Design, Synthesis, And Evaluation of Functionalized Diamino-butylbenzamides As Selective Dopamine D3 Receptor Ligands

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Design, synthesis, and evaluation of functionalized diamino-butylbenzamides as selective dopamine D3 receptor ligands

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Abstract: Substance use disorder remains a major, unmet medical need. Cocaine is one of the most commonly abused recreational drugs and in 2018, there were over 5.5 million current cocaine users. There are no approved therapies for the treatment of cocaine use disorder, but the D₃ dopamine receptor has been identified as a potential therapeutic target. We have identified a series of functionalized diamino-butylbenzamides that are potent D₃ binders with moderate to high selectivity for D₃ over D₂.

Graphical Abstract:

Keywords: Dopamine, D₃ dopamine receptor, D₂ dopamine receptor, substance use disorder, cocaine

Introduction: Substance use disorder (SUD) is a major societal and medical issue that a significant negative impact on SUD patients, as well as their family and caregivers. According to the 2018 National Survey on Drug Use and Health, approximately 32 million people over the age of 12 in the US reported consuming an illicit drug in the past 30 days, which equates to just over 19% of the US population. In addition, the US is home to over 8 million people suffering from addiction to illicit drugs such as cocaine (**1**), heroine, and methamphetamine. The estimated annual societal costs of SUD exceed \$600 billion,[1] and an increased risk of a range of conditions is definitively linked to SUD. Patients suffering from this condition experience increased rates of HIV and hepatitis infection, cardiovascular disease, lung disease, stroke, and cancer.[2]

Cocaine, which can be injected or snorted as the HCl salt or smoked as the free base (crack cocaine), is one of the most commonly abused drugs. Over 5.5 million people in the US

described themselves as current user of cocaine in 2018[1]. The pharmacological impact of cocaine is partially driven by its ability to interact with dopamine transporters (DAT) in the brain and lower dopamine uptake. This interferes with the normal operation of the mesocorticolimbic dopamine (MCL-DA) system, which is more commonly known as the reward system.[3] Synaptic levels of dopamine rise as a result of cocaine's suppression of DAT activity, which leads to an amplification of dopamine signaling and creates the euphoric linked to cocaine abuse. As cocaine use continues, changes in DAT and dopamine receptors occur, which causes long-term neuroadaptation of several brain circuits.[4] Chronic exposure to cocaine is also linked to negative impacts on executive function and decision-making processes, as well as increased risks of emotional affect such as anxiety, panic, paranoia, and violent behavior. Patient attempting to end their use of cocaine experience withdrawal symptoms including serious deficits, mainly in working memory, planning, and cognitive flexibility.[5] Cocaine overdose is also a serious risk for patients, and scale of this issue in the US was outlined in the 2011 Drug Abuse Warning Network report. This study found that in 2011, >500K patients required emergency room services as a result of cocaine overdose.

The magnitude of this problem is clear, but unfortunately therapeutic options are limited. In some patients, behavioral interventions such as contingency management programs provide rewards for abstinence is effective,

while others respond to cognitive-behavioral therapies that teach recovering addicts to recognize

and avoid situations that will trigger the desire to use cocaine. These methods are, however, far too costly and resources intensive to meet the needs of the patient population in need of treatment.[7] In principle, behavioral interventions and cognitive-behavioral therapies could be paired with a suitable pharmacological intervention, but there are no FDA approved medications for the treatment of cocaine addiction, also known as cocaine use disorder (CUD). Research efforts focused on this issue have led to exploration of novel compounds, as well as effort to repurpose previously approved therapies.[8] The AMPA/Kainate receptor antagonist Topiramate (2)[9] and the antiepileptic irreversible inhibitor of GABA transaminase Vigabatrin (3),[10] for example have also been examined in clinical trials, but neither have been approved for use as treatments for cocaine addiction. The alcoholism treatment Disulfiram (4) has shown promise in a limited patient population, but its mechanism of action is unknown.[11] Positive results in animal studies with compounds that selectively target the neurokinin-1 receptor,[12] the cysteine glutamate antiporter,[13] and the 5-HT_{2c} serotonin receptor [14] have also been reported.

In an effort to develop novel therapies with the potential to address the needs of patients suffering from CUD, we have focused our efforts on the possible positive impact of modulating dopamine (5) signaling. This neurotransmitter is

synthesized in the brain and periphery. In the periphery dopamine (5) can act as a vasodilator, modulate renal sodium excretion, and impact urine output, while in the central nervous system, dopamine (5) has a significant impact on movement, behavioral motivations, and learning.[15] The pharmacological impact of dopamine is mediated by a family of related G-protein coupled receptors (GPCRs) which are designated as designated D₁, D₂, D₃, D₄, and D₅. There are two

sub-families of dopamine (5) receptors, the D₁-like sub-family (D₁ and D₅) and D₂-like sub-family (D₂, D₃ and D₄). These classifications are based on pharmacological properties, amino acid homology, and genetic organization.[16] Multiple lines of evidence suggest that D₃ may be a viable therapeutic target for the treatment of CUD. Chronic and acute exposure to cocaine, for example, leads to increased D₃ expression in the nucleus accumbens. This area of the brain is responsible for cognitive processing including behaviors associated with CUD.[17] In addition, D₃ is highly expressed in key brain regions that have been definitively linked to cocaine addiction. The role of D₃ in CUD has been described in multiple review articles.[18] There is also evidence implicating D₂ signaling as a possible therapeutic target for the treatment of CUD, but there are significant risks associated with D₂ modulation (e.g. extrapyramidal symptoms, catalepsy).[19] In order to avoid these potential risks, we have focused our efforts on the development of novel D₃ ligands that are highly selective for D₃ over D₂.

We have recently described the identification of a series of N-(4-(4-phenyl piperazin-1-yl)butyl)-4-(thiophen-3-yl)benzamides that bind to D₃ selectively over D₂. As part of these studies, we identified (6) as a highly potent D₃ ligand (K_i = 0.5 nM) that demonstrated a significant selectivity for D₃ over D₂ (K_i = 743 nM).[20] As part of our broader effort to understand the source of D₃/D₂ selectivity, we determined that while D₃ and D₂ binding are

driven primarily by the aryl piperazine portion of the molecule (via Asp^{3.32}), D₃/D₂ selectivity is primarily driven by the phenyl thiophene region.[21] Preliminary rat pharmacokinetic studies revealed that (6) has a long terminal T_{1/2} (10.9 h), but solubility studies revealed that this

compound is poorly soluble in aqueous media ($S_{ol} < 2$ mmol). While there are examples of poorly soluble, FDA approved medication, we viewed this as issue that should be addressed in next generation compounds. We have therefore continued our efforts to identify novel, selective D₃ ligands.

Results and Discussion: Our recent efforts have focused on a related series of compounds (**7**) in which the amide of (**6**) has been inverted. These compounds are readily prepared using the chemistry described in scheme 1. In this process, 5-bromopentanoyl chloride (**8**) is reacted with a suitably substituted aniline (**9**) in the presence of triethyl amine to provide amide (**10**). Displacement of the primary bromide with a functionalized cyclic diamine (**11**) in refluxing acetone and in the presence of potassium carbonate provide the final screening targets. Table 1 describes the in vitro screening results for D₃ and D₂, as well as solubility and rat liver microsomal stability.

In our first series of compounds (**7a-7i**), we maintained the thiophen-3-yl moiety in order minimize the number of changes between our new series and compounds typified by (**6**). As noted in table 1, electron donating (**7a**) and electron withdrawing groups (**7b**, **7c**) in the 2-position provide potent D₃ binders ($K_i = 3.1$ nM, 6.2 nM, and 20 nM) with moderate selectivity over D₂ (21-40 fold). However, rat liver microsome (RLM) stability of these compounds was low to moderate (19.3 to 5.6 minutes). Potent D₃ activity was also observed when electron withdrawing groups were located in the 3-position, but selectivity over D₂ was not uniform. Specifically, while the 3-CN analog (**7d**) was highly selective for D₃ ($D_3 K_i = 14.9$ nM, $D_2 K_i = 8119$ nM), the 3-CF₃ analog (**7e**) demonstrated similar D₃ binding ($K_i = 5$ nM) but was far less selective for D₂ ($K_i = 82.1$ nM). RLM stability for both (**7d**) and (**7e**) was high ($T_{1/2} = 60$ min). Dichlorination (**7f**, **7g**, and **7h**) also provided potent D₃ binders ($D_3 K_i = 2-44$ nM) that

displayed moderate selectivity over D2 (108 to 392-fold), and were highly stable in the presence of RLM ($T_{1/2}$ = 60 min). Solubility of this initial set of analogs (**7a-7f**) was low.

Now that we had established that D₃ activity could be retained when the central amide was inverted, we turned our attention towards identifying structural changes that might improve solubility. We initially examined the impact of replacing the thiophen-3-yl ring with a 2-pyridine ring (**7i – 7o**) while maintaining previously employed aryl substituents. With the exception of the 2,4-di-Cl (**7o**) and 3,5-di-Cl (**7p**), solubility improved (184 – 10.2 mM). Importantly, D₃ binding potency was high (4.5 nM – 36 nM) and selectivity over D₂ was moderate to high (20 to 277 fold). RLM stability was low when the substituents were placed in the 2-position of the aryl piperazine (**7i-7k** RLM $T_{1/2}$ = 3.7 to 7.7 min), but improved when electron withdrawing substituents were placed in the 3-position (**7l,7m** RLM $T_{1/2}$ = 10.9, 20.9 min) or when the aryl piperazine contained 2 chlorine substituents (**7n-7p**, RLM $T_{1/2}$ = 15.1, 18.2, 60 min.).

We also explored replacing the central piperazine with a homopiperazine as a method of increasing solubility while maintaining D₃ potency and selectivity. Examples (**7q**) to (**7x**) incorporate this piperazine bioisostere, preserve the thiophen-3-yl ring, and employ the same set of aryl substituents on the aryl piperazine ring. As noted in table 1, D₃ potency and selectivity decreased when the piperazine is replaced by a homopiperazine. Solubility of the monosubstituted aryl piperazine analogs (**7q-7u**), but no significant improvement in solubility was observed with the dechlorinated analogs (**7v – 7x**). High RLM stability was observed when that aryl piperazine unit was substituted with 3CF₃ (**7u** RLM $T_{1/2}$ = 24.1 min), 2,4-di-Cl (**7w**, RLM $T_{1/2}$ = 44.5 min), and 3,5-di-Cl (**7x**, RLM $T_{1/2}$ = 43.2 min).

Conclusion: In summary, we have identified a new series of potent D3 binders that display moderate to high selectivity over D2. In addition, exemplary compounds have high RLM stability and aqueous solubility within an acceptable range. Additional efforts will be focused on further optimizing the physicochemical properties of this series of compounds while maintaining the D₃/D₂ selectivity observed in better performing compounds.

Experimental methods and materials: Reagents were purchased from Fisher Scientific, VWR International, Sigma Aldrich, and Combi-Blocks, Inc. Chromatographic purification of compounds (normal phase and reverse phase) was carried out on a Teledyne Isco Combiflash RF system. H-NMR spectra were obtained on a Bruker 400-MHz NMR. Chemical shift values (δ values) were reported in ppm relative to TMS. For multiplicity, s = singlet, d = doublet, t = triplet, m = multiplet. Purity (%) and mass spectral data were determined with a Waters Agilent 1200 HPLC/MS (Zorbax SB-C18, 2.1 x 30 mm, 3.5 μ m, 100% water/0.1% formic acid to 100% acetonitrile/0.1% formic acid over 4.0 minutes, 1.0 mL/min.) with a diode array detector from 210-400 nm and Agilent 6130 quadrupole MS. All compounds were purified to 95% purity or greater as determined by HPLC/MS and ¹H-NMR. Melting points were recorded on a capillary melting point apparatus.

Preparation of 5-bromo-N-(4-(thiophen-3-yl)phenyl)pentanamide (**10a**): The 4-(thiophen-3-yl)aniline (0.42 g, 2.36 mmol) was stirred with 5-bromovaleryl chloride (0.77 ml, 2.60 mmol, 1.1 eq) in the presence of anhydrous N,N-diisopropylethylamine (0.5 ml, 5.20 mmol, 2.2 eq) in anhydrous dichloromethane for 16 hours. After 16 hours, the reaction mixture was stripped of

solvent and the residual material was purified by normal phase chromatography, eluting with 40% dichloromethane in hexane to provide 5-bromo-N-(4-(thiophen-3-yl)phenyl)pentanamide.(640mg, 79%). ^1H NMR (400 MHz, CDCl_3) δ 8.01 (s, 1H), 7.88 (d, 1H, $J = 3.2$ Hz), 7.78 (d, 2H, $J = 7.6$ Hz), 7.65 (d, 2H, $J = 7.4$ Hz), 7.42 (d, 1H, $J = 3.1$ Hz), 3.49 (t, 2H, $J = 6.8$ Hz), 2.39 (t, 2H, $J = 7.1$ Hz), 1.79 (m, 2H), 1.59 (m, 2H). MS (LC/MS, $\text{M}+\text{H}^+$): 339.55

Preparation of 5-bromo-N-(4-(pyridin-2-yl)phenyl)pentanamide (PJC07-109) (**10b**): A solution of 4-(pyridin-2-yl)aniline (0.1955 g, 1.15 mmol) and 5-bromovaleryl chloride (0.23 ml, 1.72 mmol, 1.5 eq) in anhydrous dichloromethane was stirred at room temperature. Anhydrous triethylamine (0.42 ml, 2.99 mmol, 2.6 eq) was added dropwise into the reaction at 0 °C. The reaction was warmed to room temperature and stirred for 16 hours. After 16 hours, the reaction mixture was stripped of solvent and the residual material was purified by normal chromatography (100% dichloromethane to 80% ethyl acetate in dichloromethane) to provide 5-bromo-N-(4-(pyridin-2-yl)phenyl)pentanamide (261 mg, 68% yield). ^1H NMR (400 MHz, CDCl_3) δ 8.35 (d, 1H, $J = 7.2$ Hz), 8.28 (d, 2H, $J = 7.4$ Hz), 7.75 (d, 2H, $J = 7.4$ Hz), 7.41, (m, 1H), 7.12 (d, 1H, $J = 6.9$ Hz), 6.95 (m, 1H), 3.49 (2H, $J = 7.1$ Hz), 2.42 (t, 2H, $J = 6.8$ Hz), 1.78 (m, 2H), 1.65 (m, 2H). MS (LC/MS, $\text{M}+\text{H}^+$): 334.

Preparation of 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (MC25-67; PJC03-54) (**7h**): A solution of 5-bromo-N-(4-(thiophen-3-yl)phenyl)pentanamide (0.35 g, 1.03 mmol), 1-(3,5-dichlorophenyl)piperazine (0.48 g, 2.06 mmol, 2.0 eq) and potassium carbonate (0.43 g, 3.09 mmol, 3.0 eq) in dry tetrahydrofuran (6 ml) was refluxed at 66°C for 48 hours. After 48 hours, the mixture was cooled and filtered to obtain a filtrate. The filtrate was purified by normal phase chromatography (10% methanol in dichloromethane) to give a partial pure product. The impure product was further purified by reverse phase chromatography (100% acetonitrile/0.1% ammonium hydroxide) to give 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (242 mg, 48% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.49 (s, 3H), 7.33 (m, 1H), 7.30-7.29 (m, 2H), 6.73 (t, J = 1.68, 1.56 Hz, 1H), 6.66 (d, J = 1.68 Hz, 2H), 3.42 (d, J = 5.52 Hz, 2H), 3.17 (broad s, 4H), 2.56 (broad s, 3H), 2.42 (broad s, 2H), 2.36 (t, J = 7.32, 7.2 Hz, 2H), 1.75 (pent, 2H), 1.59 (m, 2H); MS (LC/MS, M+H⁺): 488.55.

Preparation of 5-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)Pentanamide (**7a**): The title compound was prepared according to the procedure for 5-(4-(3,5-

dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(2-methoxyphenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)-piperazine. The product was first purified by normal phase chromatography (10% methanol in dichloromethane) and was then further purified by reverse phase chromatography (30% acetonitrile/0.1% formic acid in water) to provide 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (32% yield) ^1H NMR (400 MHz, CDCl_3) δ 8.49 (s, 2H), 7.62 (d, J = 8.52 Hz, 2H), 7.51 (d, J = 8.56 Hz, 2H), 7.36-7.32 (m, 3H), 7.01 (sext, J = 4.56, 3.6, 0.8 Hz, 1H), 6.88 (d, J = 4.12 Hz, 2H), 6.85 (d, J = 8 Hz, 2H), 7.62 (d, J = 8.52 Hz, 2H), 7.51 (d, J = 8.56 Hz, 2H), 7.36-7.32 (m, 3H), 7.01 (sext, J = 4.56, 3.6, 0.8 Hz, 1H), 6.88 (d, J = 8.52 Hz, 2H), 6.85 (d, J = 8 Hz, 2H), 3.84 (s, 3H), 3.26 (broad s, 4H), 3.09 (broad s, 4H), 2.88 (broad s, 2H), 2.45 (broad s, 2H), 1.82 (s, 4H); MS (LC/MS, $\text{M}+\text{H}^+$): 450.70.

Preparation of 5-(4-(2-cyanophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7b**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(2-cyanophenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The product was first purified by normal phase chromatography (10% methanol in dichloromethane) and was then further purified by reverse phase chromatography (30% acetonitrile/0.1% formic acid in water) to provide 5-(4-(2-cyanophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (53% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.58-7.42 (m, 6H), 7.31-7.28 (m, 4H), 7.03-6.95 (m, 2H),

3.40 (broad s, 4H), 3.14 (broad s, 4H), 2.89 (broad s, 2H), 2.41 (broad s, 2H), 1.79 (broad s, 4H); MS (LC/MS, M+H⁺): 445.70.

Preparation of N-(4-(thiophen-3-yl)phenyl)-5-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (**7c**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(2-(trifluoromethyl)phenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The product was first purified by normal phase chromatography (10% methanol in dichloromethane) and was then further purified by reverse phase chromatography (100% acetonitrile/0.1% ammonium hydroxide) to provide N-(4-(thiophen-3-yl)phenyl)-5-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (27% yield) ¹H NMR (400 MHz, CDCl₃) δ 7.54 (dd, J = 1, 0.92 Hz, 1H), 7.47 (broad s, 5H), 7.41 (t, J = 7.4 Hz, 1H), 7.32-7.26 (m, 4H), 7.13(t, J = 7.6 Hz, 1H), 2.88 (t, J = 4.68 Hz, 4H), 2.52 (broad s, 4H), 2.34 (sext, J = 7.88, 7.6, 7.52, 7.28 Hz, 4H), 1.72 (pent, J = 7.52 Hz, 2H), 1.55 (pent, J = 7 Hz, 2H); MS (LC/MS, M+H⁺): 488.65.

Preparation of 5-(4-(3-cyanophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7d**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(3-cyanophenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The product was first purified by normal phase chromatography (10% methanol in dichloromethane) and was then further purified by reverse phase chromatography (85% acetonitrile/0.1% ammonium hydroxide in water) to provide 5-(4-(3-cyanophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (34% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.49 (s, 4H), 7.33-7.28 (m, 4H), 7.25-7.21 (m, 1H), 7.03-7.00 (m, 3H), 3.16 (t, $J = 5$ Hz, 4H), 2.55 (t, $J = 4.76$ Hz, 4H), 2.42-2.33 (m, 4H), 1.72 (pent, $J = 7.44$ Hz, 2H), 1.57 (pent, $J = 6.92$ Hz, 2H); MS (LC/MS, $\text{M}+\text{H}^+$): 445.70.

Preparation of N-(4-(thiophen-3-yl)phenyl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (**7e**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(3-(trifluoromethyl)phenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The product was purified by normal phase chromatography (100% ethyl acetate to 10% methanol in dichloromethane) to provide N-(4-(thiophen-3-yl)phenyl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (45% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.48 (s, 4H), 7.33-7.28 (m, 4H), 7.26 (s, 1H), 7.02-6.95 (m, 3H), 3.17 (t, $J = 5.08$ Hz, 4H), 2.54

(t, J = 6.12 Hz, 4H), 2.36 (m, 4H), 1.73 (pent, J = 7.48 Hz, 2H), 1.57 (pent, J = 7.04 Hz, 2H); MS (LC/MS, M+H⁺): 488.60.

Preparation of 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7f**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(2,3-dichlorophenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The product was purified by normal phase chromatography (100% ethyl acetate) to provide 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (65% yield) ¹H NMR (400 MHz, CDCl₃) δ 7.48 (s, 4H), 7.33-7.27 (m, 3H), 7.10-7.04 (m, 3H), 6.87 (dd, J = 4.92, 2.32 Hz, 1H), 3.02 (s, 4H), 2.63 (s, 4H), 2.45 (t, J = 7.44 Hz, 2H), 2.34 (t, J = 7.4 Hz, 2H), 1.74 (pent, J = 7.68 Hz, 2H), 1.58 (pent, J = 8.04 Hz, 2H); MS (LC/MS, M+H⁺): 488.60.

Preparation of 5-(4-(2,4-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7g**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(2,4-

dichlorophenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The product was purified by normal phase chromatography (100% ethyl acetate) to provide 5-(4-(2,4-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (42% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.59 (s, 1H), 7.52-7.46 (m, 4H), 7.33-7.28 (m, 4H), 7.11 (dd, J = 6.16, 2.44 Hz, 1H), 6.89 (d, J = 8.64 Hz, 1H), 3.02 (s, 4H), 2.64 (s, 4H), 2.45 (t, J = 7.2 Hz, 2H), 2.36 (t, J = 7.24 Hz, 2H), 1.74 (m, 2H), 1.61 (m, 2H); MS (LC/MS, $\text{M}+\text{H}^+$):488.60.

5-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (**7i**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide, except 1-(2-methoxyphenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine (71% yield). ^1H NMR (400 MHz, DMSO) δ 8.62 (dd, 1H, J = 7.4, 2.2 Hz), 8.05 (d, 2H, J = 7.7 Hz), 7.91 (d, 1H, J = 7.1 Hz), 7.83 (m, 1H, J = 6.1, 2.1 Hz), 7.22 (d, 2H, J = 7.4 Hz), 7.29 (m, 1H), 6.91 (m, 2H), 6.87 (m, 2H), 3.82 (s, 3H), 3.38 (m, 8H), 2.98 (m, 2H), 2.45 (m, 2H), 1.67 (m, 2H), 1.52 (m, 2H). MS (LC/MS, $\text{M}+\text{H}^+$): 445.

Preparation of 5-(4-(2-cyanophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (**7j**):

The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide, except 2-(piperazin-1-yl)benzonitrile was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture was purified by normal phase chromatography (10% methanol/0.1% ammonium hydroxide in dichloromethane) and normal amine phase (RediSep Rf Gold® Amine #: 69-2203-507) chromatography (100% hexane to 100% ethyl acetate) to provide 5-(4-(2-cyanophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (41% yield): ¹H NMR (400 MHz, CDCl₃) δ 8.60-8.59 (d, J = 4.64 Hz, 1H), 7.92-7.89 (d, J = 8.64 Hz, 2H), 7.67-7.62 (m, 2H), 7.60-7.58 (d, J = 8.44 Hz, 2H), 7.50-7.48 (dd, J = 6.56, 1.56, 1.4 Hz, 1H), 7.46 (broad s, 1H), 7.43-7.38 (td, J = 6.64, 6.48, 1.56, 1.08 Hz, 1H), 7.15-7.12 (m, 1H), 6.93-6.92 (d, J = 7.92 Hz, 2H), 3.19-3.17 (t, J = 4.76, 4.52 Hz, 4H), 4.63 (broad s, 4H), 2.45-2.42 (t, J = 7.44, 7.24 Hz, 2H), 2.38-2.34 (t, J = 7.48, 7.28 Hz, 2H), 1.78-1.70 (quint, J = 7.52, 7.48, 7.36 Hz, 2H), 1.61-1.54 (quint, J = 8.08, 7.52, 7.28, 6.88 Hz, 2H); MS (LC/MS, M+H⁺): 440.80.

Preparation of N-(4-(pyridin-2-yl)phenyl)-5-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (**7k**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide, except 1-(2-(trifluoromethyl)phenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture was purified by normal phase chromatography (10% methanol/0.1%

ammonium hydroxide in dichloromethane) to give an impure product. The impure product was further purified by normal amine phase (RediSep Rf Gold® Amine #: 69-2203-507) chromatography (100% hexane to 100% ethyl acetate) to provide N-(4-(pyridin-2-yl)phenyl)-5-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (32% yield): ¹H NMR (400 MHz, CDCl₃) δ 8.59 (d, J = 4.64 Hz, 1H), 7.90 (d, J = 8.68 Hz, 2H), 7.68-7.64 (m, 2H), 7.62-7.58 (m, 2H), 7.54 (d, J = 7.8 Hz, 1H), 7.43 (t, J = 7.76, 7.56 Hz, 1H), 7.29 (d, J = 8 Hz, 1H), 7.16-7.11 (m, 2H), 2.92 (broad s, 4H), 2.59 (broad s, 4H), 2.39 (broad s, 2H), 2.37 (t, J = 7.08, Hz, 2H), 1.75 (broad s, 2H), 1.60 (broad s, 2H); MS (LC/MS, M+H⁺): 483.8.

Preparation of N-(4-(pyridin-2-yl)phenyl)-5-(4-(3-cyanophenyl)piperazin-1-yl)pentanamide (**7I**):

The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide, except 1-(3-cyanophenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine (37% yield). ¹H NMR (400 MHz, DMSO) δ 8.71 (d, 1H, J = 5.4 Hz), 8.11 (d, 2H, J = 7.5 Hz), 7.97 (d, 1H, J = 7.6 Hz), 7.91 (dt, 1H, J = 6.9, 1.8 Hz), 7.78 (d, 2H, J = 7.1 Hz), 7.47 (m, 2H), 7.36 (dt, 1H, J = 6.1, 1.5 Hz), 7.24 (m, 1H), 3.41 (bs, 8H), 3.35 (m, 2H), 2.46 (m, 2H), 1.64 (m, 4H). MS (LC/MS, M+H⁺): 440.

Preparation of N-(4-(pyridin-2-yl)phenyl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (**7m**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide, except 1-(3-(trifluoromethyl)phenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture was purified by normal phase chromatography (10% methanol/0.1% ammonium hydroxide in dichloromethane) to give an impure product. The impure product was further purified by normal amine phase (RediSep Rf Gold® Amine #: 69-2203-507) chromatography (100% hexane to 100% ethyl acetate) to provide N-(4-(pyridin-2-yl)phenyl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (43% yield): ¹H NMR (400 MHz, CDCl₃) δ 8.58 (dd, J = 1.08, 0.92 Hz, 1H), 7.89 (d, J = 8.68 Hz, 2H), 7.68-7.62 (m, 3H), 7.61-7.58 (d, J = 8.56 Hz, 2H), 7.49-7.47 (d, J = 6.36 Hz, 1H), 7.42-7.38 (dt, J = 6.6, 6.28, 1.64, 1.24 Hz, 1H), 7.19-7.11 (m, 1H), 6.92 (t, J = 7.84, 7.56 Hz, 2H), 3.18 (t, J = 4.8, 4.6 Hz, 4H), 2.62 (t, J = 4.52, 4.4 Hz, 4H), 2.43 (t, J = 7.44, 7.28 Hz, 2H), 2.35 (t, J = 7.48, 7.32 Hz, 2H), 1.76-1.69 (quint, 2H), 1.60-1.53 (quint, 2H); MS (LC/MS, M+H⁺): 483.

Preparation of 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (**7n**): A solution of 5-bromo-N-(4-(pyridin-2-yl)phenyl)pentanamide (0.191 g, 0.573 mmol), 1-(2,3-dichlorophenyl)piperazine (0.11 ml, 0.573 mmol) and potassium carbonate (0.24 g, 1.72 mmol, 3.0 eq) in acetone (6 ml) was refluxed at 56°C for 24 hours. After 24 hours, the reaction was cooled and filtered to give a filtrate. The filtrate was purified by normal phase chromatography (100% dichloromethane to 100% ethyl acetate to 10% methanol in dichloromethane) to give an impure product. The impure product was further purified by normal amine phase (RediSep Rf Gold® Amine #: 69-2203-507) chromatography (100% hexane to 100% ethyl acetate) to provide 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (104 mg, 36% yield): ¹H NMR (400 MHz, CDCl₃) δ 8.58 (dd, J = 3.24, 1.2, 0.6 Hz, 1H), 7.89 (d, J = 8.68 Hz, 2H), 7.68-7.62 (m, 3H), 7.61-7.58 (m, 2H), 7.19-7.11 (m, 1H), 7.10-7.04 (m, 2H), 6.88-6.85 (dd, J = 4.88, 2.36, 2.32 Hz, 1H), 3.02 (broad s, 4H), 2.61 (broad s, 4H), 2.43 (t, J = 7.44, 7.24 Hz, 2H), 2.36 (t, J = 7.48, 7.28 Hz, 2H), 1.77-1.70 (quint, 2H), 1.61-1.54 (quint, 2H); MS (LC/MS, M+H⁺): 484.70.

Preparation of 5-(4-(2,4-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (**7o**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide, except 1-(2,4-dichlorophenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture was purified by normal phase chromatography (10% methanol/0.1% ammonium

hydroxide in dichloromethane) and normal amine phase (RediSep Rf Gold® Amine #: 69-2203-507) chromatography (100% hexane to 100% ethyl acetate) to provide 5-(4-(2,4-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (31% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.61-8.59 (td, J = 2.24, 1.28, 0.96, 0.64 Hz, 1H), 7.90 (d, J = 8.68 Hz, 2H), 7.69-7.64 (m, 2H), 7.64-7.58 (m, 2H), 7.50 (broad s, 1H), 7.28 (d, J = 2.4 Hz, 1H), 7.15-7.10 (m, 2H), 6.88 (d, J = 8.64 Hz, 1H), 3.02 (broad s, 4H), 2.63 (broad s, 4H), 2.46 (t, J = 7.52, 7.2 Hz, 2H), 2.38 (t, J = 7.44, 7.28 Hz, 2H), 1.79-1.72 (quint, 2H), 1.64-1.57 (quint, 2H); MS (LC/MS, M+H⁺): 484.

Preparation of 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide (**7p**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(4-(pyridin-2-yl)phenyl)pentanamide, except 1-(2,4-dichlorophenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine (46% yield) (400 MHz, DMSO) δ 8.69 (DD, 1H, J = 5.9, 1.5 Hz), 8.11 (d, 2H, J = 7.2 Hz), 7.97 (d, 1H, J = 7.2 Hz), 7.91 (dt, 1H, J = 5.5, 1.2 Hz), 7.78 (d, 2H, J = 7.1 Hz), 7.37 (m, 1H), 6.98 (bs, 2H), 6.92 (bs, 1H), 3.51 (m, 8H), 3.41 (m, 2H), 2.48 (m, 2H), 1.71 (m, 2H), 1.62 (m, 2H). MS (LC/MS, M+H⁺): 484.

Preparation of 5-(4-(2-methoxyphenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7q**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(2-methoxyphenyl)-1,4-diazepane was substituted for 1-(3,5-dichlorophenyl)piperazine. The reaction mixture was first purified by normal phase chromatography (10% methanol in dichloromethane) to give a partially pure desired product. The impure product was further purified by reverse phase chromatography (50% acetonitrile/0.1% formic acid in water) to provide 5-(4-(2-methoxyphenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (48% yield) ^1H NMR (400 MHz, CDCl_3) δ 7.62 (broad s, 2H), 7.47 (broad s, 2H), 7.31-7.29 (m, 3H), 6.89 (broad s, 1H), 6.82-6.78 (m, 3H), 3.74 (s, 3H), 3.33 (broad s, 6H), 3.19 (broad s, 2H), 3.16 (broad s, 2H), 2.44 (broad s, 2H), 2.28 (broad s, 2H), 1.81 (broad s, 2H); MS (LC/MS, $\text{M}+\text{H}^+$): 464.

Preparation of 5-(4-(2-cyanophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7r**): The title compound was prepared according to the procedure for - (4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 2-(1,4-

diazepan-1-yl)benzonitrile was substituted for 1-(2-methoxyphenyl)-1,4-diazepane to provide 5-(4-(2-cyanophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (44% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.69 (broad s, 1H), 7.45-7.43 (d, J = 8.64 Hz, 2H), 7.40-7.38 (d, J = 8.68 Hz, 2H), 7.35-7.33 (dd, J = 6.12, 1.64 Hz, 1H), 7.25-7.13 (m, 4H), 6.71-6.69 (d, J = 8.64 Hz, 1H), 6.64-6.61 (dt, J = 7.28, 7.2, 0.52, 0.36 Hz, 1H), 3.53-3.50 (m, 2H), 3.44-3.41 (t, J = 6, 5.96 Hz, 2H), 2.76-2.73 (t, J = 4.56, 4.52 Hz, 2H), 2.59-2.57 (t, J = 5.28, 5.2 Hz, 2H), 2.43-2.39 (t, J = 7.32, 7.16 Hz, 2H), 2.28-2.23 (t, J = 7.44, 7.32 Hz, 2H), 1.92-1.87 (m, 2H), 1.65-1.58 (quint, J = 7.56, 7.44, 7.36, 7.28 Hz, 2H), 1.49-1.42 (quint, J = 7.88, 7.52, 7.04 6.84 Hz, 2H); MS (LC/MS, $\text{M}+\text{H}^+$): 459.

Preparation of N-(4-(thiophen-3-yl)phenyl)-5-(4-(2-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)pentanamide (**7s**): The title compound was prepared according to the procedure for -(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide, except 1-(2-(trifluoromethyl)phenyl)-1,4-diazepane was substituted for 1-(2-methoxyphenyl)-1,4-diazepane to provide N-(4-(thiophen-3-yl)phenyl)-5-(4-(2-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)-pentanamide (51% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.64-7.56 (m, 6H), 7.52-7.48 (t, J = 7.32, 7.08 Hz, 2H), 7.42-7.34 (m, 4H), 7.21-7.17 (t, J = 7.64, 7.56 Hz, 1H), 3.19-3.14 (m, 4H), 2.90-2.87 (d, J = 5.88, 5.72 Hz, 2H), 2.83-2.80 (m, 2H), 2.65-2.62 (t, J = 7.32, 7.16 Hz, 2H), 2.46-2.43 (t, J = 7.48, 7.32 Hz, 2H), 1.96-1.90 (quint, J = 6.12, 6.08, 5.88 Hz, 2H), 1.86-1.78

(quint, $J = 7.56, 7.44, 7.28$ Hz, 2H), 1.68-1.61 (quint, $J = 7.8, 7.6, 6.96, 6.92$ Hz, 2H); MS (LC/MS, $M+H^+$): 502.

Preparation of 5-(4-(3-cyanophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7t**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)-pentanamide except 3-(1,4-diazepan-1-yl)benzonitrile was substituted for 1-(3,5-dichlorophenyl)piperazine to provide 5-(4-(3-cyanophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (39% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.52-7.46 (m, 4H), 7.33-7.28 (m, 3H), 7.21-7.17 (t, $J = 9.08, 7.04$ Hz, 2H), 6.88-6.86 (d, $J = 7.48$ Hz, 1H), 6.79-6.77 (d, $J = 7.72$ Hz, 1H), 3.55 (broad s, 2H), 3.42-3.38 (t, $J = 7.72, 3.24$ Hz, 2H), 2.86 (broad s, 2H), 2.72 (broad s, 2H), 2.62 (broad s, 2H), 2.36-2.32 (t, $J = 7, 6.84$ Hz, 2H), 2.04 (broad s, 2H), 1.70 (broad s, 2H), 1.61 (broad s, 2H); MS (LC/MS, $M+H^+$): 459.

Preparation of N-(4-(thiophen-3-yl)phenyl)-5-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)pentanamide (**7u**): The title compound was prepared according to the procedure for 5-(4-(3,5-

dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)-pentanamide, except 1-(3-(trifluoromethyl)phenyl)-1,4-diazepane was substituted for 1-(3,5-dichlorophenyl)piperazine to provide N-(4-(thiophen-3-yl)phenyl)-5-(4-(3-(trifluoromethyl)phenyl)-1,4-diazepan-1-yl)pentanamide (49% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.66-7.64 (d, J = 7.6 Hz, 2H), 7.56-7.54 (d, J = 7.76 Hz, 2H), 7.39-7.28 (m, 4H), 7.00-6.99 (d, J = 7.48 Hz, 1H), 6.86 (s, 1H), 6.82-6.80 (d, J = 8.24 Hz, 1H), 3.76 (broad s, 2H), 3.49 (broad s, 2H), 3.14 (broad s, 2H), 3.03 (broad s, 2H), 2.91 (broad s, 2H), 2.45 (broad s, 2H), 2.29 (broad s, 2H), 1.80 (broad s, 4H); MS (LC/MS, M+H⁺): 502.

Preparation of 5-(4-(2,3-dichlorophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7v**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)-pentanamide, except 1-(2,3-dichlorophenyl)-1,4-diazepane was substituted for 1-(3,5-dichlorophenyl)piperazine to provide 5-(4-(2,3-dichlorophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (38% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.99 (broad s, 1H), 7.60-7.57 (d, J = 8.6 Hz, 2H), 7.54-7.52 (d, J = 8.64 Hz, 2H), 7.39-7.38 (m, 1H), 7.37-7.34 (m, 2H), 7.09-7.07 (t, J = 3.32, 3 Hz, 2H), 6.98-6.95 (dd, J = 3.32, 3.28, 3.08 Hz, 1H), 3.28-3.25 (m, 4H), 2.83-2.78 (m, 4H), 2.58-2.55 (t, J = 7.32, 7.12, Hz, 2H), 2.44-2.40 (t, J = 7.48, 7.36 Hz, 2H); MS (LC/MS, M+H⁺): 503.

Preparation of 5-(4-(2,4-dichlorophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7w**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)-pentanamide, except 1-(2,4-dichlorophenyl)-1,4-diazepane was substituted for 1-(3,5-dichlorophenyl)piperazine to provide 5-(4-(2,4-dichloro-phenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (22% yield) ^1H NMR (400 MHz, CDCl_3) δ 7.67-7.65 (d, J = 8.12 Hz, 2H), 7.55-7.53 (d, J = 8.12 Hz, 2H), 7.39-7.34 (m, 4H), 7.19-7.16 (dd, J = 6.2, 2.44, 2.4 Hz, 1H), 7.00-6.97 (d, J = 8.68 Hz, 1H), 3.39 (broad s, 6H), 3.22 (broad s, 2H), 3.12 (broad s, 2H), 2.49 (broad s, 2H), 2.26 (broad s, 2H), 1.85 (broad s, 4H); MS (LC/MS, $\text{M}+\text{H}^+$): 503.

Preparation of 5-(4-(3,5-dichlorophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (**7x**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(4-(thiophen-3-yl)phenyl)-pentanamide, except 1-(3,5-dichlorophenyl)-1,4-diazepane was substituted for 1-(3,5-dichlorophenyl)piperazine to provide 5-(4-(3,5-dichlorophenyl)-1,4-diazepan-1-yl)-N-(4-(thiophen-3-yl)phenyl)pentanamide (41%

yield). ¹H NMR (400 MHz, CDCl₃) δ 7.64-7.62 (d, J = 8.12 Hz, 2H), 7.55-7.53 (d, J = 8.2 Hz, 2H), 7.40-7.35 (m, 3H), 6.75 (s, 1H), 6.51 (d, J = 1.36 Hz, 2H), 3.73 (broad s, 2H), 3.43-3.40 (d, J = 5.48, 5.4 Hz, 2H), 3.20 (broad s, 2H), 3.11 (broad s, 2H), 2.99 (broad s, 2H), 2.45 (broad s, 2H), 2.32 (broad s, 2H), 1.80 (broad s, 2H); MS (LC/MS, M+H⁺): 503.

Computational values: TPSA and cLogP values were calculated using the Dotmatics software suite (Dotmatics LLC The Old Monastery, Windhill Bishops, Stortford Herts, CW23 2ND UK).

Competitive radioligand-binding studies. For competitive binding studies, transfected HEK293 cell homogenates were suspended in homogenization buffer and incubated with radioligand [¹²⁵I]IABN, in the presence or absence of inhibitor at 37 °C for 60 min with [¹²⁵I]IABN (total volume = 150 ul) as previously described.**Error! Bookmark not defined.**

Competitive radioligand studies were performed to determine the concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC₅₀ value). The final radioligand concentration was approximately equal to the K_d value for the binding of the radioligand. For each competition curve, triplicates were performed using two concentrations of inhibitor per decade over five orders of magnitude. Binding was terminated by the addition of cold wash buffer (10 mM Tris–HCl/150 mM NaCl, pH = 7.5) and filtration over a glass-fiber filter (Pall A/B filters, #66198). A Packard Cobra Gamma Counter was used to measure the radioactivity of [¹²⁵I]IABN.

The competition curves were modeled for a single binding site using

$$B_s = B_o - ((B_o + L)/(IC_{50} + L))$$

where B_s is the amount of ligand bound to receptor and B_o is the amount of ligand bound to receptor in the absence of competitive inhibitor. L is the concentration of the competitive

inhibitor. The IC₅₀ value is the concentration of competitive inhibitor that inhibits 50% of the total specific binding. IC₅₀ values were determined using non-linear regression analysis with Table Curve 2D v 5.01 (Jandel, SYSTAT, Systat Software, Inc., San Jose, CA, USA). The values for Bns and Bo were constrained using experimentally derived values. The IC₅₀ values were converted to equilibrium dissociation constants (K_i) using the Cheng and Prusoff (1973) correction. Mean K_i values $\pm$ S.E.M. are reported for at least three independent experiments.

Microsomal Stability Analysis. Test compounds were assessed for microsomal stability by incubating them at 37 °C in the presence of rat liver microsomes and an NADPH regenerating system.**Error! Bookmark not defined.** Microsomal protein content was adjusted to give accurate rates of substrate consumption. Analysis was performed by Liquid Chromatography-tandem mass spectrometry (LC/MS/MS) analysis.

Table 1: *In vitro* pharmacological and ADME screening data for (7a) – (7x)

Entry	Ar ¹	Ar ²	N	D ₃	D ₂	D ₂ /D ₃	Sol	RLM
				K _I (nM)			μM	T _{1/2} min.
7a	2-OMe-Ph	thiophen-3-yl	1	3.1	108	34.8	7.6	5.6
7b	2-CN-Ph	thiophen-3-yl	1	6.2	250	40.3	8.9	6
7c	2-CF ₃ -Ph	thiophen-3-yl	1	20.5	438	21.4	2	19.3
7d	3-CN-Ph	thiophen-3-yl	1	14.9	8119	544.9	2	60
7e	3-CF ₃ -Ph	thiophen-3-yl	1	5	82.1	16.4	2	60
7f	2,3-Di-Cl-Ph	thiophen-3-yl	1	2	216	108.0	2	60
7g	2,4-Di-Cl-Ph	thiophen-3-yl	1	44.2	7500	169.7	2	60
7h	3,5-Di-Cl-Ph	thiophen-3-yl	1	16.5	6482	392.8	2	60
7i	2-OMe-Ph	2-Pyrdine	1	21.2	865	40.8	184	7.7
7j	2-CN-Ph	2-Pyrdine	1	8	715	89.4	123	5.8
7k	2-CF ₃ -Ph	2-Pyrdine	1	23.7	477.6	20.2	27	3.7
7l	3-CN-Ph	2-Pyrdine	1	11.4	1228	107.8	10.2	20.9

7m	3-CF ₃ -Ph	2-Pyrdine	1	9.7	717.3	73.9	23.5	10.9
7n	2,3-Di-Cl-Ph	2-Pyrdine	1	22	663.7	30.2	17.2	15.1
7o	2,4-Di-Cl-Ph	2-Pyrdine	1	36	10000	277.8	2.5	18.2
7p	3,5-Di-Cl-Ph	2-Pyrdine	1	4.5	353.1	78.5	2	60
7q	2-OMe-Ph	thiophen-3-yl	2	28.6	562	19.7	122	2
7r	2-CN-Ph	thiophen-3-yl	2	35.3	976	27.6	31.7	8.5
7s	2-CF ₃ -Ph	thiophen-3-yl	2	265	1912	7.2	35	ND
7t	3-CN-Ph	thiophen-3-yl	2	74.7	2038	27.3	30.9	2
7u	3-CF ₃ -Ph	thiophen-3-yl	2	92.2	6182	67.0	20.4	24.1
7v	2,3-Di-Cl-Ph	thiophen-3-yl	2	12.3	383	31.1	6.8	2.7
7w	2,4-Di-Cl-Ph	thiophen-3-yl	2	244	1486	6.1	2.7	44.5
7x	3,5-Di-Cl-Ph	thiophen-3-yl	2	253	8178	32.3	2	43.2

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