A Structure-Activity Study of *N*-((*trans*)-4-(2-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (SB269652); A Bitopic Ligand That Acts as a Negative Allosteric Modulator of the Dopamine D_2 Receptor

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A Structure-Activity Study of *N*-((*trans*)-4-(2-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (SB269652); A Bitopic Ligand That Acts as a Negative Allosteric Modulator of the Dopamine D_2 Receptor

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Abstract

We recently demonstrated that SB269652 (**1**) engages one protomer of a dopamine D_2 receptor (D_2R) dimer in a bitopic mode to allosterically inhibit the binding of dopamine at the other protomer. Herein, we investigate structural determinants for allostery, focusing on modifications to three moieties within **1**. We find that orthosteric 'head' groups with small 7-substituents were important to maintain the limited negative cooperativity of analogues of **1**, and replacement of the tetrahydroisoquinoline head group with other D_2R 'privileged structures' generated orthosteric antagonists. Additionally, replacement of the cyclohexylene linker with polymethylene chains conferred linker length dependency in allosteric pharmacology. We validated the importance of the indolic NH as a hydrogen-bond donor moiety for maintaining allostery. Replacement of the indole ring with azaindole conferred a 30-fold increase in affinity whilst maintaining negative cooperativity. Combined, these results provide novel SAR insight for bitopic ligands that act as negative allosteric modulators of the D_2R.

Introduction

The dopamine D_2 receptor (D_2R), a class A G protein-coupled receptor (GPCR), is a therapeutic target for central nervous system disorders including schizophrenia and Parkinson's disease.[1] To date, drug discovery at this target has focused on the orthosteric binding site, with clinically marketed antipsychotics being either antagonists/inverse agonists or partial agonists at the D_2R.[2] Unfortunately, such approaches are associated with significant side effects; in particular, high occupancy orthosteric blockade of the D_2R provided by first generation antipsychotics, while effective in treating the positive symptoms of the disease, is associated with extrapyramidal side effects.[2]

It has become apparent that many class A GPCRs have at least one other topographically distinct, allosteric binding site that can be targeted by small molecules.[3-6] Ligands that behave as allosteric modulators may offer advantages over purely orthosteric ligands, including increased receptor subtype selectivity and maintenance of spatiotemporal patterns associated with the signals of the endogenous ligand.[7] These features make negative allosteric modulators an attractive prospect for the treatment of schizophrenia, where partial blockade by a negative allosteric modulator with limited negative cooperativity, may represent a safer therapeutic option for the positive symptoms of schizophrenia.[8] More recently, concomitantly targeting both orthosteric and allosteric sites with bitopic ligands, in which allosteric and orthosteric pharmacophores have been linked together, has been explored as a means of developing more selective GPCR ligands.[9-11]

SB269652 (**1**)[12,13] was recently described as the first small molecule negative allosteric modulator of the D_2R.[13] This was somewhat surprising, given that **1** contains structural features of numerous orthosteric D_2-like receptor ligands.[12,14-16] Indeed, the 1,2,3,4-tetrahydroisoquinoline (THIQ) 'head' group of **1** contains a basic tertiary amine that is expected to form a salt bridge with the conserved aspartate ($Asp^{3.32}$, Ballosteros-Weinstein

nomenclature[17]) of aminergic GPCRs, and would thus compete with the binding of dopamine. The lipophilic 1*H*-indole-2-carboxamide 'tail' group is characteristic of the lipophilic appendages that are a feature of numerous subtype selective D_2-like receptor ligands.[14] Such appendages have been proposed to extend into a secondary pocket away from the orthosteric pocket.[14,18,19]

We have recently confirmed that **1** displays limited negative allosteric cooperativity at the D_2R with a sub-micromolar binding affinity, but found that truncated derivatives of **1** containing a THIQ moiety act in a competitive manner with dopamine.[20] We demonstrated that **1** acts via a novel mechanism, engaging one protomer of a D_2R dimer in a dual orthosteric/allosteric (or bitopic) mode, to negatively modulate dopamine binding and function at the other protomer (**Figure 1**).[20] This mechanism allowed us to reconcile an orthosteric mode of interaction with the allosteric pharmacology of SB26965. Furthermore, the interaction of the 1*H*-indole-2-carboxamide moiety of **1** with a secondary, or allosteric, pocket between the extracellular face of transmembrane domains (TMs) 2 and 7, was shown to be a requirement for the allosteric pharmacology of **1**. These findings presented a novel mode of action for small molecules at this therapeutically important target.[21] Additionally, we were able to use **1** as a probe to identify D_2R dimers in rat striatal tissue, adding to previous evidence that this receptor can function as a dimer or oligomer.[22-26] It should be noted that the above mechanism differs from previous studies that attempted to target D_2R dimers/oligomers using bivalent ligands that simultaneously occupy binding sites on two adjacent receptors.[27,28]

<Insert Figure 1>

Currently, there is limited SAR data associated with **1**, indicating scope for further structural interrogation (**Figure 2**). We therefore undertook a study to identify novel negative allosteric modulators for the D_2R, based on the structure of **1**. In particular, given the similarity of **1** to other D_2-like selective ligands[14,29] that display competitive pharmacology with dopamine, it is important to understand the key structural features of **1** that underlie its distinct allosteric pharmacology. Through modifications to the scaffold of **1**, as illustrated in **Figure 2**, we aimed to identify key molecular features that were responsible for changes in functional affinity and negative allosteric cooperativity, thereby enhancing our understanding of the nature of this bitopic mechanism and of allostery at the D_2R.

<Insert Figure 2>

Synthesis

We generated a focused library of analogues of **1** to examine the effect on functional affinity and allosteric pharmacology of the scaffold, as depicted in **Figure 2**. These included replacement of the 7-cyano group with various substituents of differing size and electronic effects (H, F, Cl, Br and CF_3); replacement of the THIQ core with D_2R privileged motifs and related bicyclic systems (2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine, 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine, 1-(2-methoxyphenyl)piperazine (2-MPP) and 1-(2,3-dichlorophenyl)piperazine (2,3-DCPP)); and the installation of polymethylene spacers in place of the cyclohexylene linker. We also explored the role of hydrogen-bond donors in **1** by examining the effect of alkylation of both the indolic and carboxamide NH moieties, and by investigating the influence of various heteroatoms on the tail group. In addition, we sought to evaluate the impact of replacing the indole moiety with monocyclic (e.g. pyrrole and

pyrrolidine) and various electron-rich and electron-deficient bicyclic (e.g. benzofuran and azaindole, respectively) heterocyclic systems.

The synthesis of all compounds generally followed previously established methods for the synthesis of **1**,[12,20] and other related compounds recently reported by our research group.[30] For the synthesis of compounds with modifications to the THIQ core, various tetrahydroisoquinolines, phenylpiperazines, or tetrahydrothienopyridine, were commercially sourced. The compound 2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine (**4**) was synthesized by a Schmidt reaction of 1-tetralone (**2**) in concentrated hydrochloric acid, favouring the alkyl-migration lactam **3** as the major product,[31] shown in **Scheme 1**. Reduction of **3** with lithium aluminium hydride gave **4** in good yield.

<Insert Scheme 1>

The preparation of the *trans*-cyclohexylene spacer unit, illustrated in **Scheme 2**, followed methods that we previously reported, to afford aldehyde **8** from 4-nitrophenylacetic acid (**5**).[30] The preparation of **1** from **8** was carried out using literature procedures.[12,20] Compound **8** was treated under reductive alkylation conditions ($NaBH(OAc)_3$) with the various secondary amine head groups (**9b-j**), which were prepared as in **Scheme 1**, or commercially supplied as the free base or hydrochloride salt. In the case where hydrochloride salts were used, *N,N*-diisopropylethylamine (DIPEA, Hünig's base) was also added to liberate the free base. This process afforded the corresponding tertiary amines (**10b-j**). Deprotection of the *N*-Boc group in TFA/DCM, followed by basic workup or 4 M HCl/1,4-dioxane, afforded the primary amines (**11a, c-h**) or the corresponding dihydrochloride (**11b**) or trihydrochloride salts (**11i-j**), respectively. The final compounds (**12b-j**) were then prepared by treatment of **11b-j** with 1*H*-indole-2-carboxylic acid in the presence of *O*-(6-chlorobenzotriazol-1-yl)-

N,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HCTU) and DIPEA, as presented in **Scheme 2**.

<Insert Scheme 2>

We next explored compounds with modifications to the spacer group of **1**, as illustrated in **Scheme 3**, using various starting points depending on commercial availability. For the synthesis of the propylene spacer, 3-bromopropylamine hydrobromide (**13a**) underwent *N*-Boc protection, before alkylation with 7-cyano-1,2,3,4-tetrahydroisoquinoline (7-CTHIQ, **9a**) in the presence of K_2CO_3 in refluxing MeCN, in moderate yield. For the synthesis of compounds with butylene, pentylene and hexylene spacers, the appropriate aminoalcohol (**13b-d**) underwent *N*-Boc protection, followed by activation of the alcohol using methanesulfonyl chloride, and subsequent nucleophilic substitution with **9a**. The *N*-Boc-protected compounds **16a-d** underwent deprotection with TFA, prior to HCTU-mediated coupling with 1*H*-indole-2-carboxylic acid to give the final products, **18a-d**.

<Insert Scheme 3>

In order to explore the requirements of the 1*H*-indole-2-carboxamide tail group for allosteric pharmacology, we generated a number of analogues of **1** bearing structural modifications to the tail moiety. To assess the role of the amide NH in a hydrogen-bond donating interaction, we synthesised the *N*-methylated indole-2-carboxamide analogue (**23**), which removes one potential hydrogen-bond donor present in **1**. For the synthesis of **23**, ethyl 2-(*trans*-4-((*tert*-butoxycarbonyl)amino)cyclohexyl)acetate (**7**), was N-methylated using an excess of sodium hydride and iodomethane in DMF over 3 days (**Scheme 4**), and ester **19** was isolated in moderate yield. This method was chosen in favor of Eschweiler-Clarke

methylation of amine **6** due to the large scale of **7** that was already synthesized, and was readily accessible for further reactions. **19** was subsequently reduced using DIBAL-H to the corresponding aldehyde (**20**), before reductive alkylation with **9a** in the presence of $NaBH(OAc)_3$ to give **21**. The *N*-Boc group was then removed with TFA in DCM to give secondary amine **22**, which was coupled with 1*H*-indole-2-carboxylic acid in the presence of HCTU to give the desired N-methylated product (**23**).

<Insert Scheme 4>

To further our understanding of a hydrogen-bonding interaction between the 1*H*-indole-2-carboxamide moiety of **1** and $Glu95^{2.65}$, as we have previously described,[20] we generated compounds which were completely devoid of a hydrogen-bond donor group on the indole moiety. To do this, we generated the benzofuran-2-carboxamide (**25a**), benzo[*d*]oxazole-2-carboxamide (**25b**), 1*H*-indene-2-carboxamide (**25c**) and 1-methyl-1*H*-indole-2-carboxamide (**25d**) analogues. We also investigated analogues which 'fine-tuned' the hydrogen-bond donating ability by modifying the electronic properties of the indole moiety, namely by the synthesis of the 1*H*-benzo[*d*]imidazole-2-carboxamide (**25e**) and 1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (**25f**) analogues. The synthesis of these compounds, depicted in **Scheme 5**, required coupling of the primary amine **11a** with the appropriate commercially available carboxylic acid analogues (benzofuran-2-carboxylic acid (**24a**), potassium benzo[*d*]oxazole-2-carboxylate (**24b**), 1*H*-indene-2-carboxylic acid (**24c**), 1-methyl-1*H*-indole-2-carboxylic acid (**24d**), benzo[*d*]imidazole-2-carboxylic acid (**24e**), 1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylic acid (**24f**) in the presence of HCTU and DIPEA, to furnish the desired final products (**25a-f**).

To explore the requirement of steric bulk and aromaticity in the tail group, we generated the ring-deleted pyrrole analogue (**26**), as seen in **Scheme 6**. Furthermore, we generated the

D- (**27b**) and L-proline (**27b**) analogues which remove all aromaticity and planarity, and introduces an ionisable nitrogen to the tail group. The synthesis of **26**, as seen in **Scheme 6**, required coupling of amine **11a** with pyrrole-2-carboxylic acid in the presence of EDCI and DMAP in DCM, which afforded **26** in moderate yield. The proline analogues (**27b** and **28b**) were synthesized by coupling of **11a** with commercially supplied *N*-Boc-D- and *N*-Boc-L-proline, respectively, in the presence of HCTU and DIPEA in DMF, and was immediately followed by *N*-Boc deprotection to afford **27b** and **28b**, as shown in **Scheme 6**.

<Insert Scheme 5>

<Insert Scheme 6>

Results and Discussion

All compounds were tested for their ability to bind the D_2R in a competition binding assay using the antagonist [^{3}H]raclopride. For competitive ligands, a value of affinity (K_B) was derived. However, some ligands were unable to completely inhibit the binding of [^{3}H]raclopride even at saturating concentrations, consistent with an allosteric mode of interaction (**Figure 3A, Tables 1-4**). In these cases, data were fit using an allosteric ternary complex to derive a value of affinity, denoted as K_B, and cooperativity with [^{3}H]raclopride (α). However, this assay does not provide information regarding the effect of compounds upon the neurotransmitter dopamine. Therefore, the activity of all compounds was tested in an assay measuring phosphorylation of ERK1/2 through activation of the long isoform of the D_2R ($D_{2L}R$) expressed in FlpIn CHO cells. This assay provides a robust, medium throughput measurement of D_2R activation. Compounds were tested for their ability to antagonize the action of increasing concentrations of dopamine. An example of these data is presented in **Figure 3B**. These data were fit with either a derivation of the operational model of

allosterism[32] (see supplementary information) to derive values of functional affinity (denoted as K_B), and allosteric cooperativity with dopamine (denoted as $\alpha\beta$, a composite parameter where α is negative cooperativity with dopamine binding, and β denotes modulation of dopamine efficacy); a Gaddum-Schild model of competitive antagonism to derive values of functional affinity (K_B); and, a Schild slope, where a Schild slope that is not significantly different from unity (Schild slope = 1) indicates competitive antagonism. For each compound, data were analysed with both models and the best fit determined by an F-test. These data are reported in **Tables 1-4** and are presented as logarithms to allow statistical comparison.[33] Values of Log$\alpha\beta$ < 0 signify negative cooperativity with dopamine. The overall correlation between the values of affinity obtained in the functional and binding assays was good (R^2 = 0.73, Supplementary figure 1). Therefore, because we were most interested in the functional effect of **1** and its analogues upon the action of dopamine, we focus upon these data for structure-activity analysis. The lead compound (**1**) acted as a negative allosteric modulator at the D_2R, with moderate functional affinity (K_B = 776 nM). The limited dextral displacement of the dopamine dose-response curve by increasing concentrations of **1** is characteristic of the action of a negative allosteric modulator, and the value of negative cooperativity (Log$\alpha\beta$ = -1.23 ± 0.14) translates to a maximal 17-fold decrease in dopamine potency (**Figure 3B, Table 1**).

<Insert Tables 1-4>

We first examined modifications to the 7-CTHIQ-containing head group of **1** (**Table 1**). Stemp et al.[12] incorporated the 7-cyano substituent to deactivate the THIQ ring system for the purposes of reducing *in vivo* metabolism, thus increasing oral bioavailability. We determined that replacement of the nitrile group with hydrogen (**12b**) resulted in a significant 9-fold increase in functional affinity (K_B = 87 nM), but no significant change to allosteric cooperativity (Log$\alpha\beta$ = -1.04 ± 0.14) at the D_2R (**Table 1**). To further explore the effect of

atomic size and electronegativity in the 7-position, we generated a series of 7-halogeno analogues of **1**. The 7-fluoro analogue (**12c**) resulted in no significant change in functional affinity (K_B =1.05 μM) compared to **1**, whilst displaying weaker negative allosteric cooperativity (Log$\alpha\beta$ = -0.69 ± 0.10). A chloro substituent at the 7-position (**12d**) had a similar effect to **12c**, with no significant change in functional affinity (K_B = 2.04 μM) and significantly diminished negative allosteric cooperativity (Log$\alpha\beta$ = -0.66 ± 0.10, Table 1) relative to **1**. While incorporation of a 7-bromo group (**12e**) resulted a significant gain in functional affinity (K_B = 138 nM), increasing concentrations of this compound caused a limitless dextral shift of the dopamine dose-response curve that was best fit by a competitive mode of interaction (**Figure 3C**) although the Schild slope was less than unity (**Table 1**). Similarly, incorporation of a trifluoromethyl group at the 7-position (**12f**) resulted in a change in pharmacology from allosteric to competitive antagonism but maintained a functional affinity (K_B = 891 nM) comparable to **1**. These modifications demonstrate that the size and degree of electronegativity of the 7-substituent may be an important determinant of both functional affinity and negative cooperativity. Indeed, a comparison of the van der Waals (vdW) radii[34] of the substituent in the 7-position suggests that smaller substituents are more optimal for maintaining allosteric pharmacology, whereas the inductive effect of the substituent[35] has less relationship to functional affinity or allosteric pharmacology. The 7-fluoro (**12c**) and 7-trifluoromethyl (**12f**) analogues both exhibit a similar inductive effect (σ_I-F = 0.45, σ_I-CF_3 = 0.38), and display similar affinity. However, the smaller fluoro substituent (vdW radius 1.47 Å) displays allosteric pharmacology whereas the larger trifluoromethyl substituent (vdW radius 2.43 Å) is a competitive antagonist. Interestingly, the 7-bromo analogue (**12e**) demonstrates a significantly greater functional affinity compared to the corresponding 7-trifluoromethyl (**12f**) and 7-chloro (**12d**) analogues, yet these substituents have comparable vdW radii. This may result from the greater degree of halogen bonding[36,37]

exhibited by bromine by virtue of its larger positive electrostatic potential compared to chlorine. The ensuing sigma hole has a greater potential to interact with electronegative atoms (such as hydroxyls in Ser, Thr and Tyr) within the orthosteric binding pocket via halogen bonding interactions.[38] It must be noted that to further explain the trends of this data, additional derivatives with substituents on various positions of the THIQ core representing all four quadrants of the Craig plot would be informative.

We next examined the effect of expanding the size of the heterocyclic ring of the THIQ scaffold, without any aromatic substitution, to tetrahydrobenzo[*c*]azepine (**12g**), which resulted in a 3-fold loss of functional affinity (K_B = 2.57 μM) and no significant change in negative allosteric cooperativity (Log$\alpha\beta$ = -0.79 ± 0.12, **Table 2**). We also examined the effect of bioisosteric replacement of the fused benzene ring for thiophene to afford the tetrahydrothieno[3,2-*c*]pyridine (**12h**), which displayed no change in functional affinity (K_B = 339 μM) but significantly reduced negative allosteric cooperativity compared to **12b** (Log $\alpha\beta$ = -0.51 ± 0.10, **Table 2**).

The strategy of 'core hopping' was also investigated, whereby the THIQ scaffold was replaced with other core substructures, (2-methoxyphenyl)piperazine (**12i**) and (2,3-dichlorophenyl)piperazine (**12j**), which are known to be privileged scaffolds for the D_2R.[28,39,40] The incorporated modification to (2-methoxyphenyl)piperazine (**12i**) resulted in the compound with the highest functional affinity of the series (K_B = 0.26 nM), yet displayed competitive pharmacology with dopamine. Similarly, modification to the 1-(2,3-dichlorophenyl)piperazine (**12j**) resulted in high functional affinity (K_B = 1.38 nM), and also behaved competitively (**Table 2**). However, these data were best fit by Schild slope of 0.57 ± 0.12 and a decrease in the maximal effect of dopamine was observed, both indicators of hemiequilibrium conditions.

Together, these results suggest that subtle modifications to the head group can have significant effects on both functional affinity and negative allosteric cooperativity. These observations are not surprising given we, and others, have shown that subtle changes to ligand-receptor interactions in the orthosteric binding site of the D_2R of ligands with similar scaffolds can modulate affinity, subtype selectivity, efficacy and even biased agonism.[18,30,41,42] When the THIQ head group was replaced with 'privileged scaffolds'[28,39,40,43,44] (**12i** and **12j**), we observed a change from allosteric to competitive pharmacology accompanied by an increase in affinity. This increase in affinity is reflected by a comparison of the literature binding affinities of the respective head groups without modification where the THIQ head group displays lower affinity (**9a**, D_2R-K_B = 2.5 μM;[20]) than either of the privileged motifs (**9i**, D_2R-K_B = 0.137 μM;[18] **9j**, D_2R-K_B = 0.680 μM[18]). Furthermore, the competitive rather than allosteric pharmacology of **12i** and **12j** is consistent with a bitopic mode of engagement for **1** at the D_2R, whereby contacts within both the conserved orthosteric and putative allosteric pocket are critical for its pharmacology. Indeed positioning of the secondary pharmacophore within the allosteric binding site may be dependent on the binding orientation of the head group within the orthosteric pocket.

The next molecular feature of **1** that we focused on was the *trans*-cyclohexylene spacer group (**Table 3**). We have previously shown the importance of the spacer group in that modification of stereochemistry around the 1,4-disubstituted cyclohexylene motif from *trans* to *cis* can cause a switch from negative allosteric cooperativity to competitive antagonism.[20] We sought to examine the importance of spacer configuration by installing simple flexible spacers with nonfunctionalized polymethylene extensions. The modification from the *trans*-cyclohexylene spacer group to the 1,3-propylene spacer (**18a**) or a 1,4-butylene spacer (**18b**) resulted in a significant increase in functional affinity (K_B = 138 nM and K_B = 81 nM, respectively) and maintenance in the level of negative allosteric cooperativity relative to **1**

(**Table 3**). Interesingly, incorporation of the 1,5-pentylene spacer (**18c**) displayed functional affinity (K_B = 132 nM) no different from the 1,3-propylene spacer (**18a**), yet displayed competitive pharmacology with dopamine. However, allosteric pharmacology was restored for the 1,6-hexylene-containing analogue (**18d**) (Log$\alpha\beta$ = -1.68 ± 0.35) and was accompanied by a further significant increase in functional affinity (K_B = 30 nM). The use of flexible linkers has also been used to explore the distance between such orthosteric and allosteric sites or even between orthosteric sites within GPCR receptor dimers.[9,11,45,46] Our findings demonstrate that flexibility in the spacer region is beneficial for enhancements to functional affinity relative to the rigid cyclohexylene spacer. This result could be expected given the ability for ligands to occupy two binding sites simultaneously could be more energetically favourable with a flexible linker.[11,47-49] However, it should be noted that no such enhancements in binding affinity were observed. Such discrepancies between binding and functional affinity has been the focus of a number of recent papers.[50,51] Indeed, we observed a similar discrepancy in a series of structurally related biased agonists at the D_2R.[30] Of particular interest, the 1,5-pentylene analogue (**18c**) displays competitive antagonism, whereas analogues containing the 1,3-propylene (**18a**), 1,4-butylene (**18b**) and 1,6-hexylene (**18d**) spacers display negative allosteric cooperativity. Although such linkers are flexible, they possess a degree of conformational constraint such that the odd numbered carbon chain of **18c** may orientate the secondary 1*H*-indole-2-carboxamide pharmacophore into an unfavourable position for interaction with the allosteric binding pocket. This is plausable given we have previously shown that orientation of **1** in the binding pocket requires a linear orientation of the allosteric moiety relative to the head group, and that a different orientation (i.e. from *trans* to *cis* orientation of the 1,4-cyclohexylene spacer) resulted in loss of allosteric pharmacology.[20] Furthermore, previous studies on bitopic ligands have demonstrated a linker-length dependency upon the novel pharmacology conferred by engagement of both

allosteric and orthosteric sites; be it a gain in affinity, subtype selectivity or biased agonism.[9,11]

We next examined the effect of modifying the 1*H*-indole-2-carboxamide tail group of **1**, which we have previously shown to form important interactions with residues in a secondary binding site of the D_2R,[20] and that these interactions are crucial for the determination of negative allosteric cooperativity (**Table 4**). Specifically, we have demonstrated the role of hydrogen bonding interactions between the indolic NH and Glu95$^{2.65}$ as a determinant of negative allosteric cooperativity and functional affinity. Therefore, we synthesized analogues of **1** with variations to the tail group to better examine this effect. The 1-methylindole analogue (**25d**) which, as previously described,[20] can no longer act as a hydrogen bond donor, displayed improved functional affinity for the D_2R (K_B = 72 nM), yet behaved as a competitive antagonist. Given there is another potential hydrogen bond donating nitrogen in the near vicinity, we generated the *N*-methylated carboxamide analogue (**23**), which resulted in improved functional affinity (K_B = 91 nM) as compared to **1** (K_B = 776 nM), and retained negative allosteric cooperativity albeit reduced (Log$\alpha\beta$ = -0.57 ± 0.10). This supported our hypothesis that a hydrogen bond between the indolic NH (and not the amide NH) and Glu95$^{2.65}$ is crucial for negative allosteric cooperativity.

However, because replacement of a hydrogen with a methyl group also introduces extra bulk, we further investigated this hypothesis by examining other tail groups (**Table 4**) that cannot act as hydrogen bond donors yet retain similar steric properties to the lead molecule, **1**. The benzofuran analogue (**25a**) displayed increased functional affinity (K_B = 18 nM) relative to **1**, yet acted as a competitive antagonist, whereas the benzoxazole (**25b**) (also incapable of hydrogen bond donation) demonstrated similar functional affinity (K_B = 18 nM) to **25a**, and was best fit by a model of competitive antagonism. However, it should be noted that in both cases the Schild slope obtained in this analysis was significantly less than unity. The indene

analogue (**25c**), resulting in removal of all heteroatoms in the tail group, caused a significant increase in functional affinity (K_B = 25 nM) relative to **1** and again behaved as a competitive antagonist. These results support the hypothesis that maintaining the hydrogen bonding interaction between the receptor (most likely with the $Glu^{2.65}$ highlighted as important for the allosteric pharmacology of **1**[20]) and the indolic NH moiety is a determining factor for negative allosteric pharmacology. Disturbance of this interaction has shown in all cases to result in both competitive antagonism and a significant increase in functional affinity relative to **1**, confirming the results of our previous study.[20] However, our data also suggests that this hydrogen bonding interaction may make the interaction of the head group with the orthosteric pocket less favourable, thus conferring a loss of affinity for such bitopic ligands.

We subsequently explored further changes to the tail group to examine the effect of modifying the acidity of the indolic NH moiety, and also the lipophilicity and size of the tail group. The benzimidazole analogue (**25e**) presented a tail moiety that is theoretically a stronger hydrogen bond donor than indole (benzimidazole pK_a = 16.4, indole pK_a = 21.0)[52] due to the anionic stabilizing effect of the second additional electronegative nitrogen atom. This modification displayed no significant change in functional affinity (K_B = 174 nM), yet displayed pharmacology consistent with competitive antagonism of dopamine. This was a surprising finding given the previously established importance of hydrogen-bond donating properties on the indolic NH moiety. The 5-membered ring of the benzimidazole system is electron-deficient compared to its electron-rich indole counterpart of **1**, and may govern the level of negative allostery or lack thereof when binding to the secondary binding pocket. Similarly, the benzimidazole NH of **25e** may be in a different relative orientation compared to the indole NH of **1** as a result of the differing alignment of intramolecular dipoles of the respective molecules. This could potentially direct the benzimidazole NH opposite relative to the amide NH and therefore impact on hydrogen-bond donating/accepting interactions with

Glu95$^{2.65}$ in the allosteric binding site. Further experiments are required to elucidate precise reasons for this finding.

Interestingly, the 7-azaindole analogue (**25f**) caused a significant increase in functional affinity (K_B = 23 nM) but with maintenance of the allosteric pharmacology (Log$\alpha\beta$ = -1.39 ± 0.16). The chemical and physical properties of 7-azaindole are considerably different to those of indole[53] and present multiple hydrogen-bonding opportunities, including H-bond donating from two different nitrogen atoms, and multiple H-bond acceptor sites.[54] Given that **25f** displays such high functional affinity whilst retaining allosteric pharmacology, it is reasonable to consider that the unique physical and electronic effects of 7-azaindole allows important hydrogen-bond donating/accepting interactions with residues in the allosteric binding site, including Glu95$^{2.65}$, which promote both high affinity and allosteric pharmacology.

We then explored the pyrrole analogue (**26**) as a ring-deleted analogue of **1** to examine the biological effect of reducing lipophilicity whilst retaining the ability for hydrogen-bond donation, and preserving similar acidity (pyrrole-pK_a = 23.0) to indole (indole-pK_a = 21.0).[52] This resulted in a significantly increased functional affinity (K_B = 3 nM), yet as a competitive antagonist. This finding indicates that the more extensive planar aromatic structures in the allosteric binding pocket may also be crucial for allosteric pharmacology, which has been previously suggested to be important for improving selectivity of compounds for D_2-like receptor subtypes.[14,19] While it would be expected that the larger, more lipophilic indole moiety of **1** would confer higher functional affinity than the pyrrole moiety we observe the opposite pattern. This is in agreement with our previous finding that the acetamide analogue, *N*-((*trans*)-4-(2-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)acetamide (MIPS1059),[20] is a competitive antagonist with significantly higher affinity than **1**.

Finally, we also synthesized the pyrrolidine analogues from D- (**27b**) and L-proline (**28b**). The most striking differences to the pyrrole analogue (**26**) are the introduction of a basic nitrogen capable of protonation at physiological pH, the lack of aromaticity and also planarity. We hypothesized that introduction of the basic nitrogen would (on protonation) result in a strong charge-reinforced hydrogen-bonding interaction with $Glu95^{2.65}$, thus maintaining allosteric pharmacology. However, in both cases of proline analogues, this modification resulted in competitive antagonists with a significant increase in functional affinity (K_B = 27 nM and 46 nM, respectively). There was no significant difference between the D- and L-proline analogues. Given the requirement of aromaticity for allosteric pharmacology previously discussed for the pyrrole analogue (**26**), it is reasonable to consider that the loss of allosteric pharmacology observed for the proline analogues (**27b** and **28b**) is also related to a lack of aromaticity, and reaffirms the importance of size and lipophilicity in the allosteric pocket. In summary, orthosteric and allosteric pharmacology is most sensitive to changes to the tail moiety, where modifications that disrupted its hydrogen-bond donor ability, or its size and lipophilicity, generally resulted in loss of allostery, and a concomitant gain in affinity. However, fine tuning of the tail group was capable of producing compounds with high functional affinity and maintenance of negative allosteric pharmacology, for example the 7-azaindole analogue (**25f**).

It must be acknowledged that, while our experimental approach allowed determination of parameters of both affinity and negative cooperativity for ligands that display allosteric pharmacology, we cannot exclude the possibility that compounds that appear to behave competitively with dopamine and [^{3}H]raclopride, may instead display very high negative cooperativity. Such high negative cooperativity would be indistinguishable from competitive antagonism. Thus, such compounds would not display the partial antagonism of the D_2R of ligands conferred by modest negative cooperativity that, in theory, may be beneficial for the

treatment of the positive symptoms of schizophrenia. Furthermore, it should be noted that **1** has the key attributes of a D_2R competitive ligand. In general the structural modifications that appear to result in competitive behaviour can be reconciled with our previously described mechanism of action for **1** whereby the interaction of the indole moiety of **1** with a secondary pocket between TM2 and TM7 confers allosteric pharmacology.

Finally, we have previously provided evidence that **1** acts as a negative allosteric modulator at a D_2R dimer. This SAR study has given insight into the structural determinants of the allosteric action of **1** at the D_2R and is consistent with the bitopic mode of interaction proposed in our first study. However, it was beyond the scope of this study to confirm the action of such novel ligands at a D_2R dimer. Future studies will focus on gaining further insight into the action of these novel THIQ analogues at D_2R dimers.

Conclusions

We have previously provided evidence that **1** binds in a bitopic manner at one protomer of a D_2R dimer and modulates the binding of dopamine at a second protomer. Furthermore, we discovered that a hydrogen bond between the indolic NH of **1** and $Glu95^{2.65}$ within the secondary pocket is a key ligand-receptor interaction for the allosteric action of **1**. In this study we designed and synthesized analogues of **1** to determine the key ligand features required for allosteric pharmacology, and successfully identified compounds with high affinity and a spectrum of negative cooperativity at the D_2R. The results of this SAR approach further validates the bitopic mode of action for **1**, whereby subtle structural modifications to any component of the scaffold can have significant consequences on the pharmacology of the resulting compound. We focused on synthetic analogues of three main portions of the molecule: the THIQ head group; the *trans*-1,4-cyclohexylene spacer group; and the 1*H*-indole-2-carboxamide tail group. The most sensitive region to chemical

modifications was the tail group, where any disturbance of the indole NH hydrogen-bond donor ability, or size and lipophilicity, resulted in a change from allosteric to apparent competitive pharmacology. The THIQ head group is crucial for maintaining allosteric pharmacology, with the requirement of a 'small' substituent in the 7-position. When the THIQ head group was replaced with higher affinity 'privileged scaffolds' we observed a change from allosteric to competitive pharmacology. We hypothesize that smaller, lower-affinity orthosteric head groups are more favourable for allosteric pharmacology because they allow correct orientation of the tail group within the secondary binding pocket. We also discovered an intriguing relationship between alkyl chain spacer length and allosteric pharmacology, in which the alternating length of the alkyl spacer may change the orientation of the secondary pharmacophore causing a switch from allosteric to competitive pharmacology. Replacement of the *trans*-1,4-cyclohexylene spacer group with a linear 1,4-butylene or 1,6-hexylene spacer group conferred an increase in functional affinity wile maintaining allosteric pharmacology. Finally, replacement of the indole moiety with a 7-azaindole, which presents multiple hydrogen-bond donating and accepting moities, conferred a 30-fold increase in affinity with maintenance of negative cooperativity with dopamine. The findings of this study provide further insight as to the bitopic binding mode of **1** at the D_2R, and present future opportunities for drug design of bitopic and allosteric molecules for the D_2R.

Experimental

General Experimental

All reagents were purchased from Accela ChemBio, Alfa Aesar, AK Scientific, AstaTech, ChemImpex, CombiBlocks, Otava Chemicals or Sigma-Aldrich and used without

purification. GR grade NH_4OH solution (28% aqueous solution), and LR grade MeOH, EtOAc, $CHCl_3$, DCM, Pet. Spirits and MeCN were purchased from Merck and used without further purification. Dry DMF was obtained from an MBraun MB-SPS-800. Davisil® silica gel (40-63μm), for flash column chromatography was supplied by Grace Davison Discovery Sciences (Victoria, Australia) and deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (USA, distributed by Novachem PTY. Ltd, Victoria, Australia).

All organic extracts collected after aqueous work-up procedures were dried over anhydrous Na_2SO_4 before gravity filtering and concentration *in vacuo* at ≤ 40°C (water bath temperature). Purification using preparative layer chromatography (PLC) was carried out on Analtech preparative TLC plates (200 mm x 200 mm x 2 mm).

All ^{1}H NMR and ^{13}C NMR spectra (DEPTQ) were recorded on a Bruker Avance III 400 Ultrashield Plus spectrometer at 400.13 and 100.62 MHz respectively. Results were recorded as follows: chemical shift values are expressed as δ units acquired in $CDCl_3$, or d_6-DMSO when specified, with tetramethylsilane (0.00 ppm) as reference for ^{1}H NMR (residual solvent peak as reference for ^{13}C NMR);[55] multiplicity (singlet (s), doublet (d), triplet (t), pentet (p), quartet (q), broad (br), multiplet (m), doublet of doublets (dd), doublet of triplets (dt), triplet of triplets (tt)); coupling constants (*J*) in Hertz; and, integration. Thin-layer chromatography was conducted on 0.2 mm plates using Merck silica gel 60 F_{254}. Flash column chromatography was performed using Merck Silica Gel 60, 230-400 mesh ASTM, or using automated CombiFlash® Rf 200 with specified solvents. High resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE (TOF) using electrospray ionization (ESI) at a cone voltage of 50 V.

LCMS were run using one of two systems to verify reaction outcome and purity. System A was the default unless otherwise stated. System A - an Agilent 6100 Series Single Quad coupled to an Agilent 1200 Series HPLC using the following buffers; buffer A: 0.1% formic

acid in H_2O; buffer B: 0.1% formic acid in MeCN. The following gradient was used with a Phenomenex Luna 3 μm C8(2) 15 x 4.6 mm column, and a flow rate of 0.5 mL/min and total run time of 12 min; 0–4 min 95% buffer A and 5% buffer B, 4–7 min 0% buffer A and 100% buffer B, 7–12 min 95% buffer A and 5% buffer B. Mass spectra were acquired in positive and negative ion mode with a scan range of 0–1000 *m/z* at 5V. UV detection was carried out at 254 nm. System B - an Agilent 6120 Series Single Quad coupled to an Agilent 1260 Series HPLC. The following buffers were used; buffer A: 0.1% formic acid in H_2O; buffer B: 0.1% formic acid in MeCN. The following gradient was used with a Poroshell 120 EC-C18 50 × 3.0 mm 2.7 μm column, and a flow rate of 0.5 mL/min and total run time of 5 min; 0–1 min 95% buffer A and 5% buffer B, from 1-2.5 min up to 0% buffer A and 100% buffer B, held at this composition until 3.8 min, 3.8–4 min 95% buffer A and 5% buffer B, held until 5 min at this composition. Mass spectra were acquired in positive and negative ion mode with a scan range of 100–1000 *m/z*. UV detection was carried out at 214 and 254 nm. All retention times (t_R) are quoted in min. Analytical reverse-phase HPLC was performed on a Waters HPLC system coupled directly to a photodiode array detector and fitted with a Phenomenex® Luna C8 (2) 100 Å column (150 mm × 4.6 mm, 5 μm) using a binary solvent system; solvent A: 0.1% TFA/H_2O; solvent B: 0.1% TFA/80% CH_3CN/H_2O. Gradient elution was achieved using 100% solvent A to 100% solvent B over 20 min at a flow rate of 1 mL/min. All compounds subjected to biological testing were found to be >95 % pure by HPLC at two wavelengths (λ = 254 and 214 nm).

General Procedure A (Reductive alkylation with *tert*-butyl (*trans*-4-(2-oxoethyl)cyclohexyl)carbamate): The secondary amine (1 eq.) and *tert*-butyl (*trans*-4-(2-oxoethyl)cyclohexyl)carbamate[30] (**8**, 1 eq.) were taken up in 1,2-DCE (10 mL). $NaBH(OAc)_3$ (1.5 eq) was added to the stirred solution at RT under nitrogen. After 16–24 h LCMS

confirmed reaction completion. The mixture was diluted with DCM (15 mL), washed with 1 M K_2CO_3 solution (3 × 20 mL), brine (15 mL), then dried and concentrated *in vacuo.* The crude material was purified by flash column chromatography to give the title compound.

General Procedure B (*N*-Boc deprotection): The *N*-Boc protected amine was taken up in a mixture of DCM (5 mL) and TFA (1 mL) at RT. After 16 h stirring, the solution was diluted with DCM (20 mL) and water (5 mL), followed by dropwise addition of NH_4OH solution (approx. 3 mL or until pH 10). The product was extracted with further DCM (2 × 15 mL), and the combined organic extracts washed with brine (20 mL), dried over Na_2SO_4 and concentrated *in vacuo*.

General Procedure C (N-Boc protection): To a stirred mixture of the amine (1.2 eq.) in DCM (50 mL/g amine) at RT, was slowly added NEt_3 (1.2 eq.), followed by dropwise addition of a solution of Boc_2O (1 eq.) in DCM (5 mL/g Boc_2O). After 1 – 24 h, the reaction was washed with 1 M $KHSO_4$ (3 × 30 mL), brine, dried and concentrated *in vacuo*.

General Procedure D (Activation of alcohols to corresponding methanesulfonate esters): To a solution of the alcohol (1 eq.) in DCM (20 mL) was added NEt_3 (2 eq.). After 5 min, a solution of methanesulfonyl chloride (1.2 eq.) in DCM (5 mL) was added dropwise. After 16 h stirring at RT, the solution was washed with 1 M NaOH (20 mL), 1 M $KHSO_4$ (20 mL), brine, then dried and concentrated *in vacuo*.

General Procedure E (HCTU-mediated amide coupling): The amine (1 eq.) was taken in dry DMF (5-10 mL), and under N_2 at RT was added the carboxylic acid (1.2 eq.) followed by HCTU (1.5 eq.) and DIPEA (5 eq.). After 16 h, the solution was concentrated *in vacuo*, then 2 M $NaHCO_3$ (20 mL) was added, and the product extracted with EtOAc (20 mL). The organic layer was then washed with 2 M $NaHCO_3$ (2 × 20 mL), brine (20 mL), then dried and concentrated *in vacuo*. The product was then purified by flash column chromatography in specified conditions.

2,3,4,5-Tetrahydro-1*H*-benzo[*c*]azepin-1-one (3).[31] 1-Tetralone (**2**) (1.00 g, 6.84 mmol) was taken up in conc. HCl (12 mL) with ice cooling to 0 °C, and sodium azide (889 mg, 13.7 mmol) was slowly added portionwise to maintain 0 °C. After complete addition, the mixture was warmed to RT, then heated at 50 °C for 16 h. The mixture was then poured into ice-cold water (15 mL), and made alkaline with slow addition of 1 M K_2CO_3 to pH 10. The product was then extracted with DCM (3 × 30 mL), and the organic extracts were combined, dried and concentrated *in vacuo*. The product was purified by flash column chromatography (EtOAc) to give pale yellow needles (675 mg, 61%, lit.[31] 52%). ^{1}H NMR δ 7.71 (dd, *J* = 7.5/1.4 Hz, 1H), 7.51 (s, 1H), 7.40 (td, *J* = 7.4/1.6 Hz, 1H), 7.34 (td, *J* = 7.5/1.4 Hz, 1H), 7.19 (dd, *J* = 7.4/0.8 Hz, 1H), 3.13 (q, *J* = 6.5 Hz, 2H), 2.87 (t, *J* = 7.1 Hz, 2H), 2.02 (p, *J* = 6.8 Hz, 2H). ^{13}C NMR δ 174.4 (C), 138.5 (C), 135.2 (C), 131.3 (CH), 128.74 (CH), 128.70 (CH), 127.0 (CH), 39.6 (CH_2), 30.7 (CH_2), 30.4 (CH_2).

2,3,4,5-Tetrahydro-1*H*-benzo[*c*]azepine (4).[56] Lithium aluminium hydride (177 mg, 4.65 mmol) was taken in dry THF (10 mL), and to the stirred solution under N_2 at 0 °C was slowly added a solution of 2,3,4,5-tetrahydro-1*H*-benzo[c]azepin-1-one (**3**) (250 mg, 1.55 mmol) in dry THF (5 mL). The solution was stirred at RT for 30 min, then heated to reflux. After 16 h, the mixture was cooled on ice, and water (15 mL) was slowly added. The solids were filtered through a plug of Celite, and then washed with Et_2O (30 mL). The product was then further extracted with Et_2O (2 × 20 mL), and the combined extracts were dried and concentrated *in vacuo* to reveal the title compound as a clear oil (197 mg, 86%, lit.[56] 60%) which required no further purification. ^{1}H NMR δ 7.17 – 7.07 (m, 4H), 3.92 (s, 2H), 3.24 – 3.15 (m, 2H), 2.98 – 2.89 (m, 2H), 1.76 – 1.66 (m, 2H), 1.41 (s, 1H). ^{13}C NMR δ 143.1 (C), 143.0 (C), 129.3 (CH), 128.4 (CH), 127.1 (CH), 126.1 (CH), 55.3 (CH_2), 53.8 (CH_2), 36.3 (CH_2), 31.1 (CH_2).

***tert*-Butyl ((*trans*)-4-(2-(3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)carbamate (10b).** Following General Procedure A using 1,2,3,4-tetrahydroisoquinoline (**9b**) (82.8 mg,

622 μmol). Flash column chromatography (EtOAc) gave the title compound as a white solid (190 mg, 85% yield). ^{1}H NMR δ 7.16 – 7.05 (m, 3H), 7.04 – 6.97 (m, 1H), 4.39 (br s, 1H), 3.60 (s, 2H), 3.38 (br s, 1H), 2.89 (t, *J* = 5.9 Hz, 2H), 2.71 (t, *J* = 5.9 Hz, 2H), 2.53 – 2.50 (m, 2H), 2.02 – 1.94 (m, 2H), 1.84 – 1.72 (m, 2H), 1.55 – 1.45 (m, 2H), 1.44 (s, 9H), 1.33 – 1.20 (m, 1H), 1.13 – 0.97 (m, 4H). ^{13}C NMR δ 155.4 (C), 134.9 (C), 134.4 (C), 128.7 (CH), 126.7 (CH), 126.2 (CH), 125.7 (CH), 79.1 (C), 56.4 (CH_2), 56.3 (CH_2), 51.1 (CH_2), 50.0 (CH), 35.4 (CH), 34.3 (CH_2), 33.6 (CH_2), 32.1 (CH_2), 29.2 (CH_2), 28.6 (CH_3). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{22}H_{34}N_2O_2$, 359.2693; found 359.2711.

***tert*-Butyl (*trans*-4-(2-(7-fluoro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl) carbamate (10c).** Following General Procedure A using 7-fluoro-1,2,3,4-tetrahydroisoquinoline hydrochloride (**9c**) (231 mg, 1.23 mmol) and DIPEA (429 μL, 2.46 mmol). Flash column chromatography (EtOAc:pet. spirits, 4:1, v/v) gave the title compound as a pale yellow solid (195 mg, 46%). ^{1}H NMR δ 7.03 (dd, *J* = 8.4/5.8 Hz, 1H), 6.81 (td, *J* = 8.5/2.6 Hz, 1H), 6.71 (dd, *J* = 9.4/2.5 Hz, 1H), 4.46 (s, 1H), 3.56 (s, 2H), 3.43 – 3.30 (m, 1H), 2.84 (t, *J* = 5.7 Hz, 2H), 2.69 (t, *J* = 5.9 Hz, 2H), 2.53 – 2.46 (m, 2H), 2.05 – 1.93 (m, 2H), 1.84 – 1.75 (m, 2H), 1.54 – 1.41 (m, 11H), 1.33 – 1.21 (m, 1H), 1.14 – 0.98 (m, 4H). ^{13}C NMR δ 162.2 (C), 159.8 (C), 155.3 (C), 136.8 (C, d, J_{CF} = 7.1 Hz), 130.0 (CH, d, J_{CF} = 7.9 Hz), 129.9 (C, d, J_{CF} = 2.8 Hz), 113.2 (CH, d, J_{CF} = 21.3 Hz), 112.9 (CH, d, J_{CF} = 21.2 Hz), 79.0 (C), 56.17 (CH_2), 56.15 (CH_2), 51.0 (CH_2), 49.9 (CH), 35.3 (CH), 34.2 (CH_2), 33.5 (CH_2), 32.0 (CH_2), 28.5 (CH_3), 28.4 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{22}H_{33}FN_2O_2$, 377.2599; found 377.2604.

***tert*-Butyl (*trans*-4-(2-(7-chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl) carbamate (10d).** Following General Procedure A using 7-chloro-1,2,3,4-tetrahydroisoquinoline (**9d**) (160 mg, 957 μmol). Purification by flash column chromatography (EtOAc:Pet. spirits, 2:1, v/v) gave the title compound as a pale yellow oil

(104 mg, 30%). ^{1}H NMR δ 7.11 (dd, *J* = 8.2/2.1 Hz, 1H), 7.06 – 7.00 (m, 2H), 4.37 (s, 1H), 3.67 (s, 2H), 3.39 (d, *J* = 13.4 Hz, 1H), 2.91 – 2.76 (m, 4H), 2.66 – 2.55 (m, 2H), 2.03 – 1.95 (m, 2H), 1.83 – 1.75 (m, 2H), 1.52 (dd, *J* = 15.4/6.9 Hz, 2H), 1.44 (s, 9H), 1.31 – 1.26 (m, 1H), 1.10 – 1.01 (m, 4H). ^{13}C NMR δ 155.3 (C), 136.8 (C), 132.9 (C), 131.1 (C), 130.0 (CH), 126.5 (CH), 126.3 (CH), 79.1 (C), 56.2 (CH_2), 55.9 (CH_2), 50.8 (CH_2), 50.0 (CH), 35.3 (CH), 34.2 (CH_2), 33.5 (CH_2), 32.1 (CH_2), 28.6 (CH_2), 28.5 (CH_3). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{22}H_{33}ClN_2O_2$, 393.2303; found 393.2306.

***tert*-Butyl (*trans*-4-(2-(7-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl) carbamate (10e).** Following General Procedure A using 7-bromo-1,2,3,4-tetrahydroisoquinoline (**9e**) (203 mg, 957 μmol). Flash column chromatography (EtOAc) gave the title compound as a pale yellow oil (110 mg, 29%). ^{1}H NMR δ 7.23 (dd, *J* = 8.1/2.0 Hz, 1H), 7.17 – 7.14 (m, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 4.37 (s, 1H), 3.56 (s, 2H), 3.37 (s, 1H), 2.82 (t, *J* = 5.9 Hz, 2H), 2.69 (t, *J* = 5.9 Hz, 2H), 2.56 – 2.44 (m, 2H), 2.04 – 1.95 (m, 2H), 1.84 – 1.73 (m, 2H), 1.53 – 1.42 (m, 11H), 1.29 – 1.22 (m, 1H), 1.12 – 0.97 (m, 4H). ^{13}C NMR δ 155.4 (C), 137.3 (C), 133.6 (C), 130.4 (CH), 129.5 (CH), 129.3 (CH), 119.2 (C), 79.1 (C), 56.2 (CH_2), 55.9 (CH_2), 50.8 (CH_2), 50.0 (CH), 35.4 (CH), 34.3 (CH_2), 33.6 (CH_2), 32.1 (CH_2), 28.7 (CH_2), 28.6 (CH_3). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{22}H_{33}BrN_2O_2$, 437.1798; found 437.1803.

***tert*-Butyl (*trans*-4-(2-(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl) cyclohexyl)carbamate (10f).** Following General Procedure A using 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (**9f**) (227 mg, 957 μmol) and DIPEA (333 μL, 1.91 mmol). Flash column chromatography (EtOAc:pet. spirits, 1:1, v/v) gave the title compound as a pale yellow solid (125 mg, 34%). ^{1}H NMR δ 7.36 (d, *J* = 8.0 Hz, 1H), 7.28 (s, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 4.37 (s, 1H), 3.64 (s, 2H), 3.38 (s, 1H), 2.94 (t, *J* = 5.7 Hz, 2H), 2.73 (t, *J* = 5.9 Hz, 2H), 2.59 – 2.48 (m, 2H), 1.98 (s, 2H), 1.85 – 1.75 (m, 2H), 1.54 – 1.42

(m, 11H), 1.33 – 1.26 (m, 1H), 1.14 – 0.96 (m, 4H). ^{13}C NMR δ 155.4 (C), 138.8 (C), 135.8 (C), 129.2 (CH), 128.1 (C, q, J_{CF} = 32.2 Hz), 124.4 (C, q, J_{CF} = 271.8 Hz), 123.6 (CH, q, J_{CF} = 3.8 Hz), 122.9 (CH, q, J_{CF} = 3.7 Hz), 79.1 (C), 56.3 (CH_2), 56.1 (CH_2), 50.7 (CH_2), 50.0 (CH), 35.4 (CH), 34.2 (CH_2), 33.5 (CH_2), 32.1 (CH_2), 29.3 (CH_2), 28.6 (CH_3). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{23}H_{33}F_3N_2O_2$, 427.2567; found 427.2586.

***tert*-Butyl (*trans*-4-(2-(4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)ethyl)cyclohexyl) carbamate (10g).** Following General Procedure A using 2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine (**9g**) (121 mg, 820 μmol). Purification by flash column chromatography (EtOAc:MeOH, 90:10, v/v) gave the title compound as a pale yellow oil (62 mg, 22%). ^{1}H NMR δ 7.21 – 7.06 (m, 4H), 4.35 (s, 1H), 3.90 (s, 2H), 3.34 (s, 1H), 3.17 – 3.06 (m, 2H), 2.95 – 2.84 (m, 2H), 2.40 – 2.31 (m, 2H), 1.99 – 1.91 (m, 2H), 1.75 – 1.65 (m, 4H), 1.47 – 1.35 (m, 11H), 1.22 – 1.11 (m, 1H), 1.09 – 0.90 (m, 4H). ^{13}C NMR δ 155.4 (C), 143.0 (C), 138.9 (C), 130.0 (CH), 129.0 (CH), 127.4 (CH), 126.1 (CH), 79.2 (C), 59.3 (CH_2), 59.0 (CH_2), 50.8 (CH_2), 50.0 (CH), 36.2 (CH_2), 35.2 (CH), 34.3 (CH_2), 33.5 (CH_2), 32.1 (CH_2), 28.6 (CH_3), 24.9 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{23}H_{36}N_2O_2$, 373.2850; found 373.2853.

***tert*-Butyl ((*trans*)-4-(2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)ethyl)cyclohexyl) carbamate (10h).** Following General Procedure A using 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine hydrochloride (**9h**) (160 mg, 912 μmol) and DIPEA (289 μL, 1.66 mmol). Purification by flash column chromatography (EtOAc:Pet. spirits, 1:1, v/v) gave the title compound as a white solid (42 mg, 14%). ^{1}H NMR δ 7.06 (d, J = 5.1 Hz, 1H), 6.71 (d, J = 5.1 Hz, 1H), 4.38 (s, 1H), 3.53 (s, 2H), 3.36 (s, 1H), 2.88 (t, J = 5.5 Hz, 2H), 2.77 (t, J = 5.7 Hz, 2H), 2.57 – 2.50 (m, 2H), 2.04 – 1.94 (m, 2H), 1.82 – 1.73 (m, 2H), 1.52 – 1.42 (m, 11H), 1.30 – 1.24 (m, 1H), 1.15 – 0.99 (m, 4H). ^{13}C NMR δ 155.4 (C), 134.0 (C), 133.6 (C), 125.4 (CH), 122.7 (CH), 79.1 (C), 55.9 (CH_2), 53.3 (CH_2), 51.1 (CH_2), 50.0 (CH), 35.4 (CH),

34.5 (CH_2), 33.6 (CH_2), 32.1 (CH_2), 28.6 (CH_3), 25.6 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{20}H_{32}N_2O_2S$, 365.2257; found 365.2274.

***tert*-Butyl (*trans*-4-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)cyclohexyl)carbamate (10i).** Following General Procedure A using 1-(2-methoxyphenyl)piperazine hydrochloride (**9i**) (284 mg, 1.24 mmol, 2 eq) and DIPEA (0.237 mL, 1.36 mmol, 2.2 eq). Purification by flash column chromatography (MeOH/DCM 0:100 to 5:95) gave the title compound as a white solid (235 mg, 91%). ^{1}H NMR δ 7.00 (ddd, *J* = 7.9/7.1/2.2 Hz, 1H), 6.95 (dd, *J* = 7.9/2.2 Hz, 1H), 6.93 – 6.90 (m, 1H), 6.86 (dd, *J* = 8.0/1.1 Hz, 1H), 4.48 – 4.20 (m, 1H), 3.86 (s, 3H), 3.50 – 2.95 (m, 5H), 2.94 – 2.25 (m, 6H), 2.11 – 1.89 (m, 2H), 1.88 – 1.68 (m, 2H), 1.67 – 1.34 (m, 11H), 1.33 – 1.16 (m, 1H), 1.16 – 0.94 (m, 4H). ^{13}C NMR δ 159.1 (C), 152.4 (C), 139.7 (C), 123.3 (CH), 121.2 (CH), 118.5 (CH), 111.3 (CH), 79.2 (C), 56.8 (CH_2), 55.5 (CH_3), 53.5 (CH_2), 53.5 (CH_2), 50.0 (CH), 35.6 (CH), 34.1 (CH_2), 33.5 (CH_2), 32.1 (CH_2), 28.6 (CH_3). *m/z* MS (TOF ES$^+$) $C_{24}H_{40}N_3O_3$ $[MH]^+$ calculated 418.3; found 418.4. LCMS t_R: 3.21 min (system B).

***tert*-Butyl ((*trans*)-4-(2-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethyl)cyclohexyl) carbamate (10j).** Following General Procedure A using 2,3-dichloropiperazine hydrochloride (**9j**) (332 mg, 1.24 mmol, 2 eq) and DIPEA (0.237 mL, 1.36 mmol, 2.2 eq). Purification by flash column chromatography (MeOH/DCM 0:100 to 5:95) gave the title compound as a white solid (258 mg, 91%). ^{1}H NMR δ 7.23 – 7.09 (m, 2H), 6.97 (dd, *J* = 7.2/2.4 Hz, 1H), 4.53 – 4.13 (m, 1H), 3.54 – 2.24 (m, 11H), 2.11 – 1.90 (m, 2H), 1.90 – 1.69 (m, 2H), 1.70 – 1.36 (m, 11H), 1.36 – 1.17 (m, 1H), 1.18 – 0.92 (m, 4H). ^{13}C NMR δ 155.4 (C), 147.2 (C), 134.2 (C), 127.7 (CH), 125.1 (CH), 124.5 (CH), 118.9 (CH), 79.0 (C), 56.6 (CH_2), 53.3 (CH_2), 53.2 (CH_2), 49.9 (CH), 35.5 (CH), 34.5 (CH_2), 33.49 (CH_2), 32.1 (CH_2), 28.6 (CH_3). *m/z* MS (TOF ES$^+$) $C_{23}H_{36}Cl_2N_3O_2$ $[MH]^+$ calculated 456.2; found 456.3; LCMS t_R: 3.55 min (system B).

(*trans*)-4-(2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine dihydrochloride (11b). Following General Procedure B using *tert*-butyl ((*trans*)-4-(2-(3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)carbamate (**10b**), followed by addition of 1 M HCl in Et_2O. The product was collected by filtration as a white solid (74 mg, 80%). ^{1}H NMR (D_2O) δ 7.64 – 7.54 (m, 3H), 7.51 – 7.49 (m, 1H), 4.84 – 4.79 (m, 1H), 4.54 (d, *J* = 15.5 Hz, 1H), 4.07 – 3.96 (m, 1H), 3.65 – 3.26 (m, 6H), 2.27 (d, *J* = 10.7 Hz, 2H), 2.11 (d, *J* = 11.8 Hz, 2H), 1.99 (dt, *J* = 11.0/5.6 Hz, 2H), 1.70 – 1.55 (m, 3H), 1.45 – 1.26 (m, 2H). ^{13}C NMR (D_2O) δ 132.1 (C), 130.0 (CH), 129.6 (CH), 128.8 (C), 128.4 (CH), 128.0 (CH), 55.3 (CH_2), 53.6 (CH_2), 50.8 (CH), 50.7 (CH_2), 34.6 (CH), 31.01 (CH_2), 30.96 (CH_2), 26.1 (CH_2).

***trans*-4-(2-(7-Fluoro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (11c).** Following General Procedure B using *tert*-butyl (*trans*-4-(2-(7-fluoro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)carbamate (**10c**) gave the title compound as a yellow oil (108 mg, 97%). ^{1}H NMR δ 7.03 (dd, *J* = 8.4/5.8 Hz, 1H), 6.81 (dd, *J* = 8.5/2.7 Hz, 1H), 6.71 (dd, *J* = 9.5/2.6 Hz, 1H), 3.58 (s, 2H), 2.84 (t, *J* = 5.8 Hz, 2H), 2.70 (t, *J* = 5.9 Hz, 2H), 2.64 – 2.56 (m, 1H), 2.54 – 2.47 (m, 2H), 1.90 – 1.82 (m, 2H), 1.82 – 1.73 (m, 2H), 1.52 – 1.37 (m, 4H), 1.31 – 1.22 (m, 1H), 1.13 – 0.94 (m, 4H). ^{13}C NMR δ 162.2 (C), 159.8 (C), 136.9 (CH, d, J_{CF} = 7.1 Hz), 130.1 (C, d, J_{CF} = 7.9 Hz), 113.3 (CH, d, J_{CF} = 21.3 Hz), 113.0 (CH, d, J_{CF} = 21.2 Hz), 56.4 (CH_2), 56.3 (CH_2, d, J_{CF} = 1.8 Hz), 51.1 (CH_2), 50.9 (CH), 36.8 (CH_2), 35.6 (CH), 34.4 (CH_2), 32.3 (CH_2), 28.5 (CH_2).

***trans*-4-(2-(7-Chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (11d).** Following General Procedure B using *tert*-butyl (*trans*-4-(2-(7-chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)carbamate (**10d**) gave the title compound as a yellow oil (62 mg, 85%). ^{1}H NMR δ 7.08 (dd, *J* = 8.2/2.1 Hz, 1H), 7.04 – 6.97 (m, 2H), 3.56 (s, 2H), 2.84 (t, *J* = 5.9 Hz, 2H), 2.69 (t, *J* = 5.9 Hz, 2H), 2.65 – 2.54 (m, 1H), 2.53 – 2.47 (m, 2H), 1.89 – 1.81 (m, 2H), 1.81 – 1.73 (m, 2H), 1.71 – 1.52 (br s, 2H), 1.51 – 1.44 (m, 2H),

1.29 – 1.22 (m, 1H), 1.05 (m, 4H). ^{13}C NMR δ 136.8 (C), 133.0 (C), 131.1 (C), 130.0 (CH), 126.5 (CH), 126.3 (CH), 56.3 (CH_2), 56.0 (CH_2), 50.9 (CH_2), 50.0 (CH), 36.7 (CH_2), 35.5 (CH), 34.4 (CH_2), 32.3 (CH_2), 28.6 (CH_2).

***trans*-4-(2-(7-Bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (11e).** Following General Procedure B using *tert*-butyl (*trans*-4-(2-(7-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)carbamate (**10e**) gave the title compound as a yellow oil (73 mg, 99%). ^{1}H NMR δ 7.22 (dd, J = 8.1/2.0 Hz, 1H), 7.17 – 7.13 (m, 1H), 6.95 (d, J = 8.2 Hz, 1H), 3.56 (s, 2H), 2.82 (t, J = 5.8 Hz, 2H), 2.74 – 2.68 (m, 2H), 2.63 – 2.55 (m, 1H), 2.56 – 2.45 (m, 2H), 1.94 – 1.73 (m, 6H), 1.53 – 1.40 (m, 2H), 1.29 – 1.22 (m, 1H), 1.15 – 0.93 (m, 4H). ^{13}C NMR δ 137.3 (C), 133.5 (C), 130.4 (CH), 129.5 (CH), 129.2 (CH), 119.1 (C), 56.3 (CH_2), 55.8 (CH_2), 50.9 (CH), 50.8 (CH_2), 36.6 (CH_2), 35.5 (CH), 34.4 (CH_2), 32.2 (CH_2), 28.7 (CH_2).

***trans*-4-(2-(7-(Trifluoromethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl) cyclohexanamine (11f).** Following General Procedure B using *tert*-butyl (*trans*-4-(2-(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)carbamate (**10f**) gave the title compound as a yellow oil (74 mg, 81%). ^{1}H NMR δ 7.35 (d, J = 8.0 Hz, 1H), 7.28 (s, J = 2.5 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 3.63 (s, 2H), 2.93 (t, J = 5.8 Hz, 2H), 2.73 (t, J = 5.9 Hz, 2H), 2.64 – 2.50 (m, 3H), 1.91 – 1.81 (m, 2H), 1.81 – 1.71 (m, 2H), 1.53 – 1.44 (m, 2H), 1.44 – 1.32 (m, 2H), 1.31 – 1.21 (m, 1H), 1.13 – 0.95 (m, 4H). ^{13}C NMR δ 138.7 (C), 135.8 (C), 129.1 (CH), 128.0 (C, q, J_{CF} = 32.1 Hz), 124.2 (C, q, J_{CF} = 224 Hz), 123.6 (CH, q, J_{CF} = 3.8 Hz), 122.8 (CH, q, J_{CF} = 3.7 Hz), 56.4 (CH_2), 56.1 (CH_2), 50.9 (CH), 50.7 (CH_2), 36.8 (CH_2), 35.5 (CH), 34.4 (CH_2), 32.3 (CH_2), 29.2 (CH_2).

***trans*-4-(2-(4,5-Dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)ethyl)cyclohexanamine (11g).** Following General Procedure B using *tert*-butyl (*trans*-4-(2-(4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)ethyl)cyclohexyl)carbamate (**10g**) gave the title compound as a yellow oil (44 mg,

99%). ^{1}H NMR δ 7.17 – 7.07 (m, 4H), 3.88 (s, 2H), 3.15 – 3.06 (m, 2H), 2.93 – 2.85 (m, 2H), 2.56 (tt, *J* = 10.8/3.8 Hz, 1H), 2.39 – 2.32 (m, 2H), 1.81 (dd, *J* = 13.9/2.0 Hz, 2H), 1.76 – 1.65 (m, 4H), 1.49 – 1.22 (m, 4H), 1.21 – 1.07 (m, 1H), 1.09 – 0.87 (m, 4H). ^{13}C NMR δ 143.0 (C), 139.4 (C), 129.8 (CH), 128.9 (CH), 127.2 (CH), 125.9 (CH), 59.5 (CH_2), 59.2 (CH_2), 51.2 (CH_2), 50.9 (CH), 36.9 (CH_2), 36.3 (CH_2), 35.4 (CH), 34.7 (CH_2), 32.3 (CH_2), 25.1 (CH_2).

***trans*-4-(2-(6,7-Dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)ethyl)cyclohexanamine (11h).** Following General Procedure B using *tert*-butyl ((*trans*)-4-(2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)ethyl)cyclohexyl)carbamate (**10h**) gave the title compound as a yellow oil (40 mg, 98%). ^{1}H NMR δ 7.06 (d, *J* = 5.1 Hz, 1H), 6.72 (d, *J* = 5.1 Hz, 1H), 3.54 (t, *J* = 1.5 Hz, 2H), 2.89 (t, *J* = 5.6 Hz, 2H), 2.77 (t, *J* = 5.7 Hz, 2H), 2.64 – 2.51 (m, 3H), 1.90 – 1.81 (m, 2H), 1.81 – 1.72 (m, 2H), 1.52 – 1.44 (m, 2H), 1.39 – 1.26 (m, 3H), 1.14 – 0.95 (m, 4H). ^{13}C NMR δ 134.1 (C), 133.6 (C), 125.4 (CH), 122.7 (CH), 56.0 (CH_2), 53.4 (CH_2), 51.2 (CH_2), 50.9 (CH), 36.9 (CH_2), 35.6 (CH), 34.7 (CH_2), 32.3 (CH_2), 25.6 (CH_2).

(*trans*)-4-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)cyclohexan-1-amine trihydrochloride (11i). *tert*-Butyl (*trans*-4-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)cyclohexyl)carbamate (**10i**) (215 mg, 0.51 mmol) was dissolved in MeOH (10 mL) at room temperature, before adding 4 M HCl/1,4-dioxane (5 mL). The mixture was stirred at room temperature for 3 hours, before concentrating under reduced pressure to give 228 mg of white solid (quantitative yield). ^{1}H NMR (d_6-DMSO) δ 11.09 (s, 1H), 8.12 (s, 3H), 7.07 – 6.86 (m, 4H), 3.63 – 3.36 (m, 4H), 3.26 – 3.00 (m, 6H), 3.00 – 2.77 (m, 1H), 1.95 (d, *J* = 10.3 Hz, 2H), 1.77 (d, *J* = 11.9 Hz, 2H), 1.71 – 1.55 (m, 2H), 1.44 – 1.14 (m, 3H), 1.14 – 0.82 (m, 2H); ^{13}C NMR (d_6-DMSO) δ 151.8 (C), 139.4 (C), 123.5 (CH), 120.8 (CH), 118.2 (CH), 112.0 (CH), 55.4 (CH_3), 53.7 (CH_2), 51.0 (CH_2), 49.2 (CH), 46.8 (CH_2), 33.8 (CH), 30.0 (CH_2), 29.9 (CH_2), 29.5 (CH_2).

(*trans*)-4-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)cyclohexan-1-amine trihydrochloride (11j). Following the same conditions as in the synthesis of (*trans*)-4-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)cyclohexan-1-amine trihydrochloride (**11i**), using *tert*-butyl ((*trans*)-4-(2-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethyl)cyclohexyl)carbamate (**10j**) as starting material, gave 227 mg of white solid (95%). ^{1}H NMR (d_6-DMSO) δ 11.30 (s, 1H), 8.11 (d, J = 3.9 Hz, 3H), 7.44 – 7.29 (m, 2H), 7.19 (dd, J = 7.1/ 2.5 Hz, 1H), 3.55 (d, J = 11.4 Hz, 2H), 3.44 – 3.31 (m, 3H), 3.25 (t, J = 11.7 Hz, 2H), 3.19 – 3.02 (m, 3H), 2.92 (d, J = 4.7 Hz, 1H), 1.95 (d, J = 10.4 Hz, 2H), 1.77 (d, J = 11.7 Hz, 2H), 1.72 – 1.57 (m, 2H), 1.47 – 1.17 (m, 3H), 1.14 – 0.86 (m, 2H); ^{13}C NMR (d_6-DMSO) δ 149.6 (C), 132.7 (C), 128.7 (CH), 126.0 (C), 125.3 (CH), 119.8 (CH), 53.6 (CH_2), 51.0 (CH_2), 49.2 (CH), 47.6 (CH_2), 33.8 (CH), 30.0 (CH_2), 29.9 (CH_2), 29.5 (CH_2).

***N*-((*trans*)-4-(2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12b).** Following General Procedure E using (*trans*)-4-(2-(3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (**11b**, 40 mg, 155 μmol) as the amine, and 1*H*-indole-2-carboxylic acid (30 mg, 186 μmol). Purification by flash column chromatography (EtOAc) gave the title compound as a white solid (19 mg, 31%). ^{1}H NMR (d_6-DMSO) δ 11.49 (br s, 1H), 8.17 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.41 (dd, J = 8.2/0.7 Hz, 1H), 7.19 – 6.98 (m, 7H), 3.82 – 3.69 (m, 1H), 3.53 (s, 2H), 2.80 (t, J = 5.7 Hz, 2H), 2.64 (t, J = 5.9 Hz, 2H), 2.49 – 2.44 (m, 2H), 1.93 – 1.77 (m, 4H), 1.46 (dd, J = 14.5/6.8 Hz 2H), 1.41 – 1.25 (m, 3H), 1.06 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.2 (C), 136.3 (C), 135.0 (C), 132.0 (C), 128.4 (C), 127.1 (CH), 126.4 (C), 125.9 (CH), 125.4 (CH), 123.1 (CH), 121.4 (CH), 119.6 (CH), 112.28 (CH), 112.23 (CH), 102.4 (CH), 55.7 (CH_2), 55.4 (CH_2), 50.6 (CH_2), 48.2 (CH), 34.8 (CH), 33.7 (CH_2), 32.3 (CH_2), 31.7 (CH_2), 28.7 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{26}H_{31}N_3O$, 402.2540; found 402.2560.

***N*-(*trans*-4-(2-(7-Fluoro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12c).** Following General Procedure E using (*trans*)-4-(2-(7-fluoro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (**11c**) (88 mg, 318 μmol) as the amine, and 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc) gave the title compound as a white solid (70 mg, 52%). ^{1}H NMR (d_6-DMSO) δ 11.50 (d, J = 1.4 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.42 (dd, J = 8.2/0.8 Hz, 1H), 7.21 – 7.08 (m, 3H), 7.02 (ddd, J = 8.0/7.1/1.0 Hz, 1H), 6.98 – 6.86 (m, 2H), 3.77 (dtt, J = 15.6/8.0/3.9 Hz, 1H), 3.53 (s, 2H), 2.77 (t, J = 5.5 Hz, 2H), 2.64 (t, J = 5.8 Hz, 2H), 2.50 – 2.42 (m, 2H), 1.94 – 1.76 (m, 4H), 1.45 (dd, J = 14.6/6.8 Hz, 2H), 1.41 – 1.23 (m, 4H), 1.14 – 1.00 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 161.4 (C), 160.2 (C), 159.0 (C), 136.3 (C), 132.0 (C), 130.3 (C, d, J_{CF} = 2.6 Hz), 130.1 (CH. d, J_{CF} = 8.0 Hz), 127.1 (C), 123.1 (CH), 121.4 (CH), 119.6 (CH), 113.0 – 112.5 (2 × CH), 112.2 (CH), 102.4 (CH), 55.4 (2 × CH_2), 50.5 (CH_2), 48.2 (CH), 34.8 (CH), 33.7 (CH_2), 32.3 (CH_2), 31.7 (CH_2), 28.0 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{26}H_{30}FN_3O$, 420.2446; found 420.2453.

***N*-(*trans*-4-(2-(7-Chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12d).** Following General Procedure E using (*trans*)-4-(2-(7-chloro-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (**11d**) as the amine, and 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc) gave the title compound as a white solid (42 mg, 43%). ^{1}H NMR (d_6-DMSO) δ 11.50 (d, J = 1.4 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.42 (dd, J = 8.2/0.8 Hz, 1H), 7.20 – 7.09 (m, 5H), 7.02 (ddd, J = 8.0/7.0/0.9 Hz, 1H), 3.76 (dtt, J = 15.2/7.7/3.7 Hz, 1H), 3.53 (s, 2H), 2.78 (t, J = 5.7 Hz, 2H), 2.64 (t, J = 5.8 Hz, 2H), 2.50 – 2.44 (m, 2H), 1.95 – 1.74 (m, 4H), 1.45 (dd, J = 14.5/6.8 Hz, 2H), 1.42 – 1.21 (m, 3H), 1.11 – 1.02 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.2 (C), 137.4 (C), 136.3 (C), 133.3 (C), 132.0 (C), 130.2 (CH), 129.8 (C), 127.1 (C), 126.1 (CH), 125.8 (CH), 123.1 (CH), 121.4 (CH), 119.6 (CH), 112.2 (CH), 102.4 (CH),

55.4 (CH_2), 55.1 (CH_2), 50.2 (CH_2), 48.2 (CH), 34.7 (CH), 33.6 (CH_2), 32.3 (CH_2), 31.7 (CH_2), 28.1 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{26}H_{30}ClN_3O$, 436.2150; found 436.2144.

***N*-(*trans*-4-(2-(7-Bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12e)**. Following General Procedure E using (*trans*)-4-(2-(7-bromo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (**11e**) as the amine, and 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (gradient EtOAc:Pet. spirits, 1:1, v/v, to 100% EtOAc) gave the title compound as a white solid (26 mg, 25%). ^{1}H NMR (d_6-DMSO) δ 11.50 (d, *J* = 1.1 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.42 (dd, *J* = 8.2/0.6 Hz, 1H), 7.33 – 7.22 (m, 2H), 7.21 – 7.13 (m, 2H), 7.09 – 6.99 (m, 2H), 3.76 (dtt, *J* = 15.3/7.7/3.7 Hz, 1H), 3.54 (s, 2H), 2.76 (t, *J* = 5.5 Hz, 2H), 2.64 (t, *J* = 5.6 Hz, 2H), 2.50 – 2.42 (m, 2H), 1.92 – 1.74 (m, 4H), 1.45 (dd, *J* = 14.4/6.9 Hz, 2H), 1.41 – 1.24 (m, 3H), 1.06 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.2 (C), 137.8 (C), 136.3 (C), 133.8 (C), 132.0 (C), 130.6 (CH), 129.0 (CH), 128.7 (CH), 127.1 (C), 123.1 (CH), 121.4 (CH), 119.6 (CH), 118.2 (C), 112.2 (CH), 102.4 (CH), 55.3 (CH_2), 55.0 (CH_2), 50.2 (CH_2), 48.2 (CH), 34.7 (CH), 33.6 (CH_2), 32.3 (CH_2), 31.7 (CH_2), 28.1 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{26}H_{30}BrN_3O$, 480.1645; found 480.1647.

***N*-(*trans*-4-(2-(7-(Trifluoromethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12f).** Following General Procedure E using (*trans*)-4-(2-(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexanamine (**11f**) as the amine, and 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc) gave the title compound as a white solid (84 mg, 79%). ^{1}H NMR (d_6-DMSO) δ 11.51 (s, 1H), 8.18 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.48 – 7.39 (m, 3H), 7.32 (d, *J* = 8.5 Hz, 1H), 7.20 – 7.11 (m, 2H), 7.05 – 6.98 (m, 1H), 3.85 – 3.70 (m, 1H), 3.62 (s, 2H), 2.88 (t, *J* = 5.3 Hz, 2H), 2.67 (t, *J* = 5.8 Hz, 2H), 2.51 – 2.45 (m, 2H), 1.94 – 1.76 (m, 4H), 1.46 (dd, *J* = 14.4/6.8 Hz, 2H), 1.42 – 1.22 (m, 3H), 1.06 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.7 (C),

139.8 (C), 136.8 (C), 136.7 (C), 132.5 (C), 129.8 (CH), 127.5 (C), 126.7 (C, q, J_{CF} = 31.5 Hz), 124.9 (C, q, J_{CF} = 272 Hz), 123.7 (CH, q, J_{CF} = 3.6 Hz), 123.6 (CH), 122.9 (CH, q, J_{CF} = 3.6 Hz), 121.8 (CH), 120.1 (CH), 112.7 (CH), 102.9 (CH), 55.8 (CH_2), 55.6 (CH_2), 50.5 (CH_2), 48.7 (CH), 35.2 (CH), 34.1 (CH), 32.8 (CH_2), 32.2 (CH_2), 29.1 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{27}H_{30}F_3N_3O$, 470.2414; found 470.2420.

***N*-(*trans*-4-(2-(4,5-Dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12g).** Following General Procedure E using (*trans*)-4-(2-(4,5-dihydro-1*H*-benzo[c]azepin-2(3*H*)-yl)ethyl)cyclohexanamine (**11g**) as the amine, and 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc, gradient to EtOAc:MeOH, 4:1, v/v), gave the product as a yellow solid which required further purification. The solid was taken in DMSO (0.5 mL), and water (3 mL) added to the solution. The product was collected by filtration, and washed with water (10 mL) to give the title compound as a white solid (15 mg, 22%). ^{1}H NMR (d_6-DMSO) δ 11.53 (d, *J* = 1.4 Hz, 1H), 8.19 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.42 (dd, *J* = 8.2/0.8 Hz, 1H), 7.20 – 7.06 (m, 6H), 7.02 (ddd, *J* = 8.0/7.0/1.0 Hz, 1H), 3.82 (s, 2H), 3.74 (dtt, *J* = 15.5/7.9/3.8 Hz, 1H), 3.08 – 2.98 (m, 2H), 2.88 – 2.80 (m, 2H), 2.37 – 2.20 (m, 2H), 1.89 – 1.79 (m, 2H), 1.73 (d, *J* = 11.5 Hz, 2H), 1.65 – 1.57 (m, 2H), 1.40 – 1.14 (m, 5H), 1.08 – 0.92 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.2 (C), 142.7 (C), 139.3 (C), 136.3 (C), 132.0 (C), 129.5 (CH), 128.6 (CH), 127.1 (C), 126.9 (CH), 125.6 (CH), 123.1 (CH), 121.4 (CH), 119.6 (CH), 112.2 (CH), 102.5 (CH), 58.5 (CH_2), 58.4 (CH_2), 50.1 (CH_2), 48.2 (CH), 35.4 (CH_2), 34.5(CH), 33.9 (CH_2), 32.3 (CH_2), 31.7 (CH_2), 24.6 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{27}H_{33}N_3O$, 416.2696; found 416.2704.

***N*-(*trans*-4-(2-(6,7-Dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12h).** Following General Procedure E using (*trans*)-4-(2-(6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl)ethyl)cyclohexanamine (**11h**) as the amine, and 1*H*-

indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc) gave the title compound as a white solid (17 mg, 28%). ^{1}H NMR (d_6-DMSO) δ 11.50 (d, J = 1.4 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.42 (dd, J = 8.2/0.7 Hz, 1H), 7.26 (d, J = 5.1 Hz, 1H), 7.20 – 7.12 (m, 2H), 7.02 (ddd, J = 8.0/7.0/0.9 Hz, 1H), 6.79 (d, J = 5.1 Hz, 1H), 3.76 (dtt, J = 15.4/7.8/3.8 Hz, 1H), 3.45 (s, 2H), 2.79 (t, J = 5.1 Hz, 2H), 2.70 (t, J = 5.6 Hz, 2H), 2.54 – 2.51 (m, 2H), 1.95 – 1.70 (m, 4H), 1.45 (dd, J = 14.5/6.8 Hz, 2H), 1.41 – 1.23 (m, 3H), 1.12 – 1.00 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.2 (C), 136.3 (C), 134.2 (C), 132.8 (C), 132.0 (C), 127.1 (C), 125.5 (CH), 123.1 (CH), 122.9 (CH), 121.4 (CH), 119.6 (CH), 112.2 (CH), 102.4 (CH), 55.0 (CH_2), 52.6 (CH_2), 50.6 (CH_2), 48.2 (CH), 34.7 (CH), 33.9 (CH_2), 32.3 (CH_2), 31.7 (CH_2), 25.1 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{24}H_{29}N_3OS$, 408.2104; found 408.2106.

***N*-((*trans*)-4-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12i).** Following General Procedure E using (*trans*)-4-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)cyclohexan-1-amine (**11i**) as the amine and 1*H*-indole-2-carboxylic acid. Flash column chromatography purification (eluent MeOH/DCM 0:100 to 5:95) gave the title compound as a pale brown solid (76mg, 63%). ^{1}H NMR (d_6-DMSO) δ 11.50 (d, J = 1.5 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.41 (dd, J = 8.2/0.8 Hz, 1H), 7.22 – 7.09 (m, 2H), 7.02 (ddd, J = 8.0/7.1/1.0 Hz, 1H), 6.98 – 6.81 (m, 4H), 3.91 – 3.60 (m, 4H), 2.95 (s, 4H), 2.73 – 2.19 (m, 6H), 2.07 – 1.68 (m, 4H), 1.55 – 1.19 (m, 5H), 1.17 – 0.92 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.2 (C), 152.0 (C), 142.0 (C), 136.3 (C), 132.0 (C), 127.1 (C), 123.1 (CH), 122.3 (CH), 121.4 (CH), 120.8 (CH), 119.6 (CH), 117.9 (CH), 112.2 (CH), 111.9 (CH), 102.4 (CH), 55.8 (CH_2), 55.3 (CH_3), 53.1 (CH_2), 50.1 (CH_2), 48.2 (CH), 34.8 (CH), 33.5 (CH_2), 32.3 (CH_2), 31.7 (CH_2). *m/z* MS (TOF ES^+) $C_{28}H_{37}N_4O_2$ $[MH]^+$ calculated 461.3; found 461.3. LCMS t_R: 3.20 min (system B).

***N*-((*trans*)-4-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)cyclohexyl)-1*H*-indole-2-carboxamide (12j).** Following General Procedure E using (*trans*)-4-(2-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethyl)cyclohexan-1-amine (**11j**) as the amine and 1*H*-indole-2-carboxylic acid. Flash column chromatography purification (eluent MeOH/DCM 0:100 to 5:95) gave the title compound as a pale brown solid (93mg, 81%). ^{1}H NMR (d_6-DMSO) δ 11.50 (d, J = 1.5 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H), 7.41 (dd, J = 8.2/0.8 Hz, 1H), 7.36 – 7.25 (m, 2H), 7.21 – 7.08 (m, 3H), 7.02 (ddd, J = 8.0/7.1/0.9 Hz, 1H), 3.75 (ddd, J = 11.7/9.8/6.0 Hz, 1H), 2.98 (s, 4H), 2.72 – 2.43 (m, 4H), 2.38 (t, J = 7.3 Hz, 2H), 2.04 – 1.66 (m, 4H), 1.53 – 1.16 (m, 5H), 1.16 – 0.92 (m, 2H); ^{13}C NMR (d_6-DMSO) δ 160.1 (C), 151.2 (C), 138.3 (C), 136.3 (C), 132.6 (C), 132.0 (C), 128.4 (CH), 127.1 (C), 124.3 (CH), 123.1 (CH), 121.4 (CH), 119.6 (CH), 119.6 (CH), 112.2 (CH), 102.4 (CH), 55.7 (CH_2), 52.9 (CH_2), 51.0 (CH_2), 48.2 (CH), 34.7 (CH), 33.5 (CH_2), 32.3 (CH_2), 31.7 (CH_2). *m/z* MS (TOF ES$^+$) $C_{27}H_{33}N_4O$ $[MH]^+$ calculated 499.2; found 499.2. LCMS t_R: 3.44 min (system B).

***tert*-Butyl (3-bromopropyl)carbamate (14a).** Following General Procedure C using 3-bromopropylamine hydrobromide (**13a**) afforded the title compound as a yellow oil (1.98 g, 91% yield). ^{1}H NMR δ 4.78 (s, 1H), 3.44 (t, J = 6.5 Hz, 2H), 3.27 (q, J = 6.3 Hz, 2H), 2.05 (p, J = 6.5 Hz, 2H) 1.44 (s, 9H). ^{13}C NMR δ 155.9 (C), 85.2 (C), 39.1 (CH_2), 32.8 (CH_2), 30.9 (CH_2), 28.5 (CH_3).

***tert*-Butyl (4-hydroxybutyl)carbamate (14b).** Following General Procedure C using 4-aminobutanol (**13b**) afforded the title compound as a clear oil (283 mg, 93%). ^{1}H NMR δ 4.65 (s, 1H), 3.67 (t, J = 6.0 Hz, 2H), 3.16 (dd, J = 12.1/6.2 Hz, 2H), 1.78 (s, 1H), 1.68 – 1.51 (m, 4H), 1.51 – 1.38 (m, 9H). ^{13}C NMR δ 156.3 (C), 79.3 (C), 62.6 (CH_2), 40.4 (CH_2), 29.9 (CH_2), 28.6 (CH_3), 26.8 (CH_2).

***tert*-Butyl (5-hydroxypentyl)carbamate (14c).** Following General Procedure C using 5-aminopentanol (**13c**) afforded the title compound as a clear oil (440 mg, 47%). ^{1}H NMR δ 4.71 (s, 1H), 3.63 (t, *J* = 6.4 Hz, 2H), 3.12 (dd, *J* = 12.8/6.4 Hz, 2H), 2.30 (s, 1H), 1.62 – 1.54 (m, 2H), 1.54 – 1.46 (m, 2H), 1.45 (d, *J* = 6.9 Hz, 9H), 1.42 – 1.35 (m, 2H). ^{13}C NMR δ 156.2 (C), 79.2 (C), 62.5 (CH_2), 40.5 (CH_2), 32.3 (CH_2), 29.9 (CH_2), 28.5 (CH_3), 23.0 (CH_2).

***tert*-Butyl (6-hydroxyhexyl)carbamate (14d).** Following General Procedure C using 6-aminohexanol (**13d**) afforded the title compound as a clear oil (446 mg, 90%). ^{1}H NMR δ 4.53 (s, 1H), 3.64 (t, *J* = 6.1 Hz, 2H), 3.12 (dd, *J* = 12.9/6.6 Hz, 2H), 1.61 – 1.53 (m, 2H), 1.53 – 1.45 (m, 3H), 1.44 (s, 9H), 1.41 – 1.31 (m, 4H). ^{13}C NMR δ 156.1 (C), 79.1 (C), 62.7 (CH_2), 40.4 (CH_2), 32.6 (CH_2), 30.1 (CH_2), 28.4 (CH_3), 26.4 (CH_2), 25.3 (CH_2).

4-((*tert*-Butoxycarbonyl)amino)butyl methanesulfonate (15b). Following General Procedure D using *tert*-butyl (4-hydroxybutyl)carbamate (**14b**) gave the title compound as a colourless oil (196 mg, 93%). ^{1}H NMR δ 4.66 (br s, 1H), 4.25 (t, *J* = 6.4 Hz, 2H), 3.22 – 3.11 (m, 2H), 3.02 (s, 3H), 1.84 – 1.74 (m, 2H), 1.68 – 1.52 (m, 2H), 1.43 (s, 9H). ^{13}C NMR δ 156.1 (C), 69.7 (CH_2), 39.8 (CH_2), 37.4 (CH_3), 28.5 (CH_3), 26.5 (CH_2), 26.3 (CH_2).

5-((*tert*-Butoxycarbonyl)amino)pentyl methanesulfonate (15c). Following General Procedure D using *tert*-butyl (5-hydroxypentyl)carbamate (**14c**) afforded the title compound as a yellow oil (521 mg, 86%). ^{1}H NMR δ 4.57 (s, 1H), 4.23 (t, *J* = 6.4 Hz, 2H), 3.13 (dd, *J* = 12.8/6.5 Hz, 2H), 3.01 (s, 3H), 1.83 – 1.73 (m, 2H), 1.56 – 1.48 (m, 2H), 1.48 – 1.36 (m, 11H). ^{13}C NMR δ 156.0 (C), 79.2 (C), 69.8 (CH_2), 40.2 (CH_2), 37.4 (CH_3), 29.5 (CH_2), 28.8 (CH_2), 28.4 (CH_3), 22.7 (CH_2).

6-((*tert*-Butoxycarbonyl)amino)hexyl methanesulfonate (15d). Following General Procedure D using *tert*-butyl (6-hydroxyhexyl)carbamate (**14d**) gave the title as a yellow oil (363 mg, 89%). ^{1}H NMR δ 4.56 (br s, 1H), 4.22 (t, *J* = 6.5 Hz, 2H), 3.11 (dd, *J* = 12.9/6.5 Hz, 2H), 3.01 (s, 3H), 1.82 – 1.70 (m, 2H), 1.55 – 1.29 (m, 15H). ^{13}C NMR δ 156.1 (C), 79.2 (C),

70.0 (CH_2), 40.5 (CH_2), 37.5 (CH_3), 30.0 (CH_2), 29.2 (CH_2), 28.5 (CH_3), 26.3 (CH_2), 25.2 (CH_2).

***tert*-Butyl (3-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)carbamate (16a).** To a solution of 1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**9a**) (100 mg, 632 µmol) in MeCN (20 mL) was added *tert*-butyl (3-bromopropyl)carbamate (**14a**) (151 mg, 632 µmol), followed by K_2CO_3 (175 mg, 1.26 mmol), and the mixture was heated at reflux. After 16 h, the solvent was removed *in vacuo*, then the product taken in EtOAc (20 mL) and washed with 1 M K_2CO_3 (2 × 15 mL), brine (15 mL) then dried and concentrated *in vacuo* to give a yellow oil. The product was then purified by CombiFlash® Rf 200 (gradient 0-100% EtOAc in Pet. Spirits, 18 min) to give the product as a colourless oil (82 mg, 41%). ^{1}H NMR δ 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 5.15 (s, 1H), 3.62 (s, 2H), 3.23 (dd, *J* = 12.2/6.0 Hz, 2H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.74 (t, *J* = 5.9 Hz, 2H), 2.59 (t, *J* = 6.8 Hz, 2H), 1.76 (p, *J* = 6.7 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR δ 156.1 (C), 140.4 (C), 136.3 (C), 130.5 (CH), 129.7 (CH), 129.6 (CH), 119.2 (C), 109.5 (C), 79.1 (C), 56.2 (CH_2), 55.8 (CH_2), 50.1 (CH_2), 39.6 (CH_2), 29.5 (CH_2), 28.5 (CH_3), 27.1 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{18}H_{25}N_3O_2$, 316.2020; found 316.2029.

***tert*-Butyl (4-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)carbamate (16b).** To a solution of 4-((*tert*-butoxycarbonyl)amino)butyl methanesulfonate (**15b**) (202 mg, 756 µmol) in DCM (15 mL) was added 1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**9a**) (131 mg, 828 µmol), followed by DIPEA (156 µL, 907 µmol). The solution was heated at reflux for 3 d, then concentrated *in vacuo* and the product purified by flash column chromatography (EtOAc) to give the title compound as a yellow oil (36 mg, 14%). ^{1}H NMR δ 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 4.99 (s, 1H), 3.62 (s, 2H), 3.15 (dd, *J* = 12.2/6.1 Hz, 2H), 2.96 (t, *J* = 5.8 Hz, 2H), 2.74 (t, *J* = 5.9 Hz, 2H), 2.54 (t, *J* = 7.0 Hz, 2H), 1.71 – 1.50 (m, 4H), 1.42 (s, 9H). ^{13}C NMR δ 156.2 (C), 140.4 (C), 136.3 (C), 130.5 (CH),

129.73 (CH), 129.65 (CH), 119.2 (C), 109.5 (CH), 79.1 (C), 57.8 (CH_2), 55.5 (CH_2), 50.3 (CH_2), 40.5 (CH_2), 29.5 (CH_2), 28.5 (CH_3), 28.0 (CH_2), 24.5 (CH_2).

***tert*-Butyl (5-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)pentyl)carbamate (16c).** Following the same conditions as in the synthesis of *tert*-butyl (4-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)carbamate (**16b**), using 5-((*tert*-butoxycarbonyl)amino)pentyl methanesulfonate (**15c**) as starting material, afforded the title compound as a yellow oil (168 mg, 52%). ^{1}H NMR δ 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.33 (s, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 4.65 (s, 1H), 3.66 (s, 2H), 3.12 (dd, *J* = 12.9/6.4 Hz, 2H), 2.97 (t, *J* = 5.8 Hz, 2H), 2.78 (t, *J* = 5.9 Hz, 2H), 2.59 – 2.49 (m, 2H), 1.62 (dt, *J* = 15.1/7.5 Hz, 2H), 1.52 (dt, *J* = 14.7/7.3 Hz, 2H), 1.47 – 1.33 (m, 11H). ^{13}C NMR δ 156.1 (C), 140.1 (C), 135.9 (C), 130.5 (CH), 129.7 (CH), 129.6 (CH), 119.1 (C), 109.5 (C), 79.1 (C), 57.9 (CH_2), 55.3 (CH_2), 50.1 (CH_2), 40.5 (CH_2), 30.0 (CH_2), 29.2 (CH_2), 28.5 (CH_3), 26.5 (CH_2), 24.6 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{20}H_{29}N_3O_2$, 344.2333; found 344.2346.

***tert*-Butyl (6-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl)carbamate (16d).** Following the same conditions as in the synthesis of *tert*-butyl (4-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)carbamate (**16b**), using 6-((*tert*-butoxycarbonyl)amino)hexyl methanesulfonate (**15d**) as starting material. The product was purified using CombiFlash® Rf 200 (gradient 0-70% EtOAc in Pet. Spirits, 20 min) to give the title compound as a pale yellow oil (81 mg, 24%). ^{1}H NMR δ 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 4.52 (s, 1H), 3.61 (s, 2H), 3.12 (dd, *J* = 13.0/6.5 Hz, 2H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.73 (t, *J* = 5.9 Hz, 2H), 2.53 – 2.47 (m, 2H), 1.63 – 1.54 (m, 2H), 1.52 – 1.42 (m, 11H), 1.40 – 1.32 (m, 4H). ^{13}C NMR δ 156.1 (C), 140.5 (C), 136.5 (C), 130.6 (CH), 129.69 (CH), 129.65 (CH), 119.3 (C), 109.5 (C), 79.2 (C), 58.3 (CH_2), 55.7 (CH_2), 50.4 (CH_2), 40.6 (CH_2), 30.2 (CH_2), 29.6 (CH_2), 28.6 (CH_3), 27.3 (CH_2), 27.2 (CH_2), 26.8 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{21}H_{31}N_3O_2$, 358.2489; found 358.2498.

2-(3-Aminopropyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (17a). Following General Procedure B using *tert*-butyl (3-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)carbamate (**16a**) gave the title compound as a yellow oil (30 mg, 46%). ^{1}H NMR δ 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 3.63 (s, 2H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.80 (t, *J* = 6.8 Hz, 2H), 2.74 (t, *J* = 5.9 Hz, 2H), 2.65 – 2.54 (m, 2H), 1.80 – 1.68 (m, 2H), 1.42 (br s, 2H). ^{13}C NMR δ 140.4 (C), 136.4 (C), 130.5 (CH), 129.64 (CH), 129.59 (CH), 119.2 (C), 109.4 (C), 56.1 (CH_2), 55.7 (CH_2), 50.3 (CH_2), 40.7 (CH_2), 30.7 (CH_2), 29.5 (CH_2).

2-(5-Aminopentyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (17c). Following General Procedure B using *tert*-butyl (5-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)pentyl)carbamate (**16c**) gave the title compound as a yellow oil (103 mg, 99%). ^{1}H NMR δ 7.39 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 3.61 (s, 2H), 2.95 (t, *J* = 5.9 Hz, 2H), 2.80 – 2.64 (m, 4H), 2.52 (dd, *J* = 8.4/6.8 Hz, 2H), 1.61 (dt, *J* = 15.1/7.4 Hz, 2H), 1.54 – 1.44 (m, 2H), 1.44 – 1.34 (m, 2H), 1.24 – 1.13 (m, 2H). ^{13}C NMR δ 140.5 (C), 136.5 (C), 130.5 (CH), 129.7 (CH), 129.6 (CH), 119.3 (C), 109.5 (C), 58.3 (CH_2), 55.7 (CH_2), 50.4 (CH_2), 42.3 (CH_2), 33.9 (CH_2), 29.6 (CH_2), 27.1 (CH_2), 24.9 (CH_2).

2-(6-Aminohexyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (17d). Following General Procedure B using *tert*-butyl (6-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl)carbamate (**16d**) gave the title compound as a yellow oil (42 mg, 99%). ^{1}H NMR δ 7.39 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 3.61 (s, 2H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.71 (dt, *J* = 13.8/6.4 Hz, 4H), 2.56 – 2.47 (m, 2H), 1.65 – 1.54 (m, 2H), 1.52 – 1.30 (m, 9H). ^{13}C NMR δ 140.5 (C), 136.5 (C), 130.5 (CH), 129.63 (CH), 129.61 (CH), 119.2 (C), 109.4 (CH), 58.3 (CH_2), 55.7 (CH_2), 50.3 (CH_2), 42.3 (CH_2), 33.8 (CH_2), 29.6 (CH_2), 27.5 (CH_2), 27.2 (CH_2), 26.9 (CH_2).

***N*-(3-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-1*H*-indole-2-carboxamide (18a).** Following General Procedure E using 2-(3-aminopropyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**17a**) as the amine, and 1*H*-indole-2-carboxylic acid. The product was purified using CombiFlash® Rf 200 (gradient 0-100% EtOAc in Pet. Spirits, 21 min) to give the title compound as a white solid (38 mg, 76%). ^{1}H NMR (d_6-DMSO) δ 11.55 (s, 1H), 8.55 (t, *J* = 5.6 Hz, 1H), 7.61 – 7.51 (m, 3H), 7.42 (dd, *J* = 8.2/0.8 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.17 (ddd, *J* = 8.2/7.0/1.1 Hz, 1H), 7.06 – 6.96 (m, 2H), 3.61 (s, 2H), 3.42 – 3.35 (m, 2H), 2.90 (t, *J* = 5.7 Hz, 2H), 2.70 (t, *J* = 5.8 Hz, 2H), 2.56 (t, *J* = 7.0 Hz, 2H), 1.81 (p, *J* = 6.9 Hz, 2H). ^{13}C NMR (d_6-DMSO) δ 161.0 (C), 140.6 (C), 136.6 (C), 136.3 (C), 131.9 (C), 130.4 (CH), 129.7 (CH), 129.5 (CH), 127.1 (C), 123.1 (CH), 121.4 (CH), 119.7 (CH), 119.1 (C), 112.3 (CH), 108.2 (C), 102.1 (CH), 55.2 (CH_2), 54.8 (CH_2), 49.8 (CH_2), 37.4 (CH_2), 29.0 (CH_2), 26.5 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{22}H_{22}N_4O$, 359.1866; found 359.1874.

***N*-(4-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)-1*H*-indole-2-carboxamide (18b).** Following General Procedure B using *tert*-butyl (4-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)butyl)carbamate (**16b**) (36 mg, 109 μmol) gave the deprotected product **17b** which was immediately reacted according to General Procedure E**.** Purification by flash column chromatography ($CHCl_3$:MeOH, 95:5, v/v) gave the product as a white solid (28 mg, 69%). ^{1}H NMR (d_6-DMSO) δ 8.44 (t, *J* = 5.7 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.57 – 7.53 (m, 2H), 7.41 (dq, *J* = 8.3/0.9 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 7.16 (ddd, *J* = 8.2/7.0/1.1 Hz, 1H), 7.08 (dd, *J* = 2.1/0.7 Hz, 1H), 7.02 (ddd, *J* = 8.0/7.0/1.0 Hz, 1H), 3.57 (s, 2H), 3.32 – 3.28 (m, 2H), 2.87 (t, *J* = 6.2 Hz, 2H), 2.66 (t, *J* = 5.8 Hz, 2H), 2.49 – 2.46 (m, 2H), 1.59 (dt, *J* = 6.7/3.5 Hz, 4H). ^{13}C NMR (d_6-DMSO) δ 161.0 (C), 140.7 (C), 136.7 (C), 136.3 (C), 131.9 (C), 130.3 (CH), 129.7 (CH), 129.4 (CH), 127.1 (C), 123.1 (CH), 121.4 (CH), 119.6 (CH), 119.1 (C), 112.3 (CH), 108.2 (C), 102.2 (CH), 57.0 (CH_2), 54.8 (CH_2), 49.7 (CH_2), 38.7

(CH_2), 28.9 (CH_2), 27.1 (CH_2), 23.9 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{23}H_{24}N_4O$, 373.2023; found 373.2022.

***N*-(5-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)pentyl)-1*H*-indole-2-carboxamide (18c).** Following General Procedure E using 2-(5-aminopentyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile **(17c)** as the amine, and 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc, gradient to EtOAc:MeOH, 20:1, v/v) gave the title compound as a pale yellow oil (129 mg, 79%). ^{1}H NMR δ 10.24 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.46 (dd, *J* = 8.3/0.8 Hz, 1H), 7.36 (dd, *J* = 7.9/1.6 Hz, 1H), 7.27 – 7.23 (m, 2H), 7.16 – 7.09 (m, 2H), 6.86 (d, *J* = 1.3 Hz, 1H), 6.60 (t, *J* = 5.8 Hz, 1H), 3.53 (dd, *J* = 12.8/6.4 Hz, 4H), 2.92 – 2.88 (m, 2H), 2.68 (t, *J* = 5.9 Hz, 2H), 2.55 – 2.46 (m, 2H), 1.75 – 1.57 (m, 4H), 1.51 – 1.40 (m, 2H). ^{13}C NMR δ 162.0 (C), 140.4 (C), 136.6 (C), 136.3 (C), 131.0 (C), 130.5 (CH), 129.60 (CH), 129.56 (CH), 127.7 (C), 124.3 (CH), 121.9 (CH), 120.6 (CH), 119.3 (C), 112.2 (CH), 109.3 (C), 102.1 (CH), 58.0 (CH_2), 55.5 (CH_2), 50.2 (CH_2), 39.7 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 26.7 (CH_2), 24.8 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{24}H_{26}N_4O$, 387.2179; found 387.2194.

***N*-(6-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)hexyl)-1*H*-indole-2-carboxamide (18d).** Following General Procedure E using 2-(6-aminohexyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile **(17d)** as the amine, and 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc) gave the title compound as a white wax (36 mg, 45%). ^{1}H NMR δ 9.92 (s, 1H), 7.63 (dd, *J* = 8.0/0.7 Hz, 1H), 7.46 (dd, *J* = 8.3/0.8 Hz, 1H), 7.37 (dd, *J* = 7.9/1.6 Hz, 1H), 7.30 – 7.26 (m, 2H), 7.18 – 7.09 (m, 2H), 6.90 – 6.81 (m, 1H), 6.33 (t, *J* = 5.7 Hz, 1H), 3.56 (s, 2H), 3.52 (dd, *J* = 13.3/7.0 Hz, 2H), 2.91 (t, *J* = 5.8 Hz, 2H), 2.70 (t, *J* = 5.9 Hz, 2H), 2.55 – 2.39 (m, 2H), 1.72 – 1.53 (m, 4H), 1.50 – 1.35 (m, 4H). ^{13}C NMR δ 161.9 (C), 140.5 (C), 136.53 (C), 136.47 (C), 131.0 (C), 130.5 (CH), 129.7 (CH), 129.6 (CH), 127.7 (C), 124.5 (CH), 121.9 (CH), 120.7 (CH), 119.3 (C),

112.2 (CH), 109.4 (C), 101.8 (CH), 58.2 (CH_2), 55.6 (CH_2), 50.3 (CH_2), 39.8 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 27.3 (CH_2), 27.1 (CH_2), 27.0 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{25}H_{28}N_4O$, 401.2336; found 401.2346.

Ethyl 2-(*trans*-4-((*tert*-butoxycarbonyl)(methyl)amino)cyclohexyl)acetate (19). To a solution of ethyl 2-((*trans*)-4-((*tert*-butoxycarbonyl)amino)cyclohexyl)acetate (**7**)[23] (200 mg, 701 μmol) in dry DMF (5 mL) at 0 °C was added sodium hydride, 60% dispersion in mineral oil (280 mg, 7.01 mmol) that had been washed with Pet. spirits (3 × 15 mL). After 30 min, iodomethane (436 μL, 7.01 mmol) was slowly added and the mixture allowed to warm to RT. After 16 h, an additional portion of iodomethane (436 μL, 7.01 mmol) was added, and after 40 h another portion (436 μL, 7.01 mmol). After an additional 24 h, the mixture was diluted with water (20 mL) and the product extracted into EtOAc (2 × 20 mL). The organic extracts were then washed with 1 M K_2CO_3 (2 × 20 mL), brine (2 × 20 mL), dried and concentrated *in vacuo*. The product was then purified by flash column chromatography (Pet. spirits:EtOAc, 4:1, v/v) to give the product as a clear oil (75 mg, 36%). ^{1}H NMR δ 4.12 (q, *J* = 7.1 Hz, 2H), 3.89 (br s, 1H), 2.71 (s, 3H), 2.19 (d, *J* = 7.0 Hz, 2H), 1.88 – 1.77 (m, 2H), 1.77 – 1.63 (m, 3H), 1.55 – 1.37 (m, 11H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.12 (m, 2H). ^{13}C NMR δ 172.9 (C), 155.7 (C), 79.2 (C), 60.3 (CH_2), 53.9 (CH), 41.6 (CH_2), 34.2 (CH), 32.1 (CH_2), 29.6 (CH_2), 28.6 (CH_3), 28.3 (CH_3), 14.4 (CH_3).

***tert*-Butyl methyl(*trans*-4-(2-oxoethyl)cyclohexyl)carbamate (20).** To a solution of ethyl 2-(*trans*-4-((*tert*-butoxycarbonyl)(methyl)amino)cyclohexyl)acetate (**19**) (75 mg, 250 μmol) in degassed toluene (10 mL) with bubbling N_2 at -78 °C was slowly added DIBAL-H, 1 M in toluene (407 μL, 501 μmol). After 30 min, the mixture was quenched with CH_3OH (5 mL) in toluene (10 mL), and warmed to RT with stirring for 15 min. Saturated potassium sodium tartrate solution (10 mL) was added and the mixture stirred vigorously for 30 min. The product was then extracted with Et_2O (3 × 20 mL), and the combined organic extracts washed

with brine (20 mL), dried and concentrated *in vacuo* to give the title compound (64 mg, 100%). ^{1}H NMR δ 9.69 (t, *J* = 2.0 Hz, 1H), 3.97 – 3.77 (m, 1H), 2.65 (s, 3H), 2.26 (dd, *J* = 6.3/2.0 Hz, 2H), 1.83 – 1.71 (m, 3H), 1.69 – 1.57 (m, 2H), 1.50 – 1.28 (m, 11H), 1.11 – 1.01 (m, 2H). ^{13}C NMR δ 202.2 (CH), 155.6 (C), 79.2 (C), 50.7 (CH_2), 32.2 (CH_2), 31.8 (CH), 29.6 (CH_2), 28.5 (CH_3), 28.3 (CH_3).

***tert*-Butyl (*trans*-4-(2-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl) (methyl)carbamate (21)**. Following General Procedure A, using 7-cyano-1,2,3,4-tetrahydroisoquinoline (**9a**) as the amine, and *tert*-butyl methyl(*trans*-4-(2-oxoethyl)cyclohexyl)carbamate (**20**) (1 eq.) as the aldehyde. Flash column chromatography (EtOAc) gave the title compound as a pale yellow oil (37 mg, 37%). ^{1}H NMR δ 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 3.94 (s, 1H), 3.61 (s, 2H), 2.94 (t, *J* = 5.8 Hz, 2H), 2.80 – 2.64 (m, 5H), 2.61 – 2.47 (m, 2H), 2.00 (s, 1H), 1.84 (d, *J* = 12.2 Hz, 2H), 1.74 – 1.63 (m, 2H), 1.55 – 1.33 (m, 12H), 1.33 – 1.18 (m, 1H), 1.14 – 1.04 (m, 2H). ^{13}C NMR δ 155.8 (C), 140.5 (C), 136.4 (C), 130.5 (CH), 129.7 (CH), 129.6 (CH), 119.2 (C), 109.5 (C), 79.2 (C), 56.2 (CH_2), 55.7 (CH_2), 54.2 (CH), 50.4 (CH_2), 35.4 (CH), 34.2 (CH_2), 32.5 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 28.6 (CH_3), 28.3 (CH_3). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{24}H_{35}N_3O_2$, 398.2802; found 398.2814.

***N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-*N*-methyl-1*H*-indole-2-carboxamide (23).** *tert*-Butyl (*trans*-4-(2-(7-cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)(methyl)carbamate (**21**) was treated to conditions in General Procedure B, then the crude product was immediately reacted following General Procedure E, using 1*H*-indole-2-carboxylic acid. Purification by flash column chromatography (EtOAc) gave the product as a white solid (12 mg, 34%). ^{1}H NMR δ 9.40 (s, 1H), 7.66 (d, *J* = 7.5 Hz, 1H), 7.44 (dd, *J* = 8.3/0.8 Hz, 1H), 7.41 (dd, *J* = 7.9/1.5 Hz, 1H), 7.33 (s, 1H), 7.31 – 7.26 (m, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 7.14 (dd, *J* = 11.1/3.9 Hz, 1H), 6.81 (br s, 1H), 4.55 (br s,

1H), 3.63 (s, 2H), 3.31 – 3.02 (m, 2H), 2.96 (t, J = 5.7 Hz, 2H), 2.75 (t, J = 5.8 Hz, 2H), 2.61 – 2.52 (m, 2H), 1.95 – 4.79 (m, 4H), 1.67 (br s, 2H), 1.55 (dd, J = 14.9/6.8 Hz, 2H), 1.40 – 1.18 (m, 4H). HPLC, t_R = 8.17 min, >99% purity. HRMS (*m/z*): $[MH]^+$ calcd. for $C_{28}H_{32}N_4O$, 441.2649; found 441.2659.

***N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)benzofuran-2-carboxamide hydrochloride (25a).** Following General Procedure E using 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) (34 mg, 120 μmol) as the amine, and benzofuran-2-carboxylic acid (**24a**). The product was purified by flash column chromatography ($CHCl_3$:MeOH, 95:5, v/v) to give the product as a yellow oil (18 mg, 35%). For pharmacological testing, the product was converted to the hydrochloride salt by taking up the product in EtOAc (1 mL) and 1 M HCl in Et_2O (1 mL). Removal of solvents revealed the hydrochloride salt as a white solid. ^{1}H NMR (d_6-DMSO) δ 11.12 (s, 1H), 8.54 (d, J = 8.2 Hz, 1H), 7.79 – 7.71 (m, 3H), 7.65 (dd, J = 8.3/0.7 Hz, 1H), 7.55 (d, J = 0.8 Hz, 1H), 7.52 – 7.40 (m, 2H), 7.37 – 7.30 (m, 1H), 4.58 (d, J = 16.0 Hz, 1H), 4.30 (dd, J = 15.6/7.9 Hz, 1H), 3.82 – 3.65 (m, 2H), 3.33 – 3.08 (m, 5H), 1.90 – 1.67 (m, 6H), 1.53 – 1.27 (m, 3H), 1.16 – 1.00 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 157.7 (C), 154.6 (C), 149.8 (C), 138.2 (C), 131.4 (CH), 131.2 (CH), 130.8 (C), 130.3 (CH), 127.7 (C), 127.1 (CH), 124.1 (CH), 123.1 (CH), 119.0 (C), 112.2 (CH), 109.8 (C), 109.7 (CH), 53.9 (CH_2), 51.3 (CH_2), 48.52 (CH_2), 48.48 (CH), 34.7 (CH), 32.2 (CH_2), 31.7 (CH_2), 30.5 (CH_2), 25.6 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{27}H_{29}N_3O_2$, 428.2334; found 428.2329.

***N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl) benzo[*d*]oxazole-2-carboxamide (25b).** Following General Procedure E, using 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) as the amine, and benzoxazole-2-carboxylic acid, potassium salt (**24b**). The product was purified by flash column chromatography ($CHCl_3$:MeOH, 98:2, v/v) to give the title compound as a clear oil

(26 mg, 16%). ^{1}H NMR δ 7.81 – 7.76 (m, 1H), 7.68 – 7.62 (m, 1H), 7.48 (td, *J* = 7.8/1.5 Hz, 1H), 7.45 – 7.40 (m, 2H), 7.34 (s, 1H), 7.21 (d, *J* = 7.9 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 4.02 – 3.93 (m, 1H), 3.67 (s, 2H), 2.99 (t, *J* = 5.4 Hz, 2H), 2.84 – 2.74 (m, 2H), 2.67 – 2.51 (m, 2H), 2.18 – 2.10 (m, 2H), 1.88 (d, *J* = 11.8 Hz, 2H), 1.57 (dd, *J* = 15.0/6.9 Hz, 2H), 1.45 – 1.09 (m, 6H). ^{13}C NMR δ 155.8 (C), 155.0 (C), 151.3 (C), 140.3 (C), 140.0 (C), 136.0 (C), 130.6 (CH), 130.0 (CH), 129.7 (CH), 127.5 (CH), 125.7 (CH), 121.2 (CH), 119.1 (C), 112.0 (CH), 109.8 (C), 56.0 (CH_2), 55.3 (CH_2), 50.2 (CH_2), 49.3 (CH), 35.2 (CH), 33.7 (CH_2), 32.8 (CH_2), 31.8 (CH_2), 29.1 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{26}H_{28}N_4O_2$, 429.2285; found 429.2275.

***N*-(trans-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-indene-2-carboxamide (25c).** Following General Procedure E using 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) as the amine, and indene-2-carboxylic acid (**24c**). Purification by flash column chromatography (EtOAc, gradient to 10:1 EtOAc:MeOH) gave the title compound as a brown solid (28 mg, 37%). ^{1}H NMR δ 7.49 – 7.45 (m, 2H), 7.43 – 7.38 (m, 2H), 7.34 – 7.28 (m, 3H), 7.19 (d, *J* = 7.9 Hz, 1H), 5.74 (d, *J* = 8.1 Hz, 1H), 3.95 – 3.81 (m, 1H), 3.66 (d, *J* = 1.4 Hz, 2H), 3.62 (s, 2H), 2.99 – 2.92 (m, 2H), 2.74 (t, *J* = 5.9 Hz, 2H), 2.59 – 2.50 (m, 2H), 2.09 (d, *J* = 9.9 Hz, 2H), 1.84 (d, *J* = 10.8 Hz, 2H), 1.57 – 1.48 (m, 2H), 1.38 – 1.08 (m, 5H). ^{13}C NMR δ 164.2 (C), 143.9 (C), 143.2 (C), 141.5 (C), 140.5 (C), 136.4 (C), 135.5 (CH), 130.5 (CH), 129.72 (CH), 129.65 (CH), 127.98 (CH), 127.97 (CH), 124.3 (CH), 122.9 (CH), 119.3 (C), 109.5 (C), 56.2 (CH_2), 55.7 (CH_2), 50.4 (CH_2), 48.8 (CH), 38.3 (CH_2), 35.4 (CH), 34.2 (CH_2), 33.3 (CH_2), 32.0 (CH_2), 29.5 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{28}H_{31}N_3O$, 426.2540; found 426.2543.

***N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1-methyl-1*H*-indole-2-carboxamide (25d).** Following General Procedure E using 2-(2-((*trans*)-4-

aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) (52 mg, 183 μmol) as the amine, and 1-methylindole-2-carboxylic acid (**24d**). The product was purified by flash column chromatography ($CHCl_3$:MeOH, 98:2, v/v) to give the product as a white solid (64 mg, 79%). ^{1}H NMR δ 7.66 – 7.56 (m, 1H), 7.44 – 7.35 (m, 2H), 7.35 – 7.27 (m, 2H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.17 – 7.06 (m, 1H), 6.80 (s, 1H), 6.02 (d, *J* = 8.0 Hz, 1H), 4.04 (s, 3H), 3.97 – 3.85 (m, 1H), 3.62 (s, 2H), 2.98 – 2.91 (m, 2H), 2.75 (t, *J* = 5.9 Hz, 2H), 2.61 – 2.50 (m, 2H), 2.18 – 2.07 (m, 2H), 1.92 – 1.81 (m, 2H), 1.53 (dd, *J* = 15.1/7.0 Hz, 2H), 1.41 – 1.31 (m, 1H), 1.31 – 1.08 (m, 4H). ^{13}C NMR δ 162.0 (C), 140.4 (C), 139.1 (C), 132.5 (C), 130.5 (CH), 129.8 (CH), 129.7 (CH), 126.1 (C), 124.1 (CH), 121.8 (CH), 120.6 (CH), 119.3 (C), 110.2 (CH), 109.5 (C), 103.4 (CH), 98.5 (C), 56.2 (CH_2), 55.7 (CH_2), 50.4 (CH_2), 48.9 (CH), 35.4 (CH), 34.1 (CH_2), 33.2 (CH_2), 32.0 (CH_2), 31.6 (CH_3), 29.5 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{28}H_{32}N_4O$, 441.2644; found 441.2648.

***N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-benzo[*d*]imidazole-2-carboxamide (25e).** Following General Procedure E, using 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) as the amine, and 1*H*-benzimidazole-2-carboxylic acid (**24e**). Purification by flash column chromatography ($CHCl_3$:MeOH, 95:5, v/v) gave the product as a yellow solid (67 mg, 58%). For further purification, the solid was taken up in MeOH (1 mL) and the remaining precipitate collected by filtration and washed with MeOH (5 mL) to give a white solid. ^{1}H NMR (d_6-DMSO) δ 8.66 (d, *J* = 8.6 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.59 – 7.49 (m, 3H), 7.34 – 7.20 (m, 3H), 3.85 – 3.71 (m, 1H), 3.57 (s, 2H), 2.88 (t, *J* = 5.4 Hz, 2H), 2.50 – 2.45 (m, 2H), 2.66 (t, *J* = 5.9 Hz, 2H), 1.87 – 1.73 (m, 4H), 1.55 – 1.37 (m, 4H), 1.35 – 1.18 (m, 1H), 1.15 – 0.96 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 157.8 (C), 145.9 (C), 142.5 (C), 140.7 (C), 136.7 (C), 134.4 (C), 130.4 (CH), 129.7 (CH), 129.4 (CH), 124.0 (CH), 122.5 (CH), 119.8 (CH), 119.1 (C), 112.5 (CH), 108.2 (C), 55.3 (CH_2), 54.8 (CH_2), 49.8 (CH_2), 48.3 (CH), 34.4

(CH), 33.6 (CH_2), 31.8 (CH_2), 31.7 (CH_2), 28.9 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{26}H_{29}N_5O$, 428.2445; found 428.2438.

***N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide dihydrochloride (25f).** Following General Procedure E using 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) as the amine, and pyrrolo[2,3-*b*]pyridine-2-carboxylic acid (**24f**). Purification by flash column chromatography (20:1, EtOAc:MeOH) gave the free base as a white solid (18 mg, 24%), which was converted to the hydrochloride salt by dissolving in DMSO (2 mL) and 1 M HCl (1 mL) then lyophilised to dryness. ^{1}H NMR (d_6-DMSO) δ 12.33 (s, 1H), 11.28 (s, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 8.40 – 8.34 (m, 1H), 8.17 (dd, *J* = 7.9/1.4 Hz, 1H), 7.78 – 7.72 (m, 2H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.23 – 7.15 (m, 2H), 4.58 (d, *J* = 15.0 Hz, 1H), 4.30 (dd, *J* = 15.8/7.9 Hz, 1H), 3.82 – 3.73 (m, 3H), 3.41 – 3.06 (m, 5H), 1.98 – 1.69 (m, 6H), 1.47 – 1.32 (m, 3H), 1.16 – 1.04 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 159.4 (C), 146.9 (C), 143.9 (CH), 137.8 (C), 132.9 (C), 131.3 (CH), 130.9 (CH), 130.7 (CH), 130.4 (C), 129.8 (CH), 120.1 (C), 118.6 (C), 116.4 (CH), 109.3 (C), 102.3 (CH), 53.5 (CH_2), 50.8 (CH_2), 48.2 (CH), 48.0 (CH_2), 34.3 (CH), 31.9 (CH_2), 31.3 (CH_2), 31.2 (CH_2), 30.0 (CH_2), 25.2 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{26}H_{29}N_5O$, 428.2445; found 428.2444

***N*-(trans-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl)-1*H*-pyrrole-2-carboxamide (26).** To a solution of 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) (50 mg, 176 μmol) in DCM (15 mL) was added pyrrole-2-carboxylic acid (22 mg, 194 μmol), 4-dimethylaminopyridine (4.3 mg, 35 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (37 mg, 194 μmol), and the brown solution stirred at RT. After 1 h, water (20 mL) was added, then the organic layer washed with 1 M K_2CO_3 (2 × 20 mL), brine (20 mL) and dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by flash column chromatography

(EtOAc, gradient to 20:1, EtOAc:MeOH, v/v) to give **32** as a white solid (27 mg, 41%). ^{1}H NMR δ 9.52 (br s, 1H), 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.33 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 6.90 (td, *J* = 2.7/1.3 Hz, 1H), 6.51 (ddd, *J* = 3.7/2.5/1.3 Hz, 1H), 6.22 (dt, *J* = 3.7/2.6 Hz, 1H), 5.66 (d, *J* = 8.2 Hz, 1H), 3.96 – 3.81 (m, 1H), 3.63 (s, 2H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.75 (t, *J* = 5.8 Hz, 2H), 2.61 – 2.49 (m, 2H), 2.13 – 2.01 (m, 2H), 1.88 – 1.80 (m, 2H), 1.52 (dd, *J* = 15.1/6.8 Hz, 2H), 1.41 – 1.07 (m, 5H). ^{13}C NMR δ 160.4 (C), 140.4 (C), 136.4 (C), 130.6 (CH), 129.8 (CH), 129.7 (CH), 126.4 (C), 121.3 (CH), 119.3 (C), 109.8 (CH), 109.6 (C), 108.3 (CH), 56.2 (CH_2), 55.7 (CH_2), 50.4 (CH_2), 48.6 (CH), 35.4 (CH), 34.1 (CH_2), 33.4 (CH_2), 32.0 (CH_2), 29.5 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{23}H_{28}N_4O$, 377.2336; found 377.2348.

(*R*)-*N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl) pyrrolidine-2-carboxamide (27b). Following General Procedure E using 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) as the amine, and (*R*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid. Purification by flash column chromatography (10:1, EtOAc:MeOH, v/v) afforded **27b** as a pale yellow oil, which was then taken up in DCM (5 mL) and deprotected following General Procedure B. The product was purified by flash column chromatography (7:1:0.1, $CHCl_3$:MeOH:NH_4OH) to give the title compound as a pale yellow oil (18 mg, 15%). ^{1}H NMR δ 7.43 (d, *J* = 8.6 Hz, 1H), 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 3.72 – 3.64 (m, 2H), 3.60 (s, 2H), 3.04 – 2.82 (m, 4H), 2.72 (t, *J* = 5.9 Hz, 2H), 2.58 – 2.47 (m, 2H), 2.16 – 2.06 (m, 1H), 1.99 – 1.83 (m, 4H), 1.83 – 1.75 (m, 2H), 1.73 – 1.64 (m, 2H), 1.50 (dd, *J* = 15.1/6.9 Hz, 2H), 1.33 – 1.26 (m, 1H), 1.21 – 1.06 (m, 4H). ^{13}C NMR δ 174.4 (C), 140.5 (C), 136.5 (C), 130.5 (CH), 129.7 (CH), 129.6 (CH), 119.3 (C), 109.5 (CH), 60.7 (CH), 56.2 (CH_2), 55.8 (CH_2), 50.4 (CH_2), 47.9 (CH), 47.4 (CH_2), 35.4 (CH), 34.2 (CH_2), 33.2 (CH_2)*, 33.1 (CH_2)*, 32.04

(CH_2)*, 32.01 (CH_2)*, 30.9 (CH_2), 29.6 (CH_2), 26.3 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{23}H_{32}N_4O$, 381.2649; found 381.2656. *Extra signals due to different rotameric forms.

(*S*)-*N*-(*trans*-4-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)cyclohexyl) pyrrolidine-2-carboxamide (28b). Following General Procedure E using 2-(2-((*trans*)-4-aminocyclohexyl)ethyl)-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (**11a**) as the amine, and (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid. Purification by flash column chromatography (10:1, EtOAc:MeOH, v/v) afforded **28b** as a pale yellow oil, which was then taken up in DCM (5 mL) and deprotected following General Procedure B. The product was purified by flash column chromatography (20:1:0.01, $CHCl_3$:MeOH:NH_4OH to 5:1:0.1 $CHCl_3$:MeOH:NH_4OH) to give the title compound as a pale yellow oil (22 mg, 23%). ^1H NMR δ 7.46 (d, *J* = 8.6 Hz, 1H), 7.40 (dd, *J* = 7.9/1.6 Hz, 1H), 7.32 (s, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 3.74 (dd, *J* = 9.1/5.4 Hz, 1H), 3.70 – 3.61 (m, 1H), 3.60 (s, 2H), 3.06 – 2.98 (m, 1H), 2.98 – 2.85 (m, 3H), 2.72 (t, *J* = 5.9 Hz, 2H), 2.58 – 2.50 (m, 2H), 2.25 – 2.10 (m, 2H), 1.98 – 1.85 (m, 3H), 1.84 – 1.76 (m, 2H), 1.76 – 1.65 (m, 2H), 1.50 (dd, *J* = 15.2/6.9 Hz, 2H), 1.36 – 1.23 (m, 1H), 1.23 – 1.05 (m, 4H). ^{13}C NMR δ 174.0 (C), 140.5 (C), 136.5 (C), 130.5 (CH), 129.7 (CH), 129.7 (CH), 119.3 (C), 109.5 (C), 60.7 (CH), 56.2 (CH_2), 55.8 (CH_2), 50.4 (CH_2), 48.0 (CH), 47.3 (CH_2), 35.4 (CH), 34.2 (CH_2), 33.3 (CH_2)*, 33.1 (CH_2)*, 32.04 (CH_2)*, 32.00 (CH_2)*, 31.0 (CH_2), 29.6 (CH_2), 26.2 (CH_2). HRMS (*m/z*): $[MH]^+$ calcd. for $C_{23}H_{32}N_4O$, 381.2649; found 381.2658. *Extra signals due to different rotameric forms.

Pharmacological Characterization

Cell Lines and Transfection: FlpIn CHO cells (Invitrogen, Carlsbad, USA) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and maintained at 37 °C in a humidified incubator containing 5% CO_2. The FlpIn CHO cells were transfected with the pOG44 vector encoding Flp recombinase and the pDEST vector encoding the wild-type long isoform of the human D_2 receptor ($D_{2L}R$) at a ratio of 9:1 using

polyethylenimine as transfection reagent. 24 h after transfection the cells were subcultured and the medium was supplemented with 700µg/ml HygroGold as selection agent. Cells were grown and maintained in DMEM containing 20 mM HEPES, 5% fetal bovine serum and 200 µg/mL Hygromycin-B. Cells were maintained at 37 °C in a humidified incubator containing 5% CO_2, 95% O_2. For radioligand binding assays when cells were approximately 90% confluent, they were harvested and centrifuged (300 g, 3 min). The resulting pellet was resuspended in assay buffer (20 mM HEPES, 6 mM $MgCl_2$, 1 mM EGTA, 1 mM EDTA; pH 7.4), and the centrifugation procedure repeated. The intact cell pellet was then resuspended in assay buffer and homogenised using a Polytron homogeniser for three 10-second intervals on the maximum setting, with 30-second periods on ice between each burst. The homogenate was made up to 30 mL and centrifuged (350 g, 5 min, 4 °C), the pellet discarded and the supernatant recentrifuged at 30,000 g for 1 hour at 4 °C. The resulting pellet was resuspended in 5 mL assay buffer and the protein content determined using the method of Bradford. The homogenate was then separated into 1 mL aliquots and stored frozen at -80 °C until required for binding assays.

[^{3}H]raclopride binding assay: Cell membranes (D_{2L}-Flp-In CHO, 10 µg) were incubated with varying concentrations of test compound in binding buffer (20 mM HEPES, 100 mM NaCl, 6 mM $MgCl_2$, 1 mM EGTA, 1 mM EDTA; pH 7.4) containing 0.5 nM of [^{3}H]raclopride to a final volume of 0.3 mL and incubated at 37 °C for 3 h. Binding was terminated by fast flow filtration over GF/B membrane unifilter plates (Perkin Elmer) using a Uniplate 96-well harvester (Perkin Elmer) followed by five washes with ice-cold 0.9% NaCl. Bound radioactivity was measured in a MicroBeta^2 LumiJET microbeta counter (Perkin Elmer).

ERK1/2 phosphorylation assay: FlpIn CHO cells stably expressing the $D_{2L}R$ were seeded into 96-well plates at a density of 50,000 cells/well. After 5-7 h, cells were washed with phosphate-buffered saline (PBS) and incubated in serum-free DMEM overnight before assaying. Initially, time-course experiments were conducted at least twice for each ligand to determine the time required to maximally promote ERK1/2 phosphorylation via the dopamine $D_{2L}R$. Concentration-response experiments in the absence or presence of ligand were performed at 37 °C in a 200 µL total volume of DMEM containing 20 mM HEPES and 0.1 % ascorbic acid. Concentration-response stimulation or inhibition curves were generated by exposure of the cells to antagonist ligand for 30 min and then dopamine for 5 min. Stimulation of cells was terminated by the removal of media and the addition of 100 µL of SureFire™ lysis buffer to each well. The plate was agitated for 1-2 min. A 4:1 v/v dilution of Lysate:SureFire™ activation buffer was made in a total volume of 50 µL. A 1:100:120 v/v dilution of AlphaScreen™ beads:activated lysate mixture:SureFire™ reaction buffer in an 11 µL total volume was then transferred to a white opaque 384-well Proxiplate™ in the dark. This plate was then incubated in the dark at 37 °C for 1.5 h after which time the fluorescence signal was measured by a Fusion-TM plate reader (PerkinElmer), using standard AlphaScreen™ settings. Data were normalized to the response generated by 10% fetal bovine serum.

Data Analysis:

Radioligand binding data: Competition-binding curves between [^{3}H]raclopride and **1** could be fit to the allosteric ternary complex model using the following equation[6]:

$$Y = \frac{\frac{[A]}{K_A}}{\frac{[A]}{K_A} + \left(\frac{1+\frac{[B]}{K_B}}{1+\alpha\frac{[B]}{K_B}}\right)} \quad (1)$$

Where Y is percentage (vehicle control) binding; [A] and [B] are the concentrations of [^{3}H]raclopride and **1**, respectively; K_A and K_B are the equilibrium dissociation constants of [^{3}H]raclopride and **1**, respectively; α is the cooperativity between **1** and [^{3}H]raclopride Values of α >1 denote positive cooperativity; values <1 (but >0) denote negative cooperativity, and values = 1 denote neutral cooperativity.

Functional data: A logistic equation of competitive agonist-antagonist interaction was globally fitted to data from functional experiments measuring the interaction between dopamine and all analogues of **1**:

$$Response = Bottom + \frac{(E_{max} - Bottom)}{1+\left(\frac{10^{-pEC_{50}}\left[1+\left(\frac{[B]}{10^{-pA_2}}\right)\right]^s}{[A]}\right)^{nH}} \quad (2)$$

Where s represents the Schild slope for the antagonist, and pA_2 represents the negative logarithm of the molar concentration of antagonist that makes it necessary to double the concentration of agonist needed to elicit the original submaximal response obtained in the absence of antagonist.

Functional data describing the interaction between all **1** analogues and dopamine analyzed according to the allosteric ternary complex model.

$$E = \frac{E_m.[A]^{nH}}{[A]^{nH} + [EC_{50}]^{nH}\left(\frac{1+\frac{[B]}{K_B}}{1+\frac{\alpha\beta[B]}{K_B}}\right)} \quad (3)$$

Where E_m is the maximum possible cellular response, [A] and [B] are the concentrations of orthosteric and allosteric ligands, respectively, and K_B are the equilibrium dissociation constant of the orthosteric and allosteric ligands, αβ is a composite cooperativity parameter between the orthosteric and allosteric ligand that includes effects upon othosteric ligand affinity and efficacy and nH is the Hill slope of the orthosteric agonist concentration-response curve. Values of α and/or β greater than 1 denote allosteric potentiation, whereas values less than 1 (but greater than 0) denote allosteric inhibition.

For each of the compounds the two equations (models) were then compared for their fit using an extra-sum-of-squares F test, whereby the simpler model was selected unless the P value was less than 0.05.

Supporting Information

Additional tables of pharmacological data are available free of charge via the Internet at http://pubs.acs.org.

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Notes

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Abbreviation List

2-MPP - 1-(2-methoxyphenyl)piperazine

2,3-DCPP - 1-(2,3-dichlorophenyl)piperazine

D_2R – dopamine D_2 receptor

DA – dopamine

DIPEA - *N,N*-diisopropylethylamine

HCTU - *O*-(6-chlorobenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate

THIQ - tetrahydroisoquinoline

TM - transmembrane

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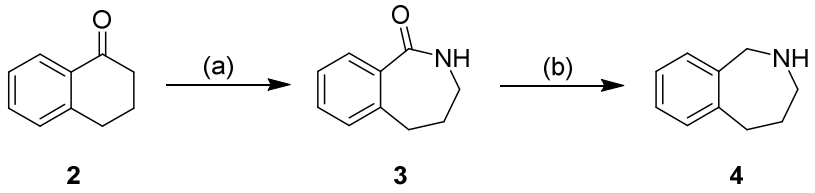
Schemes

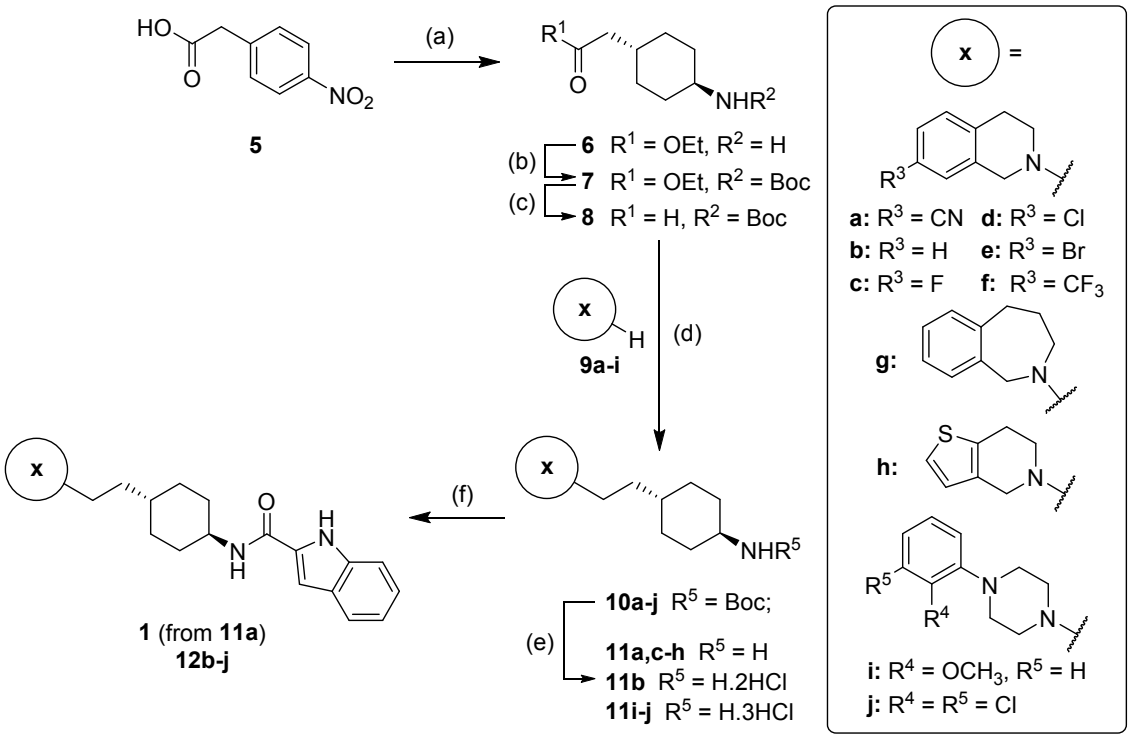
Scheme 1. Synthesis of 2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine.[a]



2 **3** **4**

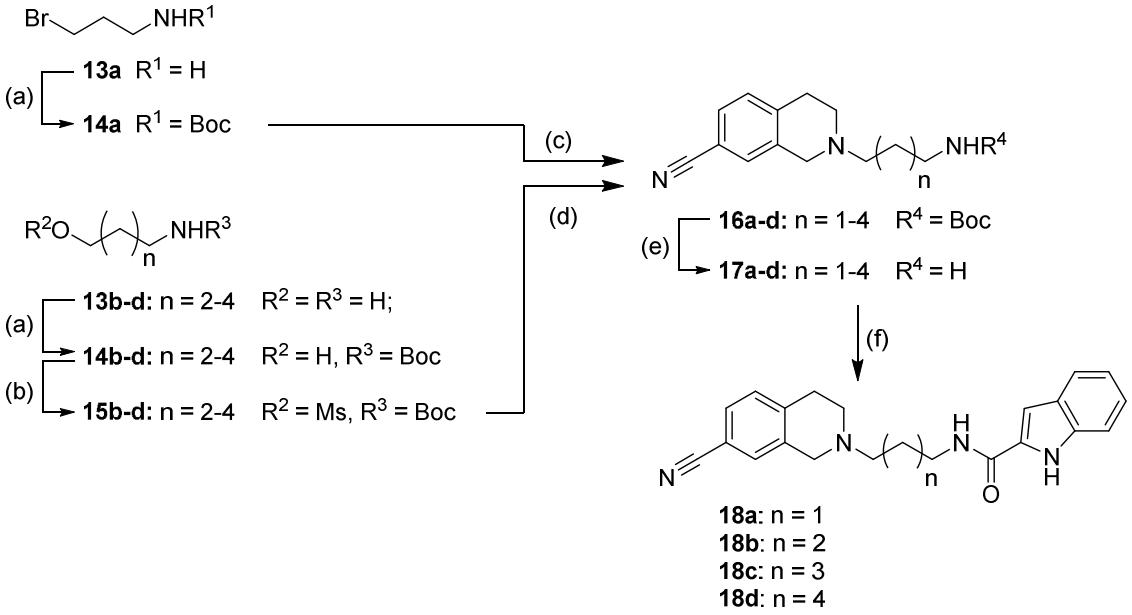
[a]Reagents and conditions: (a) concentrated HCl, NaN_3, 0-50 °C, 16 h, basic workup, 61%; (b) $LiAlH_4$, THF, reflux, 16 h, 86%.

Scheme 2. Synthesis of the *trans*-cyclohexylene spacer unit and compounds with modifications to the 7-CTHIQ head group.[a]



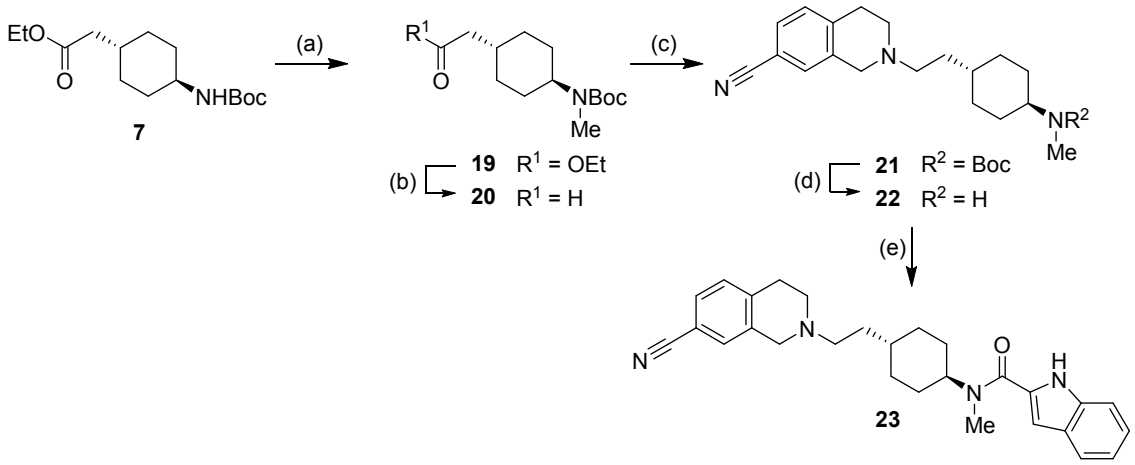
[a]Reagents and conditions: (a) 10% Pd/C, H_2, 60 psi, rt, 3 d, then; EtOH, conc. HCl, reflux, 2 h, 81%; (b) Boc_2O, NEt_3, DCM, rt, 2 h, 96%; (c) DIBALH, toluene, −78 °C, 30 min, 99%; (d) $NaBH(OAc)_3$, 1,2-DCE, RT, 16-24 h, 14-91%; (e) TFA, DCM, RT, 16 h, followed by NH_4OH, 80-99% (**11a, c-h** as free base; **11b** as dihydrochloride salt following acidification with HCl/Et_2O) and 4 M HCl/1,4-dioxane, rt, 95-100% (**11i-j** as trihydrochlride salt); (f) 1*H*-indole-2-carboxylic acid, HCTU, DIPEA, DMF, RT, 16 h, 22-81%.

Scheme 3. Synthesis of **1** derivatives (**18a-d**) with polymethylene spacer units.[a]



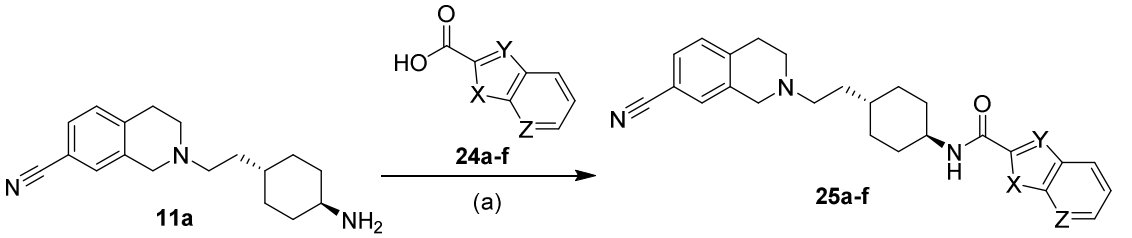
[a]Reagents and conditions: (a) Boc_2O, Et_3N, DCM, RT, 24 h, 47–93%; (b) MsCl, Et_3N, DCM, 16 h, RT, 86–93%; (c) **9a**, K_2CO_3, MeCN, reflux, 16 h, 41%; (d) **9a**, NEt_3, DCM, RT, 16 h, 14–52%; (e) TFA, DCM, RT, 2-16h, followed by NH_4OH, 46-99%; (f) 1*H*-indole-2-carboxylic acid, HCTU, DIPEA, DMF, RT, 16 h, 45–79%.

Scheme 4. Synthesis of the *N*-methylated indole-2-carboxamide analogue of **1** (**23**).[a]



[a]Reagents and conditions: (a) MeI, NaH, DMF, 0 °C, 3d, 36%; (b) DIBAL-H, PhMe, -78 °C, 100%; (c) **9a**, $NaBH(OAc)_3$, 1,2-DCE, RT, 37%; (d) TFA, DCM, RT, 16 h, followed by NH_4OH, 98%; (e) indole-2-carboxylic acid, HCTU, DIPEA, DMF, RT, 16 h, 34%.

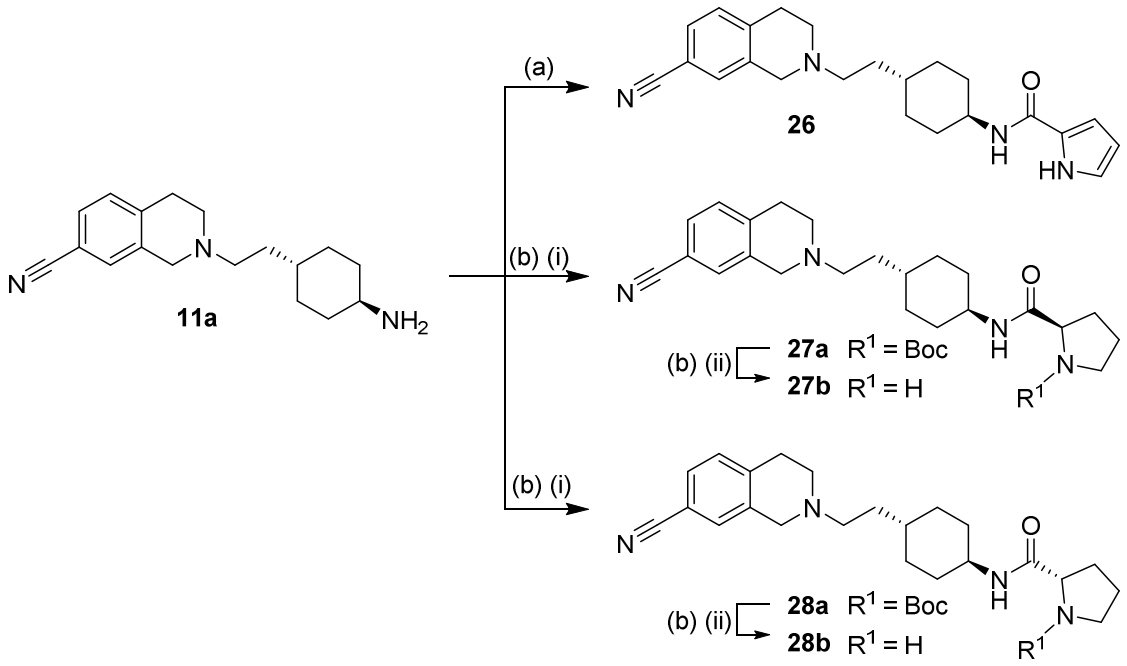
Scheme 5. Synthesis of analogues of **1** with modifications to the hydrogen-bond donating ability of the 1*H*-indole-2-carboxamide tail group (**25a-f**).[a]



a: X=O, Y=CH, Z=CH **b**: X=O, Y=N, Z=CH
c: X=CH_2, Y=CH, Z=CH **d**: X=NCH_3, Y=CH, Z=CH
e: X=NH, Y=N, Z=CH **f**: X=NH, Y=CH, Z=N

[a]Reagents and conditions: (a) benzofuran-2-carboxylic acid (**24a**), benzoxazole-2-carboxylic acid potassium salt (**24b**), indene-2-carboxylic acid (**24c**), 1-methylindole-2-carboxylic acid (**24d**), 1*H*-benzimidazole-2-carboxylic acid (**24e**), pyrrolo[2,3-*b*]pyridine-2-carboxylic acid (**24f**), HCTU, DIPEA, DMF, RT, 16 h, 16-79%.

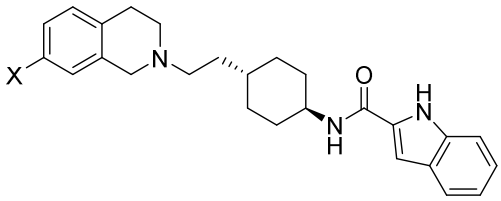
Scheme 6. Synthesis of the ring-deleted, pyrrole analogue of **1** (**26**), and the D- (**27b**) and L-proline (**28b**) analogues of **1** to investigate the impact of steric bulk and ionization on the tail group.[a]



[a]Reagents and conditions: (a) pyrrole-2-carboxylic acid, EDCI, DMAP, DCM, RT, 1 h, 41%; (b) (i) *N*-Boc-D-proline (for **27a**) or *N*-Boc-L-proline (for **28b**), HCTU, DIPEA, DMF, RT, 16 h; (ii) TFA, DCM, RT, 16 h, followed by NH_4OH, 15-23%.

Tables and captions

Table 1. Functional Parameters for Analogues of 1 with chemical modifications to the 7-cyano head group



		Radioligand binding: [^{3}H]raclopride			pERK1/2 phosphorylation			
Compound	**X =**	**pK_B[a] (K_B, nM)**	**Logα[b] (α)**	**n**	**pK_B[c] (K_B, nM)**	**Log$\alpha\beta$[d] ($\alpha\beta$)**	**Schild slope[e]**	**n**
1	-CN	6.45 ± 0.02 (355)	-0.68 ± 0.15 (0.21)	3	6.11 ± 0.06 (776)	-1.23 ± 0.14 (0.059)	n/a	6
12b	-H	6.53 ± 0.14 (295)	-1.35 ± 0.06* (0.045)	3	7.06 ± 0.07* (87)	-1.04 ± 0.14 (0.091)	n/a	3
12c	-F	6.61 ± 0.08 (245)	-0.95 ± 0.06 (0.11)	3	5.98 ± 0.14 (1047)	-0.69 ± 0.10* (0.20)	n/a	3
12d	-Cl	6.83 ± 0.24 (148)	-0.85 ± 0.06 (0.14)	3	5.68 ± 0.22 (2090)	-0.66 ± 0.10* (0.22)	n/a	4
12e	-Br	6.77 ± 0.16 (170)	n/a	3	6.86 ± 0.07* (138)	n/a	0.64 ± 0.05	3
12f	-CF_3	6.12 ± 0.15 (759)	n/a	3	6.05 ± 0.17 (891)	n/a	0.74 ± 0.11	3

[a] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined by radioligand binding.

[b] Estimate of the logarithm of the net cooperativity factor between the modulator and [^{3}H]raclopride ± standard error of the mean (s.e.m.) determined by radioligand binding.

[c] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay

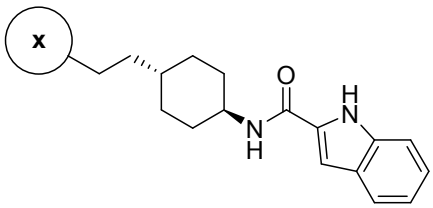
[d] Estimate of the logarithm of the net cooperativity factor between the modulator and dopamine ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay

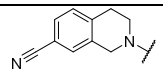
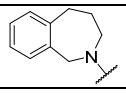
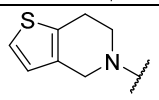
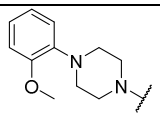
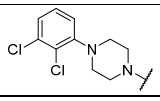
[e] Schild slope ± standard error of the mean (s.e.m.)

n/a compound displayed competitive pharmacology with [^{3}H] racloproide and/or dopamine therefore no value of cooperativity factor was derived.

* Statistically different from corresponding **1** parameter ($p < 0.05$, one-way ANOVA, Dunnett's post-hoc test)

Table 2. Functional Parameters for Analogues of 1 with chemical modifications to the THIQ orthosteric core



		Radioligand binding: [^{3}H]raclopride			pERK1/2 phosphorylation			
Compound	**X =**	**pK_B[a] (K_B, nM)**	**Logα[b] (α)**	**n**	**pK_B[c] (K_B, nM)**	**Log$\alpha\beta$[d] ($\alpha\beta$)**	**Schild slope[e]**	**n**
1		6.45 ± 0.02 (355)	-0.68 ± 0.15 (0.21)	3	6.11 ± 0.06 (776)	-1.23 ± 0.14 (0.059)	n/a	6
12g		5.60 ± 0.21* (2510)	-0.74 ± 0.22 (0.18)	3	5.59 ± 0.13 (2570)	-0.79 ± 0.12 (0.16)	n/a	4
12h		5.65 ± 0.26 (2240)	-1.56 ± 0.08* (0.028)	3	6.47 ± 0.24 (339)	-0.51 ± 0.10* (0.31)	n/a	3
12i		8.41 ± 0.11* (3.4)	n/a	3	9.59 ± 0.14* (0.257)	n/a	0.83 ± 0.02	3
12j		8.28 ± 0.09* (5.25)	n/a	3	8.86 ± 0.13* (1.38)	n/a	0.57 ± 0.12	3

[a] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined by radioligand binding.

[b] Estimate of the logarithm of the net cooperativity factor between the modulator and [^{3}H]raclopride ± standard error of the mean (s.e.m.) determined by radioligand binding.

[c] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay.

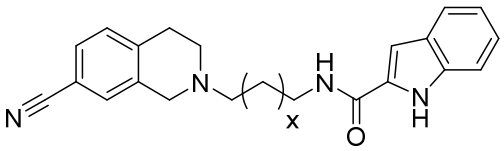
[d] Estimate of the logarithm of the net cooperativity factor between the modulator and dopamine ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay.

[e] Schild slope ± standard error of the mean (s.e.m.).

n/a compound displayed competitive pharmacology with [^{3}H]racloproide and/or dopamine therefore no value of cooperativity factor was derived.

* Statistically different from corresponding **1** parameter ($p < 0.05$, one-way ANOVA, Dunnett's post-hoc test)

Table 3. Functional Parameters for Analogues of 1 with chemical modifications to the cyclohexylene spacer



		radioligand binding: [^{3}H]raclopride			pERK1/2 phosphorylation			
Compound	X =	**pK_B[a] (K_B, nM)**	**Logα[b] (α)**	**n**	**pK_B[c] (K_B, nM)**	**Log$\alpha\beta$[d] ($\alpha\beta$)**	**Schild slope[e]**	**n**
1	-	6.45 ± 0.02 (355)	-0.68 ± 0.15 (0.21)	3	6.11 ± 0.06 (776)	-1.23 ± 0.14 (0.059)	n/a	6
18a	1	6.43 ± 0.29 (372)	-1.03 ± 0.38 (0.093)	3	6.86 ± 0.07* (138)	-1.38 ± 0.11 (0.042)	n/a	3
18b	2	6.80 ± 0.28 (158)	-0.94 ± 0.10 (0.11)	3	7.09 ± 0.14* (81.2)	-1.32 ± 0.09 (0.048)	n/a	4
18c	3	6.32 ± 0.17 (479)	n/a	3	6.88 ± 0.09 (132)	n/a	1.27 ± 0.05	3
18d	4	6.57± 0.20 (269)	-1.05 ± 0.10 (0.089)	3	7.52 ± 0.28* (30.1)	-1.68 ± 0.35 (0.021)	n/a	3

x no of carbon atoms within methylene linker

[a] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined by radioligand binding.

[b] Estimate of the logarithm of the net cooperativity factor between the modulator and [^{3}H]raclopride ± standard error of the mean (s.e.m.) determined by radioligand binding.

[c] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay.

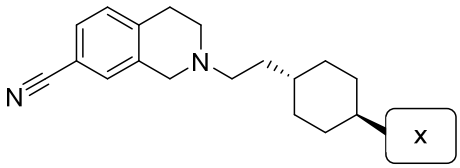
[d] Estimate of the logarithm of the net cooperativity factor between the modulator and dopamine ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay.

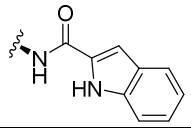
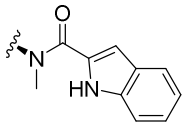
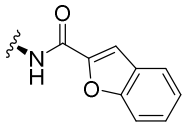
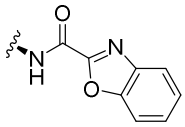
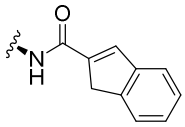
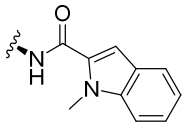
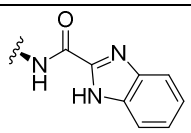
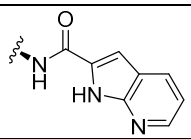
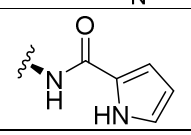
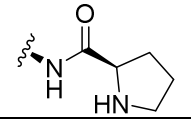
[e] Schild slope ± standard error of the mean (s.e.m.).

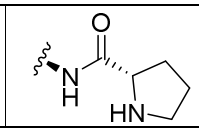
n/a compound displayed competitive pharmacology with [^{3}H]racloproide and/or dopamine therefore no value of cooperativity factor was derived.

* Statistically different from corresponding **1** parameter ($p < 0.05$, one-way ANOVA, Dunnett's post-hoc test)

Table 4. Functional Parameters for Analogues of 1 with chemical modifications to the 1*H*-indole-2-carboxamide allosteric tail



		radioligand binding: [^{3}H]raclopride			pERK1/2 phosphorylation			
Compound	**X =**	**pK_B[a] (K_B, nM)**	**Logα[b] (α)**	**n**	**pK_B[c] (K_B, nM)**	**Log$\alpha\beta$[d] ($\alpha\beta$)**	**Schild slope[e]**	**n**
1		6.45 ± 0.02 (355)	-0.68 ± 0.15 (0.21)	3	6.11 ± 0.06 (776)	-1.23 ± 0.14 (0.059)	n/a	6
23		6.93 ± 0.31 (117)	-0.41 ± 0.15 (0.39)	3	7.04 ± 0.26* (91.2)	-0.57 ± 0.10* (0.27)	n/a	3
25a		6.55 ± 0.23 (282)	n/a	3	7.74 ± 0.17* (18.2)	n/a	0.51 ± 0.01	3
25b		6.58 ± 0.15 (263)	n/a	3	7.74 ± 0.20* (18.2)	n/a	0.66 ± 0.06	5
25c		6.82 ± 0.06* (151)	n/a	3	7.64 ± 0.22* (22.9)	n/a	0.90 ± 0.07	3
25d		6.82 ± 0.20 (151)	n/a	3	7.14 ± 0.09* (72.4)	n/a	0.98 ± 0.06	3
25e		7.09 ± 0.12 (81.3)	n/a	3	6.76 ± 0.15 (174)	n/a	1.06 ± 0.07	3
25f		7.34 ± 0.05* (45.7)	-1.46 ± 0.11* (0.035)	3	7.63 ± 0.13* (23.4)	-1.39 ± 0.16 (0.041)	n/a	5
26		7.24 ± 0.21* (57.5)	n/a	3	8.48 ± 0.23* (3.31)	n/a	0.99 ± 0.07	5
27b		6.74 ± 0.18 (363)	n/a	3	7.34 ± 0.17* (45.7)	n/a	1.04 ± 0.07	5

28b		7.63 ± 0.12* (23.4)	n/a	3	7.56 ± 0.27* (27.5)	n/a	1.37 ± 0.14	3

[a] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined by radioligand binding.

[b] Estimate of the logarithm of the net cooperativity factor between the modulator and [^{3}H]raclopride ± standard error of the mean (s.e.m.) determined by radioligand binding

[c] Estimate of the negative logarithm of the equilibrium dissociation constant ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay.

[d] Estimate of the logarithm of the net cooperativity factor between the modulator and dopamine ± standard error of the mean (s.e.m.) determined in an pERK1/2 functional assay.

[e] Schild slope ± standard error of the mean (s.e.m.)

n/a compound displayed competitive pharmacology with [^{3}H]racloproide and/or dopamine therefore no value of cooperativity factor was derived.

* Statistically different from corresponding **1** parameter ($p < 0.05$, one-way ANOVA, Dunnett's post-hoc test)

Figures and captions

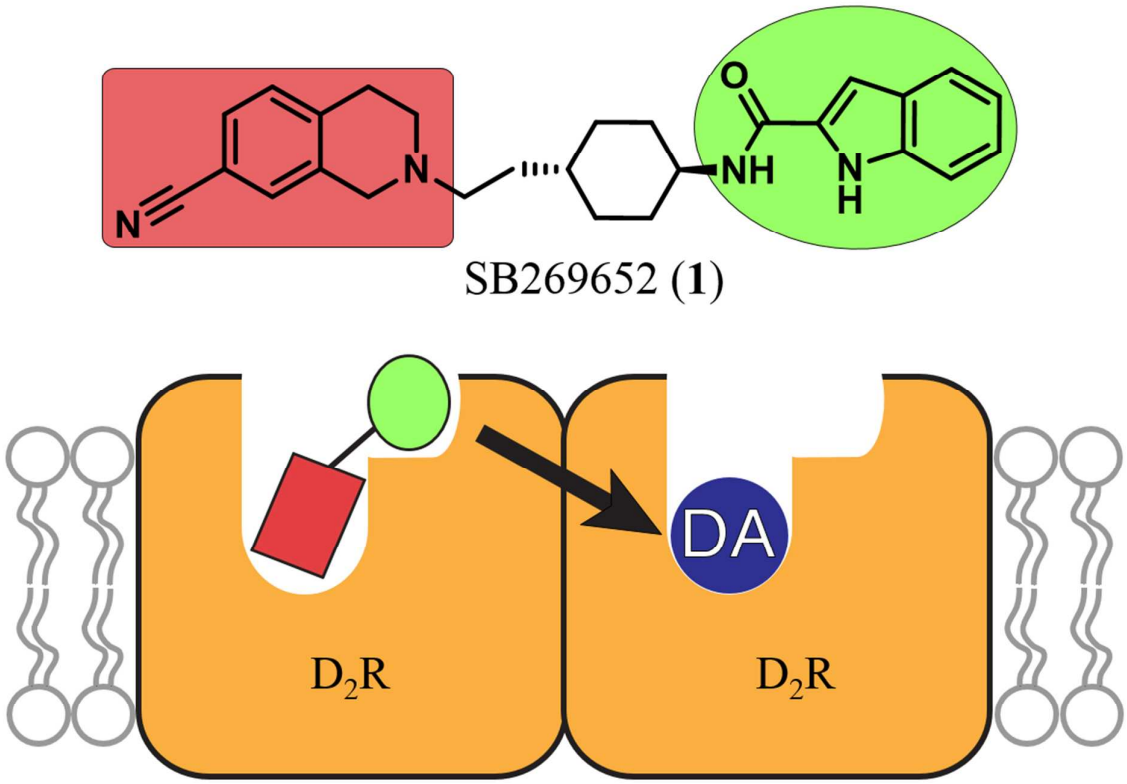


Figure 1. The proposed binding mode of **1**, which displays allosteric pharmacology despite containing structural features of a competitive ligand. **1** was found to act via a novel mechanism, engaging one protomer of a D_2R dimer in a bitopic mode, and negatively modulating dopamine (DA) binding and function at the other protomer.[20] The red box represents the orthosteric-binding portion, while the green oval represents the allosteric-binding portion of **1**.

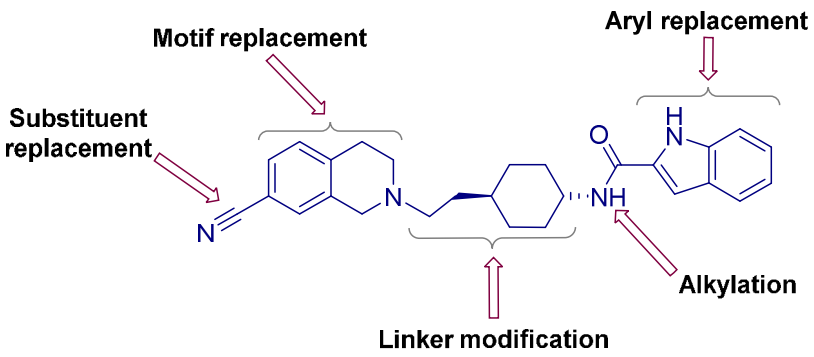


Figure 2. General overview of structural modifications of **1** investigated in this study.

Please refer to pdf labelled 'figure 3' for high resolution image.

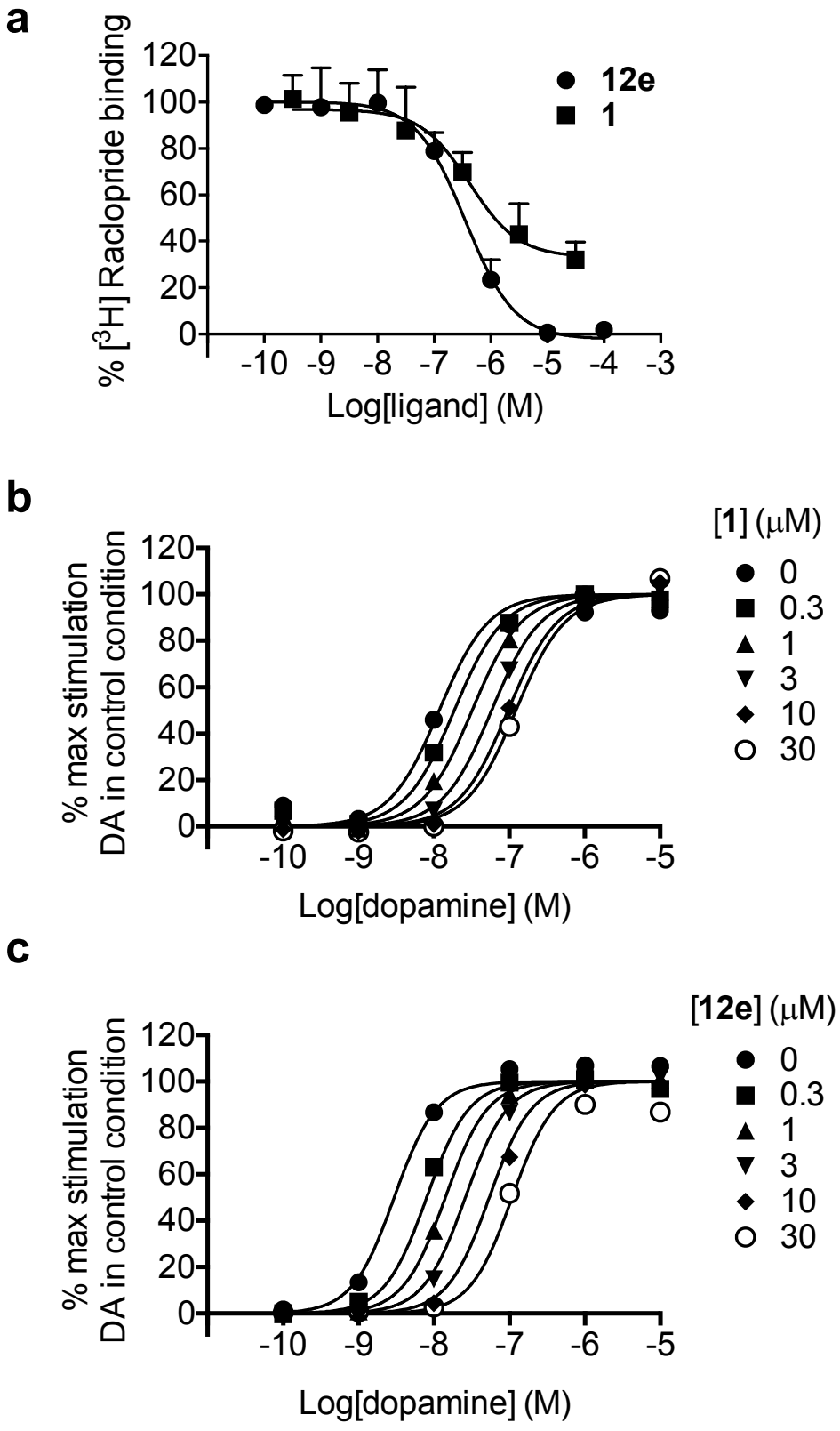


Figure 3:

a) Competition binding experiments between analogues of **1** and the radiolabeled antagonist [^{3}H]raclopride using membranes of FlpIn CHO expressing the $hD_{2L}R$ reveal that some compounds (such as **12e** shown here) were able to fully inhibit raclopride binding consistent with a competitive interaction. These data could be used to derive a value of affinity (K_B). Other compounds, exemplified by **1**, were unable to fully displace [^{3}H]raclopride binding consistent with an allosteric interaction. These data could be fit with an allosteric ternary complex model to derive values of affinity (K_B) and negative cooperativity with [^{3}H]raclopride.

b) The action of increasing concentrations of **1** upon a dopamine concentration-response curve was measured in an assay measuring ERK1/2 phosphorylation using whole cells expressing the $hD_{2L}R$. Increasing concentrations of **1** caused a limited dextral displacement of the dopamine dose-response curve and data were analyzed according to an operational model of allosterism.

c) The action of increasing concentrations of **12e** upon a dopamine concentration-response curve was measured in an assay measuring ERK1/2 phosphorylation using whole cells expressing the $hD_{2L}R$. Increasing concentrations of **12e** caused a limitless dextral displacement of the dopamine dose-response curve and data were analyzed according to a Gaddum/Schild model of competitive pharmacology.

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