregation is independent of oxidising effects.³⁸

Therefore, we have demonstrated that the pyridazine ring of the ATPZ scaffold is not imperative for activity. Aliphatic amides were demonstrated to be well tolerated at the 3-position of the thiophene providing an excellent handle for further SAR exploration. Aromatic interactions were similarly shown not to be crucial for inhibition of tau aggregation, with the adamantane analogue demonstrating similar results to that of the 4-fluorophenyl oxime allyl ether analogue **30**.³⁸

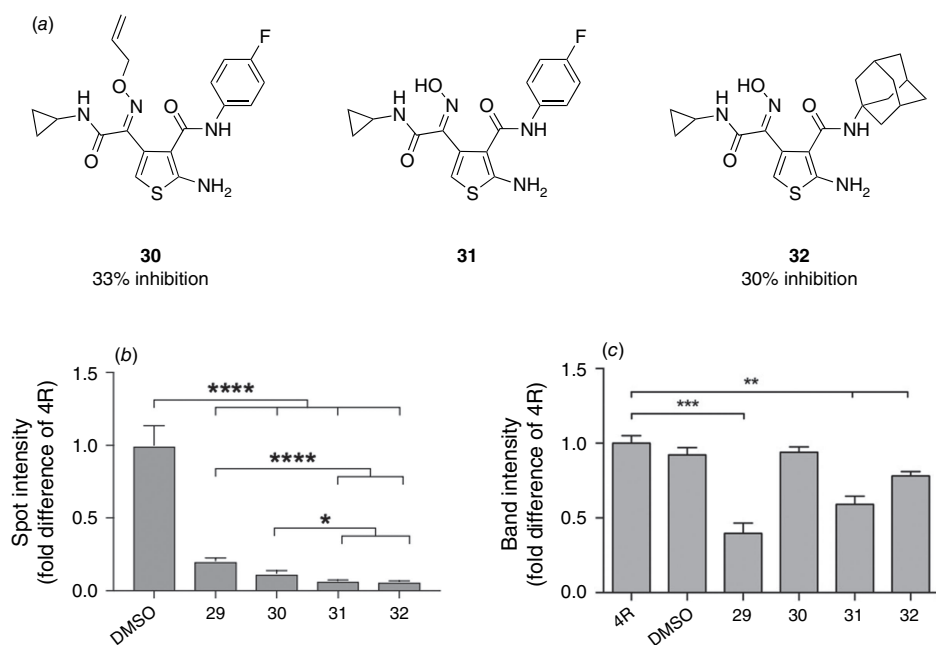


Fig. 7. Biological assessment of tau aggregation and redox properties. (a) Analogues assessed with percentage inhibition values determined from a thioflavin T assay. (b) A decrease in spot intensity is consistent with a decreased amount of fibrillar tau aggregates. Quantified from seven independent experiments (*, $P < 0.05$; ****, $P < 0.0001$). (c) Lower band intensities of IAA-OF Oregon green indicate increased oxidation. Quantified from five independent experiments and normalised to total recombinant tau proteins, stained in Coomassie blue (*, $P < 0.01$; ***, $P < 0.001$). Reprinted (adapted) with permission from Moir *et al.*³⁸ Copyright 2017 The Royal Society of Chemistry.

Synthetic cannabinoids

Cannabinoid (CB) receptors are G-protein coupled receptors (GPCRs) that are categorised into two subtypes: CB₁ and CB₂. Responsible for the mediation of the endocannabinoid system, CB receptors play a key role in synaptic plasticity and homeostatic processes in the brain.³⁹ Endogenously, CB receptors are activated by endocannabinoids such as anandamide and 2-arachidonoylglycerol. However, numerous exogenous ligands, both natural and synthetic, have been shown to act as CB receptor agonists.³⁹ Activation of the CB₁ receptor is responsible for the psychotropic effects associated with recreational cannabis, whereas CB₂ receptor agonists have been highlighted as potential therapeutic targets in a multitude of preclinical models. Expressed throughout the CNS on immune cells such as microglia and astrocytes, activation of the CB₂ receptor has been demonstrated to result in the resolution of inflammation following an assault or injury.⁴⁰

Synthetic cannabinoids (SCs) are the most rapidly emerging class of designer drugs and notably have been for the past 20 years.⁴¹ In 2022–23, synthetic cannabinoid receptor agonists (SCRAs) made up 40% of new substances reported to the United Nations Office on Drugs and Crime.⁴¹ SCRAs intentionally target the endocannabinoid system, activating the CB₁ receptor to mimic the intoxicating effects of known recreational drugs, namely cannabis.⁴² Owing to the rapid emergence of new SCs, the implementation of appropriate legal restrictions is significantly delayed because of a lack of reference materials and analytical data. The lack of data surrounding key synthetic intermediates and major metabolites also prompts significant health dangers and issues within the public health sector.

Over the past 24 years, adamantane-derived SCRAs have been consistently identified in illicit material. First detected by the European Monitoring Centre for Drugs and Drug Addiction in 2010, adamantan-1-yl(1-pentyl-1*H*-indol-3-yl)methanone (AB-001, **34**) had no synthetic details or pharmacological evaluation in scientific literature.⁴³ Soon after, *N*-(adamantan-1-yl)-1-pentyl-1*H*-indole-3-carboxamide (APICA, **35**) was also detected. Despite their novel structures, it was suspected that AB-001 and APICA were products of clandestine rational drug design as they both exploited known SAR data for previously reported cannabimimetic indoles.

Our study in 2013 represented the first attempt to characterise and evaluate the pharmacology of two novel synthetic cannabimimetics of abuse, AB-001 (**34**) and APICA (**35**).⁴³ The synthetic pathway proposed allowed for an efficient method to substituted analogues of the adamantyl-indole scaffold, and their corresponding intermediates for the development of reference standards of potentially emerging SCRAs. Preliminary SAR into the AB-001 and APICA chemotypes was also explored. All compounds (**36–41**, Fig. 8) were identified to be full agonists at CB₁ and CB₂ receptors *in vitro* (with the exception of SDB-002 **41**), demonstrating that structural heterogeneity is well tolerated at the 3-indole position.⁴³ Interestingly, these results did not correlate to *in vivo* assays, with only APICA (**35**) demonstrating activity. It was therefore inferred that the nature of the functional group joining the adamantane and indole units determined whether a compound had cannabimimetic activity or not, whereas distance between the two groups was found to have no effect.

The pharmacology and toxicity of fluorinated SCRA scaffolds have been highlighted as an area of concern.

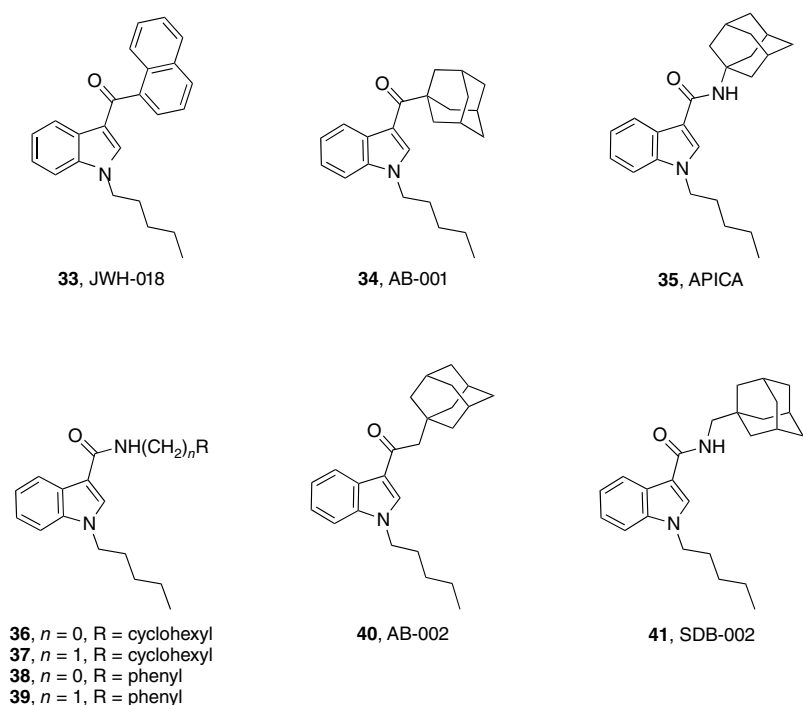


Fig. 8. Synthetic cannabimimetics of abuse identified in seizures and preliminary SAR of the indole chemotype.

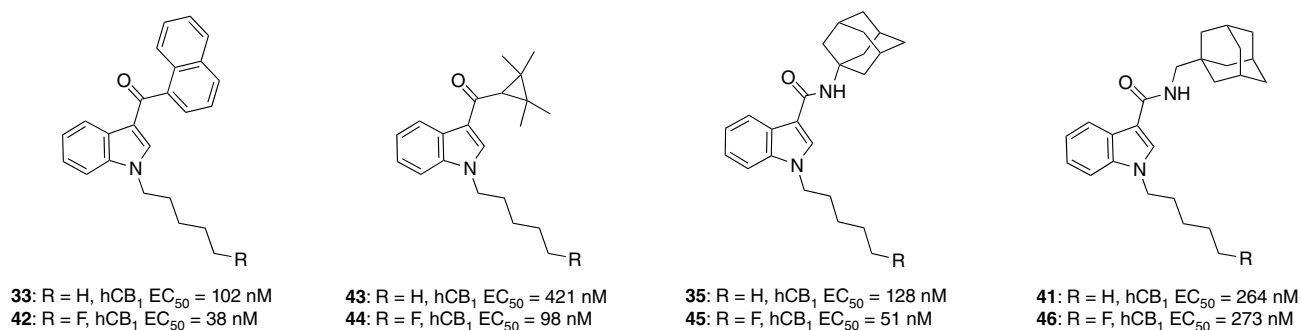


Fig. 9. A sample set of the fluorinated analogues was analysed for functional activity. Fluorination generally resulted in increased potency at the hCB₁ receptor. Data extracted from Shi *et al.*⁴⁴ EC₅₀ determined by a FLIPR membrane potential assay in AtT-20 cells expressing human CB₁ or CB₂ receptors.

Fluorinated *N*-pentyl indoles are susceptible to thermolytic defluorination due to the smoking route of administration and metabolic oxidative defluorination *in vivo*.⁴⁴ Consequently, pharmacological evaluation of such compounds is imperative to assess any potential toxicity. A series of 5-fluoropentyl analogues (42, 44–46, Fig. 9) were synthesised from a series of different cannabimimetic chemotypes including APICA (35) which incorporates the adamantane motif.⁴⁴ *In vitro* potency at the CB₁ receptor was found to increase for fluorinated analogues, except for 46. Although 46 did not improve potency *v.* its non-fluorinated analogue 41 it did improve efficacy at both the CB₁ and CB₂ receptors (hCB₁ maximum: 87 *v.* 53% respectively). Compounds 42–46 were found to dose-dependently decrease body temperature and heart rate in rats. However, this did not correlate to *in vivo*

potency in which no obvious trends were observed among the fluorinated and non-fluorinated analogues. It was suggested that the former discrepancy may be a correlation between the compound's PK properties, species-related differences in ligand affinity and efficacy or an error in measuring parameters such as body temperature to identify small differences.⁴⁴

Major metabolites of the adamantane-containing APICA (35) were synthesised and pharmacologically characterised by our group in 2017.⁴⁵ The adamantane motif is known to undergo oxidation during Phase 1 metabolism. Other major sites of metabolism include the *N*-alkyl group and carboxamide, with 1-adamantylamine identified as an APICA biomarker. All metabolites were shown to have comparable cannabimimetic activity to APICA at both CB receptors.⁴⁵

This suggested that they could be contributing to the physiological effects of the leads. Selectivity for the CB₂ receptor was observed for metabolites exploring oxidation on the *N*-pentyl chain regardless of the heterocyclic core. Oxidation of the adamantane bridgehead carbon demonstrated an increased potency at both cannabinoid receptors. To confirm whether the metabolites included in this study act synergistically with their parent compound requires further investigation into their biodistribution.

Forensic samples of street seizures have confirmed that new modifications to the SC scaffold are consistently occurring. SC scaffolds with an ester linker and azaindole motif were reported in 2013 and 2015 respectively. Owing to the isomeric relationship between azaindole and indazoles, routine methods of characterisation such as mass spectrometry were unable to distinguish between the two.⁴⁶ The indazole (47) and azaindole (48) derivatives of APICA were synthesised through previously reported methods (Fig. 10). Total ion chromatograms (TICs) and electron ionisation (EI) mass spectra were reported for all compounds and intermediates synthesised. Through gas chromatography–mass spectrometry (GC-MS) analysis we were able to reliably differentiate between each compound of the carboxamide series exclusively on retention times, despite each derivative producing similar EI fragmentation patterns (Fig. 10).⁴⁶ Fragmentation of the C–N amide bond and cleavage of the pentyl chain was commonly observed. Similarly, carboxylates incorporating the azaindole scaffold could be distinguished based on retention times and fragmentation ratios.⁴⁶ Substantial SAR data was also elucidated within this study with several compounds having no activity at either CB receptor, demonstrating the importance of the bicyclic ring system. However, no consistent trends were observed for the change of linker within each scaffold.

Selective agonists for the CB₂ receptor are crucial to avoid the psychotropic effects associated with the activation of the CB₁ receptor.⁴⁷ Multiple efforts have been made to synthesise CB₂ selective analogues; however, most have failed in the clinic due to species receptor differences and lack of selectivity. A study in 2017 by Shi *et al.* identified that for the APICA SC scaffold, CB₂ selectivity could be achieved by shifting the amide at the 3-position to the 2-position of the indole.⁴⁸ By incorporating an adamantyl amide at the 2-position (49) the lipophilicity was increased and only resulted in low to moderate potency. We had previously shown that the substitution of the adamantane for bulky amino acid derived amides and cumyl amides at the 3-position restores the potency and decreases the lipophilicity of the compounds.⁴³ Within our development of an EI mass spectrometry method for the distinguishment between azaindole and indazole derivatives we also observed that the azaindole scaffold reported some CB₂ selectivity.⁴⁶ Combining the former SAR knowledge, a hybrid strategy (Fig. 11) was established for the development of selective

CB₂ agonists from non-selective synthetic cannabinoid designer drugs.⁴⁷

Building off the 2-amide strategy reported by Shi *et al.*, CB₁ activity was found to be suppressed within analogues incorporating the 7-azaindole core, and the addition of a polar hydroxy group at the terminal position of the pentyl chain (50).^{47,48} By contrast, the hybrid structure 51 lost all activity at both subtypes (Fig. 11). Substitution of the 2-amide adamantane for amino acid (52) and cumyl amides mirrored that at the 3-position with analogues retaining potency and selectivity while improving the PK properties and solubility. Polar amino acid substituents were demonstrated to disrupt CB₂ activity due to unfavourable intramolecular H-bonding interactions. The following strategies were all supported through molecular modelling data.⁴⁷

Adamantane-derived SCRAAs are consistently identified in illicit material and thereby remain a key interest within academia. Characterising their synthetic pathway and pharmacology is imperative for both law enforcement and public safety. As demonstrated above, our group has greatly contributed to this knowledge by deducing novel synthetic pathways and analytical methods to distinguish between structural isomers of both SCs and their metabolites. The lipophilicity issues corresponding to the adamantyl group were addressed through substitution with cumyl amides. Retaining the adamantyl motif, effective modifications were also reported to confer CB₂ selectivity, providing invaluable data for the development of novel CB₂ agonists for the treatment of chronic pain.⁴⁹

The P2X7 receptor

The P2X7 receptor (P2X7R) is a non-selective, LGIC that is upregulated on immune cells following a peripheral or central insult.⁵⁰ Activated by ATP, the P2X7R mediates the processing and release of pro-inflammatory cytokines, interleukin 1 β (IL-1 β), and thus plays a role in a variety of cellular actions. Therapeutic intervention through targeting the P2X7R has demonstrated success in eliminating or reducing symptoms of a multitude of neuroinflammatory diseases such as AD and multiple sclerosis.⁵⁰ However, despite multiple pursuits, only a few have proceeded to clinical trials with many failing due to unfavourable ADMET properties.

In 2014, our group synthesised the first molecule to possess CNS-modifying activity with a carborane motif (Fig. 12).⁵¹ Similar to that of adamantane, carboranes are bioisosteres of phenyl rings, however, their unique structure and composition present an exciting avenue for drug discovery.⁵² Structurally, carboranes are pseudoaromatic polyhedral clusters consisting of boron, carbon and hydrogen atoms.⁵² They were originally utilised within boron neutron capture therapy and can exist as a neutral, lipophilic *closo* cluster or the anionic, hydrophilic *nido* species. Within our study, the carborane motif was utilised as a bioisosteric

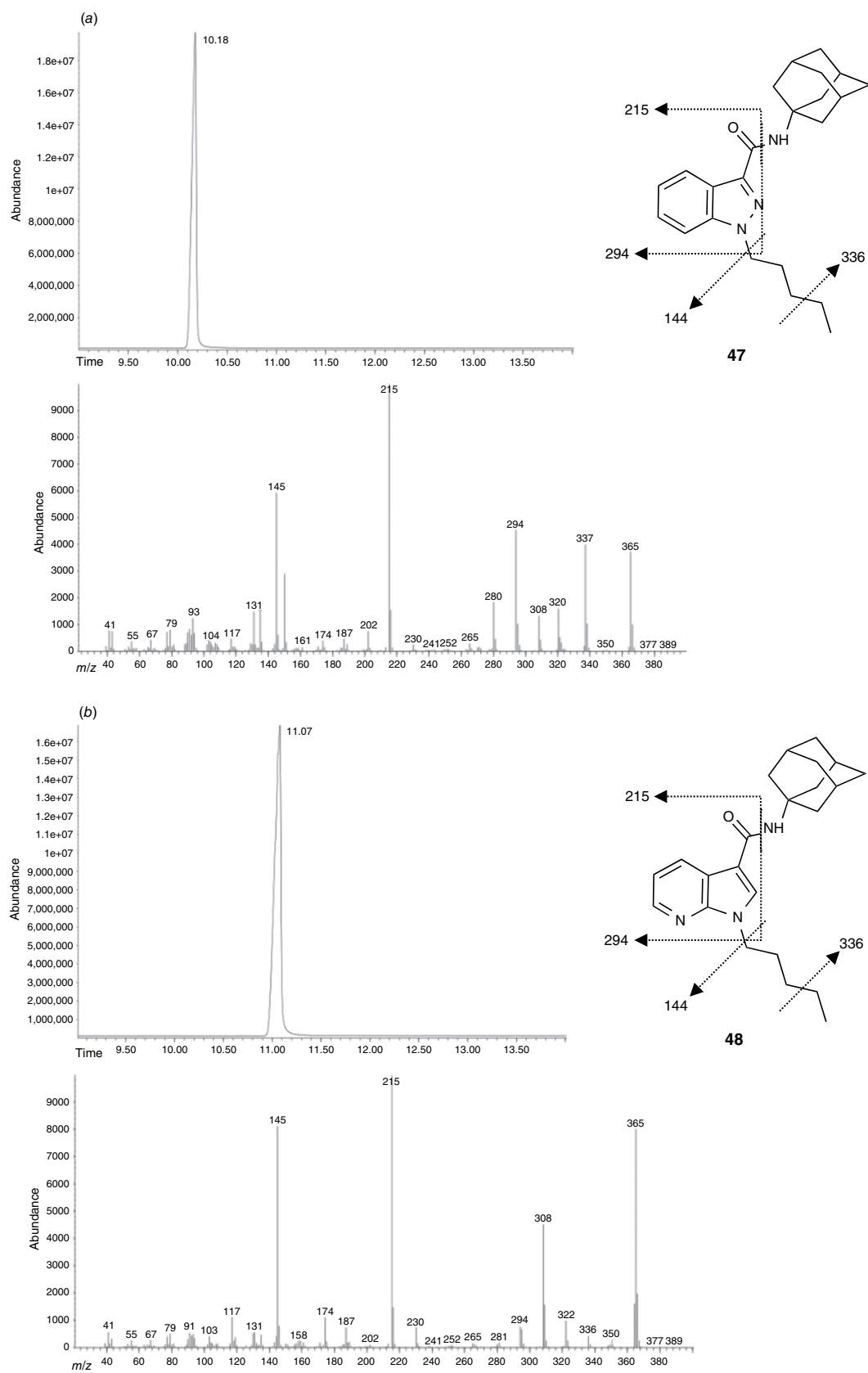


Fig. 10. (Caption on next page)

Fig. 10. GC-MS analyses showing the total ion chromatograms (TICs; time recorded in minutes, top) and electron ionisation mass spectra (EIMS, bottom) for indazole **47** (a) and azaindole **48** (b). Reprinted (adapted) from an Open-Access article distributed under the terms of the CC BY license.⁴⁶

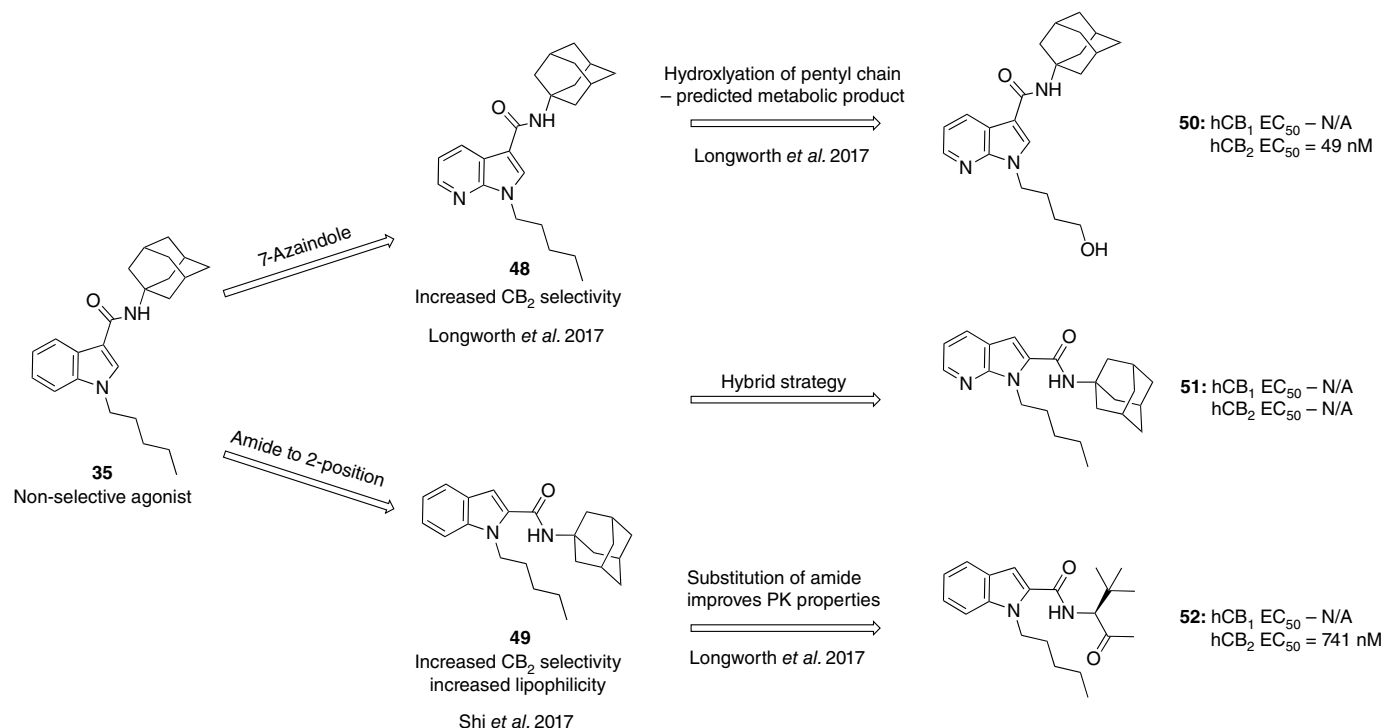


Fig. 11. Strategies to develop CB₂ selective agonists from non-selective synthetic cannabinoids such as APICA.^{45,47,48} Data extracted from Moir *et al.*⁴⁷ Half maximal effective concentration (EC₅₀) determined by a FLIPR membrane potential assay in AtT-20 cells expressing human CB₁ or CB₂ receptors.

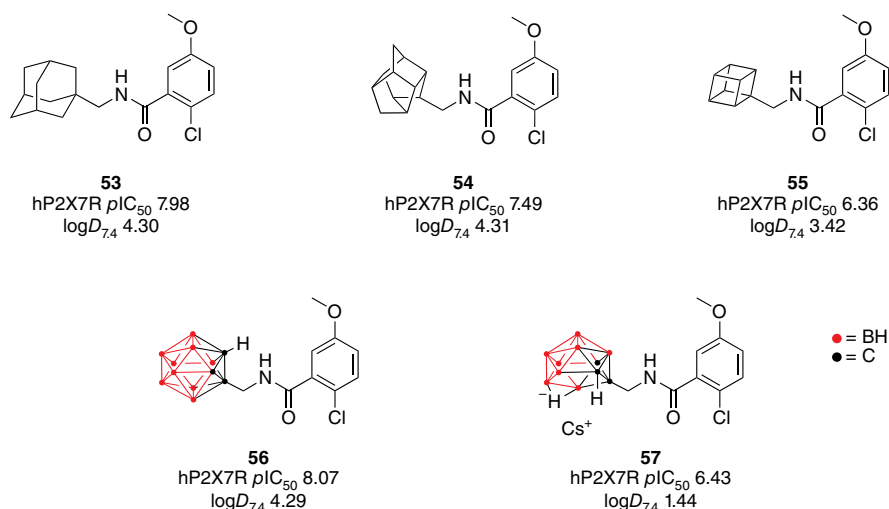


Fig. 12. Exploring the effect of polycyclic cages with varying lipophilic and steric properties on P2X7R antagonism. Reprinted (adapted) with permission from Banister *et al.*⁴³ Copyright 2014 American Chemical Society.

replacement of the adamantane within the AstraZeneca benzamide P2X7 antagonist **53**.^{51–53} Previous SAR studies of the benzamide chemotype identified that removing the three-dimensionality of the polycycle for simpler cycloalkane

functional groups was detrimental to activity.⁵³ Therefore, to explore the SAR further, a series of polycyclic cages such as cubane and trishomocubane were included in the library. *In vitro* activity was assessed in a functional dye uptake assay in

THP-1 cells utilising BzATP (100 μM) to induce pore formation.

Reducing the size of the polycyclic cage reduced potency at the hP2X7R accordingly.⁵¹ The trishomocubane analogue **54** was found to reduce potency by approximately one-third (hP2X7R $p\text{IC}_{50}$ 7.49), whereas the cubane analogue **55** saw a decrease in over one order of magnitude (hP2X7R $p\text{IC}_{50}$ 6.36). Although equivalent in size, the *closo*-1,2-carborane cage is more lipophilic compared to adamantane due to the low polarity of the B–H bonds, whereas the *nido*-7,8-carboranyl **57** has significantly less lipophilic properties due to its anionic charge.⁵⁴ Both analogues were shown to maintain potency, with the *closo* derivative **56** displaying a slight improvement compared to the adamantane lead (Fig. 12).

The analogues' CNS activity was validated through animal behavioural tests to measure their *in vivo* efficacy and BBB permeability.⁵¹ A forced-swim test (FST) was implemented as a method of evaluating the antidepressant potential of the benzamide analogues. Compounds **55** and **57** were shown to have significant antidepressant activity (16 and 13% more mobile than wild-type respectively), despite being the least potent analogues *in vivo*.⁵¹ Compounds **53**, **54** and **56** demonstrated no activity within the FST, therefore suggesting insufficient BBB permeability. This was rationalised by their experimental $\log D_{7,4}$ values being much greater than the range reported for successful CNS drugs ($c \log P < 3$). Furthermore, the compounds' drug-likeness' was further evaluated by determining their ligand-lipophilicity efficiency (LLE; $\text{LLE} = p\text{IC}_{50} - \log D$) value.⁵¹ The *nido*-7,8-carborane **57** reported a LLE of 4.99, making it the most promising drug candidate of the reported benzamides ($\text{LLE} \geq 5$). Despite reporting comparable *in vivo* and *in vitro* results to **57**, the cubanyl **55** derivative is suspected to be less bioavailable due to its high lipophilicity and thereby is less likely to be a good drug candidate ($\text{LLE} = 2.94$).⁵¹

Expanding on the AstraZeneca benzamide scaffold **53**, a series of hybrid structures with the reported cyanoguanidine chemotype **58** by Abbott Laboratories was synthesised and evaluated at the hP2X7R.^{53,55,56} This study investigated whether combining favourable motifs from two distinct compound classes would retain activity at the hP2X7R. Choosing to keep the adamantyl moiety from the AstraZeneca scaffold as a hydrophobic portion, the hybrid molecules include various substituted aryl groups connected by a cyanoguanidine linker (e.g. **59**, Fig. 13).⁵⁵ SAR studies on this hybrid skeleton structure have spanned multiple years and remain under investigation in our group.

The optimal distance of the cyanoguanidine linker to the adamantyl ($L_1 = 0-2$) and aryl ($L_2 = 0-2$) groups was initially explored.⁵⁵ Variations to L_1 were not tolerated with only $L_1 = 1$ maintaining activity, whereas L_2 allowed for more variance. The effect of L_2 was found to vary in conjunction with the electronics of the aryl moiety. Benzene derivatives were found to be more potent when $L_2 = 0$, although a methylene linker was favoured for heteroaromatic analogues. This was thought to be due to the electron-deficient pyridinyl analogues being unable to effectively participate in cation– π interactions in the active site when directly attached ($L_2 = 0$).⁵⁵ This notion was further supported through the comparison of the 5-quinolinyl analogue **59** (Fig. 13) and the 3-pyridyl analogue **60** (Fig. 13). Compound **59** features the quinoline directly attached ($L_2 = 0$), however, sufficient cation– π interactions can still occur by the phenyl portion. The drug-likeness of the analogues were assessed through a lipophilicity efficiency (LiPE; $\text{LiPE} = p\text{IC}_{50} - c \log P$) score. Despite having better *in vitro* potency, the 5-quinolinyl derivative **59** yielded a lower LiPE value to that of the 3-pyridyl derivative **60** due to its inherent higher calculated $\log P$.⁵⁵ As a direct result, **60** was deemed the more 'drug-like' candidate based on *in silico* physicochemical and PK properties and thereby utilised within *in vivo* studies. Compound **60** demonstrated

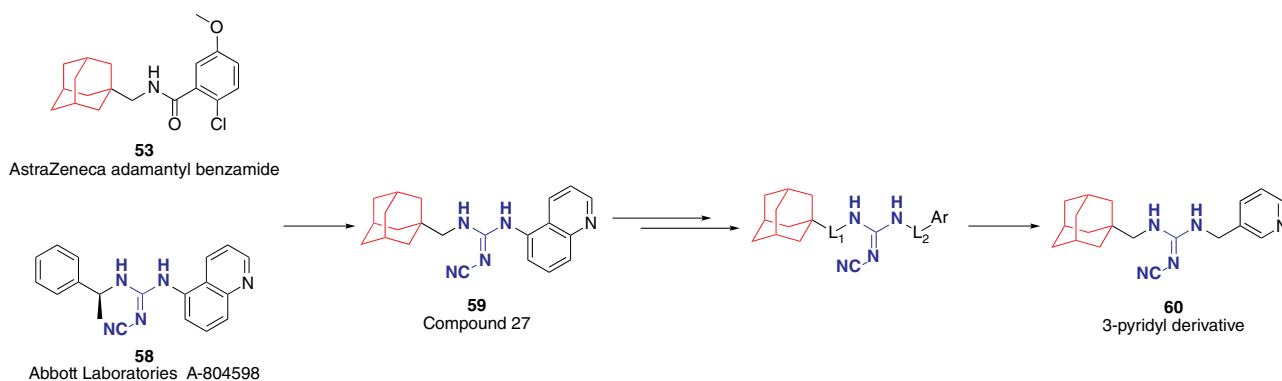


Fig. 13. Investigational SAR study of adamantyl-cyanoguanidine hybrid compounds^{53,55,56} first explores the linker length between the cyanoguanidine linker and the adamantyl (L_1) and aryl (L_2) groups.⁵⁵

unfavourable *in vivo* PK properties, however, was able to demonstrate the ability of the hybrid analogues to act centrally and produce antidepressant activity in the FST, thereby validating the hybrid approach and prompting further SAR studies on the adamantyl-cyanoguanidine core structure for the development of potent CNS-permeable P2X7R antagonists.⁵⁵

To optimise lipophilicity and thereby increase the potential for clinical translation, we next explored the cyanoguanidine linker.⁵⁷ Chemical modification to the cyanoguanidine moiety was made to explore its significance. Such substitutions included the introduction of a urea, thiourea and guanidine motif (Fig. 14). A methylene group between the linker motif and the adamantyl remained constant, whereas the spacing of the aryl analogue (*n*) varied between 0 and 2. Of the 10 analogues synthesised, no derivative improved potency *v.* the lead, highlighting the importance of the cyanoguanidine moiety for potent P2X7R inhibition within this chemotype.⁵⁷

Expanding on this, key pharmacophoric features of the linker were further explored through bioisosteric replacement and systematic deconstruction of the cyanoguanidine motif.⁵⁸ Retaining the methyl adamantane and the superior 5-quinoline heterocycle, 12 additional analogues were synthesised. The library explored the effects of hydrogen bond donors (HBDs) and acceptors (HBAs), tolerance of larger functional groups and conformational restriction (Fig. 15). *In vitro* evaluation of the compound library reported all compounds to be less potent than the lead, however, key structural information was extrapolated from the study.⁵⁸ Mirroring the results of the formerly described study, the urea (**61**, 152 nM) and thiourea (**62**, 120 nM) analogues resulted in a seven-fold reduction in potency compared to the lead (**59**, 18 nM). Larger functional groups, such as trifluoromethanesulfonyl (**63**, 478 nM) demonstrated moderate potency, whereas the primary sulfonamide HBD **64** (2730 nM) was not tolerated. Interestingly, the introduction of a HBA to form the carbamate analogues **65**

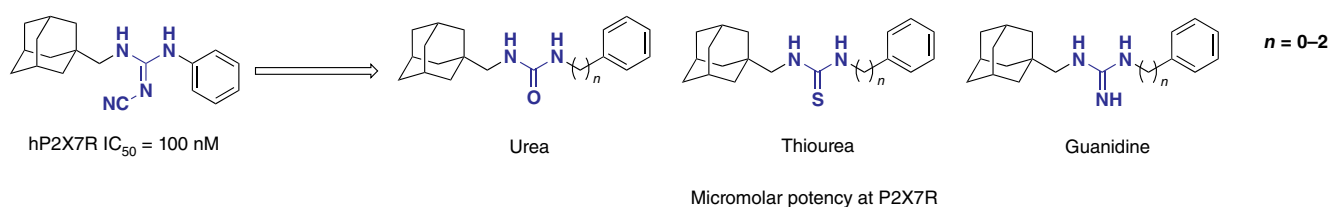


Fig. 14. Substitution of the cyanoguanidine linker to optimise lipophilicity resulted in a significant decrease in hP2X7R antagonism.

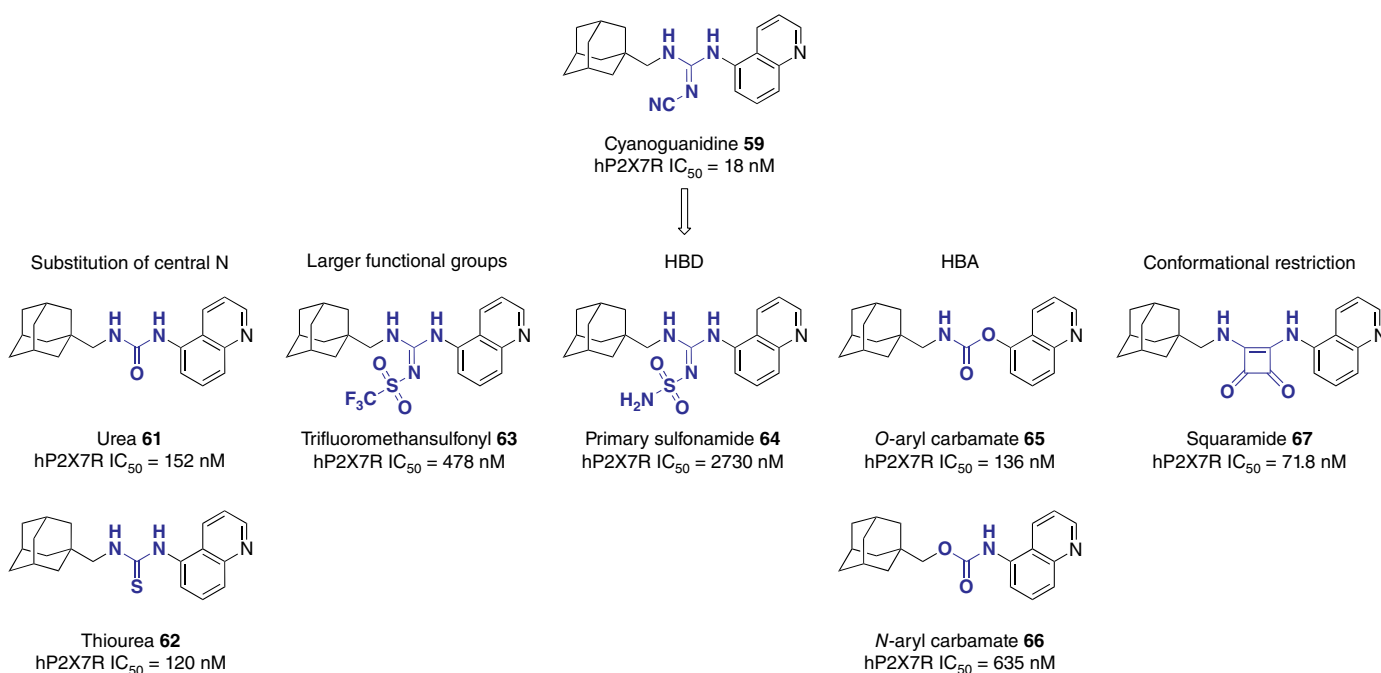


Fig. 15. SAR evaluation of the linker motif. Data extracted from Garrett *et al.*⁵⁸ IC₅₀ assessed by inhibition of hP2X7R-mediated pore formation using the YO-PRO-1 dye uptake assay in human THP-1.

and **66** yielded very different results based on orientation. The *O*-aryl carbamate **65** (136 nM) retained potency, whereas the *N*-aryl carbamate **66** (635 nM) saw a significant reduction. Conformational restriction of the linker motif was explored through the non-linear squaramide functionality. The squaramide analogue **67** (71.8 nM) demonstrated a moderate increase in potency relative to the urea analogue **61**, however, it still displayed a four-fold reduction compared to the lead (**59**).⁵⁸

Despite the rigorous efforts made to discover potent and selective P2X7R antagonists, many fail to proceed to clinical translation due to poor ADMET properties. Extensive SAR studies exploring the chemical space of the AstraZeneca benzamide scaffold have continuously highlighted the importance of the adamantyl moiety on activity.^{53,55} However, the compounds have high lipophilicity and poor metabolic stability as a result. Through a systematic approach, our group explored two common bioisosteric strategies. Firstly, we replaced the benzene ring with heteroaromatic motifs. Substituting for azabenzene and diazenes successfully reduced the lipophilicity, however, also significantly reduced the potency at the hP2X7R.⁵⁹ The bridgehead carbons of the adamantane cage are known to be prone to oxidation. Introducing carbon–fluorine bonds is a common approach to overcome this in drug discovery. The highly electronegative fluorine atom forms a strong, polar covalent bond with carbon that reduces lipophilicity and blocks metabolically labile sites. The trifluoroadamantane derivative **68** was well tolerated with only a small loss of

activity, yet significantly improved physicochemical and PK properties compared to the lead benzamide (Table 1).⁵⁹ For example, it was identified to have a half-life six times longer than that of the lead (**53**) in rat liver microsomes and was effective across several isoforms of the hP2X7R. These data identified **68** as a potential preclinical ligand to validate models of CNS disorders and as a suitable PET radiotracer.

Combining the formerly mentioned SAR data of the hybrid scaffold and AstraZeneca's benzamide, the role of polycycles and the cyanoguanidine linker was explored by incorporating them with other known P2X7R antagonists.⁶⁰ For example, the original AstraZeneca adamantyl benzamide **53** was modified to include a cyanoguanidine linker. Aiming to improve the lipophilicity of these compounds, different polycycles were introduced. Substituting the amide for a cyanoguanidine linker in the benzamide scaffold to give **69** resulted in a decrease in potency (162 v. 2–10 nM for the 2-chloro-5-methoxy analogue, Fig. 16) across the board.⁶⁰ Reducing the polycycle size significantly reduced activity (3467 v. 57 nM, Fig. 16), mirroring observations from our previous study.⁶⁰ This further confirms the important role adamantane plays in the functional activity of P2X7R inhibitors.

Despite the extensive research into P2X7R antagonists, a severe lack of mechanistic pharmacological characterisation has been noted.⁶¹ Therefore, the suitability of previously discussed antagonists as clinical tools or drug candidates has been restricted. In 2022, our group determined the mechanism of action of our lead compound **59**, and other previously reported

Table 1. Pharmacokinetic data of the lead benzamide and its trifluoroadamantyl derivative **68**.

Pharmacokinetic properties		
	53	68
$\log D_{7.4}$	4.23	2.83
hP2X7R IC ₅₀ (nM)	10.5	33.9
LLE	3.75	4.64
rLiver t _{1/2} (min)	7.8	46.8
rBrain t _{1/2} (min)	9.6	75.6
rPlasma t _{1/2} (min)	28.2	103
Cl (mL min ⁻¹ kg ⁻¹)	86.90	41.81
CYP1A2 (%)	25.7	5.48
CYP2C9 (%)	75.0	9.30
CYP2C19 (%)	84.4	12.9
CYP2D6 (%)	-47.3	-29.1
CYP3A4 (%)	84.4	89.9

$\log D_{7.4}$ data were determined experimentally by HPLC. hP2X7R IC₅₀ values were the mean values ($n > 4$) \pm standard deviation derived from a dye uptake assay with THP-1 cells. LLE = $pIC_{50} - \log D$, where pIC_{50} was from dye uptake and $\log D$ was from HPLC. Reprinted (adapted) with permission from Wilkinson *et al.*⁵⁹ Copyright 2017 American Chemical Society.

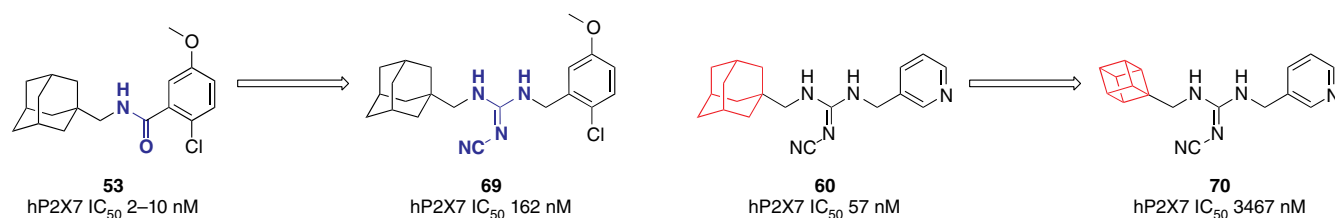


Fig. 16. Series of hybrid structures to confirm key SAR data. Adamantane was confirmed to be an important motif within P2X7R antagonists. Data extracted from Callis *et al.*⁶⁰ Values refer to pore-formation inhibition and were obtained from a fluorescent dye uptake assay in THP-1 cells.

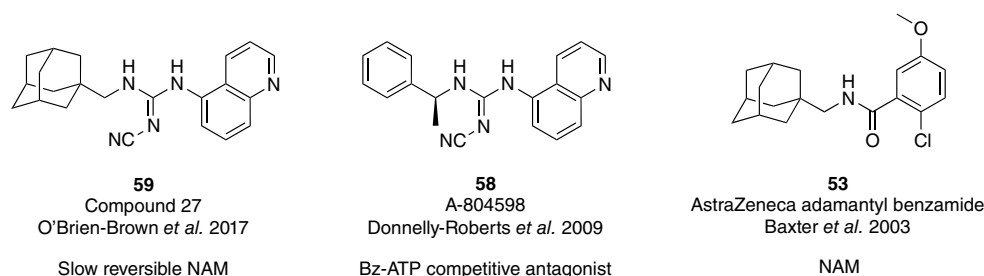


Fig. 17. Pharmacological evaluation of our lead compound and reported P2X7 antagonists to determine the mechanism of action.^{53,55,56}

antagonists (**53** and **58**, Fig. 17).^{53,55,56,62} The adamantyl-cyanoguanidine **59** was determined to be a slowly reversible negative allosteric modulator (NAM) of the hP2X7R highlighting its potential for use in *in vitro* and *in vivo* studies.⁶² Furthermore, our characterisation also identified that the adamantane moiety promotes binding in the allosteric site of the hP2X7R.

The adamantyl-cyanoguanidine scaffold **59**, initiated from our exploratory hybrid approach, resulted in a largely successful novel P2X7R antagonist chemotype.⁵⁵ Since its conceptualisation our group has conducted extensive SAR studies on the aryl and linker motifs.^{55,57,58} The adamantane functional group has been a consistent feature in new analogues and has thus been identified as a privileged structure for the P2X7R. Metabolic soft spots, such as the bridgehead carbons, have been shown to be easily resolved through a hydrogen-to-fluorine bioisosteric replacement.⁵⁹ Our studies have also shown adamantane's superiority over other polycycles such as cubane, within multiple P2X7R antagonist chemotypes.⁶⁰

Summary and future directions

The adamantane motif and its analogues continue to be useful tools in drug discovery campaigns to alter PK properties and exploit precise substitution orientation to explore three-dimensionality. Since 2011, our group has published and patented work surrounding the synthesis and validation of adamantyl-containing compounds. We have demonstrated the effects of adamantane within multiple SAR studies on unique targets and validated these *in vivo*. Most

notably, we have confirmed adamantane to be a privileged scaffold of the P2X7R and supplied a multitude of reference materials and data to assist in SC identification by law enforcement. Current work within the group continues to include adamantane as a key benchmark of steric allowance and tolerance of hydrophobic motifs. However, a key gap in our current utility of adamantane is exploiting the three-dimensionality of the scaffold beyond the bridgehead carbons. Utilising synthetic strategies, that are becoming readily available, to access more diverse substitution patterns will provide us with further avenues to explore the potential of this moiety in CNS drug discovery.

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