Design, Synthesis, and Evaluation of Functionalized 5-(4-arylpiiperazin-1-yl)-N-Quinoliny-pentanamides as Selective Dopamine D3 Receptor Ligands

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Design, synthesis, and evaluation of functionalized 5-(4-arylpiperazin-1-yl)-N-quinolinyl-pentanamides as selective dopamine D₃ receptor ligands

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Abstract: Dopamine (**1**) plays a key role in normal physiological pathways in both the central nervous system and the periphery. The physiological impact of this neurotransmitter is mediated through its interaction with family of G-protein-coupled receptors (GPCRs). These receptors are designated as D₁, D₂, D₃, D₄, and D₅ and divided into two sub-families, the D₁-like sub-family (D₁ and D₅) and D₂-like sub-family (D₂, D₃ and D₄) based on pharmacological properties, amino acid homology, and genetic organization. Aberrant D₃ activity has been linked to multiple diseases and conditions such as depression, schizophrenia, substance use disorder, inflammatory diseases, and Parkinson's disease (PD). As part of our on-going program focused on the identification of novel D₃ ligands, we have identified a novel series of 5-(4-arylpiperazin-1-yl)-N-quinolinyl-pentanamides that are high affinity ligands for this receptor.

Graphical Abstract:

Keywords: Dopamine, D₃ dopamine receptor, D₂ dopamine receptor

Introduction: In 1910, long before the pharmacological role of dopamine (**1**) (Figure 1) would be determined, George Barger and James Ewens described the first laboratory synthesis of this compound.[1] Nearly 50 years later, the presence of this compound in the human brain was reported by Katharine Montagu,[2] and shortly thereafter, Arvid Carlsson and Nils-Åke Hillarp determined that dopamine (**1**) functions as a neurotransmitter.[3] In the decades that followed, it was determined that dopamine's (**1**) impact on physiological systems is mediated by a family of

G-protein coupled receptors (GPCRs) that are designated as D₁, D₂, D₃, D₄, and D₅. There are two sub-families, the D₁-like (D₁ and D₅) sub-family and the D₂-like (D₂, D₃ and D₄) sub-family, that are based

Figure 1: Structures of Dopamine (1), Aripiprazole (2), risperidone (3), haloperidol (4), and pimozide (5)

on genetic organization, amino acid homology and pharmacological properties.[4] These receptors are expressed and functional in the both the central nervous system and the periphery, and their activation has been linked to wide range of physiological processes such as vasodilation, modulation of renal sodium excretion, altering urine output, learning, movement, and behavioral motivations.[5] Aberrant dopamine receptor activity, on the other hand, has been linked to numerous disease states and conditions including depression, schizophrenia, substance use disorder,[6] inflammatory diseases,[7] and Parkinson's disease (PD).[8] Multiple institutions and pharmaceutical companies have devoted significant resource to the development of novel dopamine receptor ligands capable of providing therapeutic relief to patients in need. Important medications such as Aripiprazole (2),[9] Risperidone (3),[10] Haloperidol (4),[11] and Pimozide (5)[12] (Figure 1) are potent dopamine receptor ligands that have proven useful for the treatment of conditions such as schizophrenia, bipolar disorder,[13] Tourette syndrome,[14] autism,[15] and tardive dyskinesia.[16].

We recently reported our effort to develop novel, selective, drug-like 5-(4-aryl)piperazin-1-yl)-pentanamides based D₃ ligands typified by (6) and (7) (Figure 2). In these studies, we

determined that aryl piperazine region plays a critical role in binding via its interaction with Asp^{3.32}, a key amino acid in the ligand binding pocket of D₃ and D₂. Selectivity for D₃ over D₂, on the other hand, was the product of the ability of D₃ to accommodate the biaryl moiety and the relative inability of D₂ to do the same.[17] We have recently extended our exploration of 5-(4-arylpiperazin-1-yl)-pentanamides in an effort to

determine if the biaryl region could be replaced with alternative moieties. Herein, we report on the synthesis and *in vitro* characterization of a series of 5-(4-arylpiperazin-1-yl)-pentanamides in which the biaryl region has been replaced with quinolines.

Results and Discussion: Our effort to determine the impact of incorporating quinolines into the 5-(4-arylpiperazin-1-yl)-pentanamide employed quinolin-3-amine and quinolin-2-amine. Target compounds were prepared as outlined in scheme 1. Quinoline amines (**6a**) or (**6b**) were reacted with 5-bromopentanoyl chloride (**7**) in the presence of triethylamine which provided the corresponding amides (**8a**) and (**8b**). Displacement of the bromine with an aryl piperazine (**9**) under basic conditions (K₂CO₃ in refluxing acetone) provided the target compounds (**10a-10k** and **11a-11f**). Aryl piperazine substituents for these studies were selected based on our previously reported collection of D₃ ligands.[18]

Table 1 includes the *in vitro* binding (K_i at D_3 and D_2) as well as the physicochemical properties (MW, TPSA, LogP), aqueous solubility, and rat liver microsomal (RLM) stability of target compounds (**10a-10k**) and (**11a-11f**). The compounds prepared and tested have MW, TPSA, and cLogP values that are consistent with drug like properties. Solubility of the quinolin-3-amine based analogs (**10a-10k**) was moderate to high (sol. = 23 – 196 μ M) with the notable exception of the 3,5-di-Cl (**10h**) and 2,4-di-Cl (**10i**) analogs (sol. = 2 – 4 μ M), while solubility of the quinolin-2-amine based analogs (**11a-11f**) was substantially lower (sol. = 2 – 42 μ M). RLM stability was low for the majority of examples prepared, but 5 compounds (**10a**, **10b**, **10g**, **10i**, **10k**) had moderate RLM stability ($T_{1/2}$ = 10 - 13 min.) and one example (**11e**) was highly stable in the presence of RLM ($T_{1/2}$ = 60 min.).

The structure activity analysis began with a series of 5-(4-arylpiperazin-1-yl)-N-(quinolin-3-yl)pentanamides (**10a-10k**). The 2-OMe aryl piperazine analog (**10a**) had high affinity for D_3 (K_i = 43.4 nM), but low selectivity for D_3 over D_2 (K_i = 176 nM). Relocating the methoxy substituent to the 4-position (**10b**) led to a substantial loss of D_3 affinity (K_i = 6896 nM) and D_2 affinity (K_i = 12,999 nM). Incorporating a 2-CF₃ moiety on the aryl piperazine (**10c**) also caused D_3 affinity to drop, but the decrease was less substantial (D_3 K_i = 94.5 nM), and selectivity over D_2 (3.7-fold) was similar to that observed with the 2-OMe analog (**10a**). D_3 affinity increased when the 2-OMe substituent was replaced with a 2-OCF₃ moiety (**10d**, D_3 K_i = 6.4 nM) and selectivity over D_2 increased to 6.7-fold. Further improvement in D_3/D_2 selectivity were observed when an electron withdrawing moiety was placed in the 3-position of the aryl piperazine. Specifically, the 3-CF₃ analog (**10e**) demonstrated high affinity for D_3 and a 17-fold separation between D_3 and D_2 affinity (D_3 K_i = 18.8 nM, D_2 K_i = 320 nM). The 3-CN analog

(**10f**) had less affinity for D₃ (K_i = 43.4 nM) than the 3-CF₃ analog (**10e**), but demonstrated improved selectivity for D₃ over D₂ (43.2-fold).

Disubstituted arylpiperazines selected based on previous work [19] produced mixed results. While the 2,3-di-Cl analog (**10g**) and the 3,5-di-Cl analog (**10h**) were both high affinity D₃ ligands (D₃ K_i = 11.9 nM and 33.7 nM respectively) and had moderate D₃/ D₂ selectivity (12.6 and 12.3-fold respectively), the 2,4-di-Cl analog (**10i**) had less affinity for D₃ (K_i = 207 nM) and decreased D₃/D₂ selectivity (5.4-fold). The 2-Me-5-F analog (**10j**) was less potent (D₃ K_i = 70.6 nM) and less selective (7.3-fold) than the 2,3-di-Cl analog (**10g**) and the 3,5-di-Cl analog (**10h**). Lastly, the 1-naphthyl analog (**10k**) demonstrated high affinity for D₃ (K_i = 7.8 nM) and moderate D₃/ D₂ selectivity (17.4-fold).

We next turned our attention to the quinolin-2-amine based analogs and prepared a series of six 5-(4-arylpiperazin-1-yl)-N-(quinolin-2-yl)pentanamides (**11a-11f**). All six analogs had high affinity for D₃ (K_i = 2.9 nM – 18.6 nM), but D₃/ D₂ selectivity was not uniform. Low D₃/ D₂ selectivity was observed with the 2-OCF₃ analog (**11a**, 7.1-fold), while the 3-CF₃ (**11b**), 2,3-di-Cl (**11c**), 2-Me-5-F (**11e**), and 1-naphthyl analog (**11f**) demonstrated moderate D₃/D₂ selectivity (14.9-fold to 25-fold). The 3,5-di-Cl analog (**11d**), however, was significantly more selective (49.8-fold). Based on its high affinity for D₃ (K_i = 11.6 nM) high, D₃/D₂ selectivity, (49.8-fold), and RLM stability (T_{1/2} = 60 min.), we elected to determine the impact of (**11d**) on Cyp3A4 activity and determine its stability in the presence of human liver microsomes (HLM). Our studies demonstrated that (**11d**) had limited capacity to inhibit Cyp3A4 (IC₅₀ = 10,000 nM) and was highly stable in the presence of HLM (T_{1/2} = 60 min.). These positive results were tempered by the observation that (**11d**) was poorly soluble in aqueous media (sol = 2 μM). Future efforts will be focused on improving the solubility of (**11d**) analogs.

Conclusion: In summary, we have prepared a series of 5-(4-arylpiperazin-1-yl)-N-arylpentanamides and evaluated their properties in a range of *in vitro* assays. Our studies demonstrate that the biaryl region of our 5-(4-arylpiperazin-1-yl)-pentanamides can be replaced with quinoline and produce high affinity D₃ ligands with varying degrees of D₃/D₂ selectivity (1.9-fold to 49.8-fold). In addition, we have identified a new lead compound (**11d**) that has 1) high affinity for D₃ ($K_i = 11.6$ nM), 2) good D₃/D₂ selectivity (49.8-fold), 3) stability in the presence of RLM and HLM ($T_{1/2} = 60$ min.), and 4) limited capacity to inhibit Cyp3A4 ($IC_{50} = 10,000$ nM). Future efforts will be focused on the further characterization of (**11d**) as a potential lead molecule and the design of additional (**11d**) analogs with the potential for increased solubility.

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. ¹H-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-(quinolin-3-yl)pentanamide (**8a**): A solution of 3-aminoquinoline (1.01 g, 7.01 mmol) and 5-bromovaleryl chloride (1.41 ml, 10.51 mmol) in anhydrous dichloromethane was stirred at room temperature. A liquid of anhydrous triethylamine (3.42 ml, 24.54 mmol) was added dropwise into the reaction at 0 °C, and the reaction mixture was stirred at room temperature for 16 hours. After 16 hours, the reaction was triturated with dichloromethane and filtered to give a filtrate. The filtrate was purified by normal phase chromatography (100% dichloromethane to 80% ethyl acetate) to provide 5-bromo-N-(quinolin-3-yl)pentanamide (62% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.83 (d, J = 2.6, 1H), 8.59 (s, 1H), 8.07 (d, J = 7.5 Hz, 1H), 7.81 (d, J = 7.1 Hz, 1H), 7.68 (m, 1H), 7.57 (m, 1H), 3.03 (t, J = 7.3 Hz, 2H), 2.51 (t, J = 7.1 Hz, 2H), 1.81 (m, 2H), 1.67 (m, 2H), MS (LC/MS, M+H⁺): 309.

Preparation of 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**): A solution of 5-bromo-N-(quinolin-3-yl)pentanamide (0.24 g, 0.781 mmol), 1-(2,3-dichlorophenyl)piperazine (0.15 ml, 0.781 mmol) and potassium carbonate (0.57 g, 4.09 mmol, 5.0 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 20% methanol in ethyl acetate) to provide 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (48% yield): ¹H NMR (400 MHz, CDCl₃) δ 8.80 (s, 2H), 8.57 (s, 1H), 8.03-8.01 (d, J = 8.4 Hz, 1H), 7.79-7.77 (d, J = 7.32 Hz, 1H), 7.64-7.60 (dt, J = 7, 5.6, 1.32 Hz, 1H), 7.55-7.51 (dt, J = 7, 6.96, 1 Hz, 1H), 7.17-7.11 (m, 2H), 6.94-6.91 (dd, J = 5.2, 2.16 Hz, 1H), 3.06 (broad s, 4H), 2.64 (broad s, 4H), 2.53-2.49 (t, J = 7.52, 7.32 Hz, 2H), 2.49-2.45 (t, J = 7.52, 7.32 Hz, 2H), 1.87-1.80 (quint, J = 7.6, 7.52, 7.44, 7.32 Hz, 2H), 1.68-1.60 (quint, J = 7.92, 7.76, 7.08, 7.04 Hz, 2H). MS (LC/MS, M+H⁺): 459.10.

Preparation of 5-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10a**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(2-methoxyphenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(2-methoxyphenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (55% yield) ¹H NMR (400 MHz, CDCl₃) δ 8.79 (d, J = 3.76 Hz, 1H), 8.78 (broad s, 1H), 8.49 (broad s, 1H),

8.04-8.02 (d, $J = 8.4$ Hz, 1H), 7.79-7.77 (dd, $J = 7.32, 0.88, 0.8$ Hz, 1H), 7.64-7.60 (dt, $J = 5.64, 5.56, 1.36$ Hz, 1H), 7.54-7.50 (dt, $J = 7, 6, 1.08, 1.04, 0.96$ Hz, 1H), 7.03-6.98 (m, 1H), 6.93-6.92 (m, 2H), 6.87-6.85 (d, $J = 7.68$ Hz, 1H), 3.86 (s, 3H), 3.09 (broad s, 4H), 2.65 (broad s, 4H), 2.51-2.48 (t, $J = 7.44, 7.32$ Hz, 2H), 2.47-2.43 (t, $J = 7.52, 7.32$ Hz, 2H), 1.86-1.79 (quint, $J = 7.6, 7.52, 7.48, 7.32$ Hz, 2H), 1.67-1.60 (quint, $J = 7.96, 7.84, 7.04, 7$ Hz, 2H); MS (LC/MS, $M+H^+$): 420.20.

Preparation of 5-(4-(4-methoxyphenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10b**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(4-methoxyphenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(4-methoxyphenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (52% yield) ^1H NMR (400 MHz, CDCl_3) δ 8.80 (d, $J = 2.4$, 1H), 8.76 (broad s, 1H), 8.66 (s, 1H), 8.03-8.00 (d, $J = 8.4$ Hz, 1H), 7.77-7.76 (d, $J = 7.36$ Hz, 1H), 7.64-7.60 (dt, $J = 7, 5.72, 1.32, 1.28, 1.24$ Hz, 1H), 7.54-7.50 (dt, $J = 7.08, 7, 0.92, 0.88$ Hz, 1H), 6.89-6.87 (d, $J = 9.24$ Hz, 2H), 6.85-6.82 (d, $J = 9.28$ Hz, 2H), 3.77 (s, 3H), 3.08-3.06 (t, $J = 5.04, 4.72$ Hz, 4H), 2.59-2.56 (t, $J = 4.88, 4.84$ Hz,

4H), 2.51-2.47 (t, J = 7.44, 7.32 Hz, 2H), 2.43-2.39 (t, J = 7.56, 7.24 Hz, 2H), 1.85-1.77 (quint, J = 7.6, 7.52, 7.48, 7.32 Hz, 2H), 1.65-1.57 (quint, J = 7.96, 7.8, 7.04 Hz, 2H); MS (LC/MS, M+H⁺): 419.20

Preparation of N-(quinolin-3-yl)-5-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (**10c**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(2-(trifluoromethyl)phenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide N-(quinolin-3-yl)-5-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (32% yield) ¹H NMR (400 MHz, MeOD-*d*₄) δ 8.89 (d, J = 2.52 Hz, 1H), 8.70 (d, J = 2.32 Hz, 1H), 7.96-7.94 (d, J = 8.44 Hz, 1H), 7.82-7.80 (d, J = 7.4 Hz, 1H), 7.65-7.58 (m, 2H), 7.56-7.51 (dt, J = 7.12, 6.12, 1, 0.92 Hz, 2H), 7.43-7.41 (d, J = 8.04 Hz, 1H), 7.26-7.22 (t, J = 7.6 Hz, 1H), 2.96-2.90 (m, 4H), 2.87-2.85 (m, 1H), 2.60 (broad s, 3H), 2.54-2.50 (t, J = 7.48, 7.2 Hz, 2H), 2.47-2.43 (t, J = 7.76, 7.68 Hz, 2H), 1.82-1.74 (quint, J = 7.64, 7.56, 7.32, 7.28 Hz, 2H), 1.69-1.61 (quint, J = 7.28, 6.8, 5.04, 5, 3.4, 2.96 Hz, 2H); MS (LC/MS, M+H⁺): 457.20.

Preparation of N-(quinolin-3-yl)-5-(4-(2-(trifluoromethoxy)phenyl)piperazin-1-yl)pentanamide (**10d**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(2-(trifluoromethoxy)phenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 N-(quinolin-3-yl)-5-(4-(2-(trifluoromethoxy)phenyl)piperazin-1-yl)pentanamide (41% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.35 (broad s, 1H), 8.83 (d, J = 2.48 Hz, 1H), 8.78 (s, 1H), 8.00-7.99 (d, J = 8.4 Hz, 1H), 7.74-7.73 (d, J = 7.48 Hz, 1H), 7.61-7.56 (dt, J = 7, 5.64, 1.36, 1.32, Hz, 1H), 7.50-7.47 (dt, J = 7.08, 7, 0.96, 0.92, Hz, 1H), 7.49-7.47 (t, J = 7.08, 7 Hz, 1H), 7.28-7.16 (m, 2H), 6.99-6.93 (m, 2H), 3.57 (s, 1H), 3.06-3.04 (t, J = 4.4, 4.24 Hz, 4H), 2.55 (broad s, 4H), 2.50-2.46 (t, J = 7.52, 7.36 Hz, 2H), 2.40-2.37 (t, J = 7.56, 7.24 Hz, 2H), 1.83-1.75 (quint, J = 7.68, 7.44, 7.36 Hz, 2H), 1.63-1.55 (quint, J = 7.92, 7.76, 7.08, 7 Hz, 2H); MS (LC/MS, M+H⁺): 473.20.

Preparation of N-(quinolin-3-yl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (**10e**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(3-(trifluoromethyl)phenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 reverse phase chromatography (30% acetonitrile/0.1% formic acid in water) and normal amine phase (RediSep Rf Gold® Amine #: 69-2203-507) chromatography (100% hexane to 100% ethyl acetate) to provide N-(quinolin-3-yl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (37% yield). ¹H NMR (400 MHz, MeOD-*d*4) δ 8.92 (d, *J* = 2.48 Hz, 1H), 8.70 (d, *J* = 2.32 Hz, 1H), 8.55 (s, 1H), 7.98-7.96 (d, *J* = 8.4 Hz, 1H), 7.85-7.83 (d, *J* = 7.56 Hz, 1H), 7.68-7.64 (dt, *J* = 5.56, 5.52, 1.44, 1.4, 1.36 Hz, 1H), 7.59-7.55 (dt, *J* = 7, 5.96, 1.08, 1 Hz, 1H), 7.19-7.17 (d, *J* = 7.44 Hz, 2H), 7.12-7.10 (d, *J* = 7.6 Hz, 1H), 3.36-3.33 (t, *J* = 6.76, 4.84 Hz, 4H), 2.97-2.94 (t, *J* = 5.08, 4.96 Hz, 4H), 2.79-2.76 (t, *J* = 7.88, 7.04 Hz, 2H), 2.57-2.54 (t, *J* = 6.96, 6.8 Hz, 2H), 1.86-1.72 (m, 4H); MS (LC/MS, M+H⁺): 457.20.

Preparation of 5-(4-(3-cyanophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10f**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 3-(piperazin-1-

yl)benzonitrile was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(3-cyanophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (39% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.80-8.78 (t, J = 3.68, 2.52 Hz, 2H), 8.06 (broad s, 1H), 8.05-8.03 (d, J = 8.56 Hz, 1H), 7.82 (dd, J = 7.2, 0.92 Hz, 1H), 7.66-7.62 (dt, J = 5.56, 5.52, 1.4 Hz, 1H), 7.57-7.53 (dt, J = 5.92, 5.8, 1.16, 1 Hz, 1H), 7.34-7.30 (m, 1H), 7.10 (m, 3H), 3.24-3.21 (t, J = 5.08, 4.96 Hz, 4H), 2.62-2.60 (t, J = 5.08, 4.96 Hz, 4H), 2.55-2.51 (t, J = 7.4, 7.28 Hz, 2H), 2.49-2.45 (t, J = 7.44, 7.28 Hz, 2H), 1.89-1.82 (quint, J = 7.6, 7.56, 7.48, 7.28 Hz, 2H), 1.70-1.63 (quint, J = 7.92, 7.84, 7.04, 7 Hz, 2H); MS (LC/MS, M+H⁺): 414.20.

Preparation of 5-(4-(3-cyanophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10f**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 3-(piperazin-1-yl)benzonitrile was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(3-cyanophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (29% yield) ^1H NMR (400 MHz, CDCl_3) δ 8.80-8.78 (t, J = 3.68, 2.52 Hz, 2H), 8.06 (broad s, 1H), 8.05-8.03 (d, J = 8.56 Hz, 1H), 7.82 (dd, J = 7.2, 0.92 Hz, 1H), 7.66-7.62 (dt, J = 5.56, 5.52, 1.4 Hz, 1H), 7.57-7.53 (dt, J = 5.92, 5.8, 1.16, 1 Hz, 1H), 7.34-7.30 (m, 1H), 7.10 (m, 3H), 3.24-3.21 (t, J = 5.08, 4.96 Hz, 4H), 2.62-2.60 (t, J = 5.08, 4.96 Hz, 4H), 2.55-2.51 (t, J = 7.4, 7.28 Hz, 2H), 2.49-2.45 (t, J = 7.44, 7.28 Hz, 2H), 1.89-1.82 (quint, J = 7.6, 7.56, 7.48, 7.28 Hz, 2H), 1.70-1.63 (quint, J = 7.92, 7.84, 7.04, 7 Hz, 2H); MS (LC/MS, $\text{M}+\text{H}^+$): 414.20.

Preparation of 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10h**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(3,5-dichlorophenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (33% yield) ^1H NMR (400 MHz, CDCl_3) δ 9.05 (s, 1H), 8.83 (d, J = 2.52 Hz, 1H), 8.74 (d, J = 1.96 Hz, 1H), 8.00-7.98 (d, J = 8.32 Hz, 1H), 7.75-7.73 (d, J = 7.48 Hz, 1H), 7.62-7.58 (dt, J = 7, 5.72, 1.32,

1.28, 1.24 Hz, 1H), 7.52-7.48 (dt, $J = 7, 0.92, 0.88$ Hz, 1H), 6.77-6.76 (m, 1H), 6.67 (d, $J = 1.68$ Hz, 2H), 3.12-3.09 (t, $J = 5.16, 4.72$ Hz, 4H), 2.50-2.46 (m, 6H), 2.36-2.33 (t, $J = 7.48, 7.24$ Hz, 2H), 1.82-1.74 (quint, $J = 7.6, 7.52, 7.48, 7.4$ Hz, 2H), 1.60-1.52 (quint, $J = 7.96, 7.72, 7.12, 7.04$ Hz, 2H); MS (LC/MS, $M+H^+$): 459.20.

Preparation of 5-(4-(2,4-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10i**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(2,4-dichlorophenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(2,4-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (35% yield). ^1H NMR (400 MHz, $\text{MeOD}-d_4$) δ 8.93 (d, $J = 2.32$ Hz, 1H), 8.72 (d, $J = 2.12$ Hz, 1H), 8.00-7.98 (d, $J = 8.44$ Hz, 1H), 7.90-7.88 (d, $J = 7.8$ Hz, 1H), 7.72-.67 (dt, $J = 7.04, 5.64, 1.32, 1.28$ Hz, 1H), 7.63-7.59 (dt, $J = 7.12, 7.04, 0.88, 0.84$ Hz, 1H), 7.46-7.45 (d, $J = 2.4$ Hz, 1H), 7.33-7.30 (dd, $J = 6.2, 2.44, 2.4$ Hz, 1H), 7.17-7.15 (d, $J = 8.68$ Hz, 1H), 3.23 (broad s, 8H), 3.02 (broad s, 2H), 2.60 (broad s, 2H), 1.85 (broad s, 4H); MS (LC/MS, $M+H^+$): 459.10.

Preparation of 5-(4-(5-fluoro-2-methylphenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10j**): The title compound was prepared according to the procedure for 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(5-fluoro-2-methylphenyl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(5-fluoro-2-methylphenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (44% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.73-8.71 (t, J = 4.4, 2.52 Hz, 2H), 7.95-7.93 (d, J = 8.32 Hz, 1H), 7.71-7.69 (d, J = 8.16 Hz, 1H), 7.56-7.52 (t, J = 8.04, 7.12 Hz, 1H), 7.46-7.42 (t, J = 7.68, 7.28 Hz, 1H), 7.02-6.99 (t, J = 7.56, 7.32 Hz, 1H), 6.62-6.57 (m, 2H), 2.84 (broad s, 4H), 2.56 (broad s, 4H), 2.46-2.40 (m, 4H), 2.14 (s, 3H), 1.79-1.72 (quint, J = 7.44, 7.4, 7.36 Hz, 2H), 1.62-1.57 (quint, J = 7.12, 7, 6.92 Hz, 2H); MS (LC/MS, M+H⁺): 421.20

Preparation of 5-(4-(naphthalen-1-yl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10k**): The title compound was prepared according to the procedure for 5-(4-(2,3-

dichlorophenyl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (**10g**), except 1-(naphthalen-1-yl)piperazine was substituted for 1-(2,3-dichlorophenyl)piperazine. The reaction mixture 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 (20% methanol in ethyl acetate) to provide 5-(4-(naphthalen-1-yl)piperazin-1-yl)-N-(quinolin-3-yl)pentanamide (38% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.71 (s, 2H), 8.11-8.09 (m, 1H), 8.05 (broad s, 1H), 7.97-7.95 (d, J = 8.36 Hz, 1H), 7.75-7.71 (m, 2H), 7.57-7.53 (5.56, 5.52, 1.4, 1.36 Hz, 1H), 7.48-7.45 (m, 2H), 7.40-7.37 (m, 2H), 7.33-7.29 (t, J = 8.12, 7.52 Hz, 1H), 7.01-6.99 (dd, J = 6.76, 0.68, 0.64 Hz, 1H), 3.08 (broad s, 4H), 2.70 (broad s, 4H), 2.50-2.48 (t, J = 7.2, 4.4 Hz, 2H), 2.47-2.44 (t, J = 7.48, 4.24 Hz, 2H), 1.84-1.76 (quint, J = 7.52, 7.48, 7.4, 7.28 Hz, 2H), 1.68-.60 (quint, J = 7.84, 7.76, 6.96, 6.92 Hz, 2H); MS (LC/MS, M+H⁺): 439.30.

Preparation of 5-bromo-N-(quinolin-2-yl)pentanamide (**8b**): A solution of 2-aminoquinoline (0.9784 g, 6.79 mmol) and 5-bromovaleryl chloride (0.91 ml, 6.79 mmol) in anhydrous dichloromethane was stirred at room temperature. Anhydrous triethylamine (2 ml, 14.26 mmol, 2.1 eq) was added dropwise into the reaction at 0°C, and the reaction mixture was stirred at room temperature for 16 hours. After 16 hours, the reaction was triturated with dichloromethane and filtered to give a filtrate. The filtrate was purified by normal phase chromatography (100% dichloromethane to 100% ethyl acetate) to provide 5-bromo-N-(quinolin-2-yl)pentanamide (73% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.51 (d, J = 8.5 Hz, 2H), 8.17 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 7.73 (m, 1H), 7.493 (m, 1H), 3.07 (t, J = 7.2

Hz, 2H), 2.51 (t, J = 7.1 Hz, 2H), 1.95 (m 2H), 1.61 (m, 2H), MS (LC/MS, M+H⁺): 308.

Preparation of 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11d**): A solution of 5-bromo-N-(quinolin-2-yl)pentanamide (0.1521 g, 0.495 mmol), 1-(3,5-dichlorophenyl)piperazine (0.12 g, 0.495 mmol) and potassium carbonate (0.85 g, 6.1 mmol, 5.0 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 20% methanol in ethyl acetate) to provide 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (31% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.45-8.43 (d, J = 8.64 Hz, 2H), 8.21-8.19 (d, J = 8.96 Hz, 1H), 7.81-7.79 (d, J = 8.64 Hz, 2H), 7.70-7.66 (dt, J = 6.04, 5.52, 1.64, 1.4, 1.28 Hz, 1H), 7.49-7.45 (dt, J = 5.88, 5.84, 1.24, 1.16, 1.08 Hz, 1H), 6.81-6.80 (d, J = 1.68 Hz, 1H), 6.75 (d, J = 1.72 Hz, 2H), 3.26-3.23 (t, J = 5.12, 4.96 Hz, 4H), 2.61-2.58 (t, J = 5.04, 4.92 Hz, 4H), 2.53-2.49 (t, J = 7.32, 7.28 Hz, 2H), 2.48-2.44 (t, J = 7.44, 7.12 Hz, 2H), 1.88-1.81 (quint, J = 7.6, 7.48, 7.24 Hz, 2H), 1.69-1.62 (quint, J = 7.8, 7.08, 7 Hz, 2H); MS (LC/MS, M+H⁺): 458.

Preparation of N-(quinolin-2-yl)-5-(4-(2-(trifluoromethoxy)phenyl)piperazin-1-yl)pentanamide (**11a**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11d**), except 1-(2-(trifluoromethoxy)phenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide N-(quinolin-2-yl)-5-(4-(2-(trifluoromethoxy)phenyl)piperazin-1-yl)pentanamide (44% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.09 (s, 1H), 8.48-8.46 (d, J = 8.88 Hz, 1H), 8.20-8.18 (d, J = 9 Hz, 1H), 7.84-7.82 (d, J = 8.48 Hz, 2H), 7.80-7.78 (d, J = 7.68 Hz, 2H), 7.69-7.64 (dt, J = 7.04, 5.68, 1.36, 1.32, 1.28 Hz, 1H), 7.48-7.44 (dt, J = 7, 0.96 Hz, 1H), 7.28-7.18 (m, 2H), 7.01-6.95 (m, 2H), 3.11-3.08 (t, J = 4.48, 4.32 Hz, 4H), 2.58 (broad s, 4H), 2.47-2.44 (t, J = 7.44, 7.4 Hz, 2H), 2.41-2.37 (t, J = 7.52, 7.24 Hz, 2H), 1.83-1.76 (quint, J = 7.68, 7.48, 7.36 Hz, 2H), 1.62-1.54 (quint, J = 7.88, 7.72, 7.12 Hz, 2H); MS (LC/MS, M+H⁺): 473.20.

Preparation of N-(quinolin-2-yl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-

yl)pentanamide (**11b**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11d**), except 1-(3-(trifluoromethyl)phenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide N-(quinolin-2-yl)-5-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)pentanamide (48% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.37-8.35 (d, J = 8.92 Hz, 1H), 8.12-8.09 (d, J = 8.96 Hz, 1H), 7.72-7.70 (d, J = 8.36 Hz, 2H), 7.60-7.56 (dt, J = 6.84, 5.72, 1.48, 1.44, 1.24 Hz, 1H), 7.40-7.36 (dt, J = 7.32, 6.88, 0.96, 0.72 Hz, 1H), 7.28-7.24 (t, J = 7.96, 7.92 Hz, 1H), 7.03-6.96 (m, 3H), 3.21-3.19 (t, J = 5.8, 4.92 Hz, 4H), 2.58-2.55 (t, J = 5, 4 Hz, 4H), 2.45-2.38 (m, 4H), 1.79-1.72 (quint, J = 7.6, 7.48, 7.44, 7.24 Hz, 2H), 1.62-1.54 (quint, J = 7.92, 7.88, 7, 6.88 Hz, 2H); MS (LC/MS, M+H⁺): 457.30.

Preparation of 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11c**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11d**), except 1-(2,3-dichlorophenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(2,3-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (37% yield). ¹H NMR (400 MHz, MeOD-*d*4) δ 8.29-8.23 (dd, J = 8.96, 8.92, 7.08 Hz, 2H), 7.85-7.82 (t, J = 7, 6.72 Hz, 2H), 7.69-7.65 (t, J = 7.52, 7.4 Hz, 1H), 7.48-7.44 (t, J = 7.56, 7.36 Hz, 1H), 7.23-7.22 (t, J = 3.64, 1.92 Hz, 2H), 7.08-7.06 (m, 1H), 3.17 (broad s, 4H), 3.04 (broad s, 4H), 2.85 (broad s, 2H), 2.57 (broad s, 2H), 1.79 (broad s, 4H); MS (LC/MS, M+H⁺): 457.10.

Preparation of 5-(4-(5-fluoro-2-methylphenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11e**): The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11d**), except 1-(5-fluoro-2-methylphenyl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(5-fluoro-2-methylphenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (52% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.43 (broad s, 1H), 8.37-8.34 (d, J = 8.92 Hz, 1H), 8.11-8.09 (d, J = 8.96 Hz, 1H), 7.75-7.70 (t, J = 8.6, 7.48 Hz, 2H), 7.61-7.57 (dt, J = 5.52, 1.48, 1.44,

1.4 Hz, 1H), 7.39-7.35 (dt, J = 5.92, 5.88, 1.08, 1.04 Hz, 1H), 7.02-6.99 (t, J = 7.84, 7.24 Hz, 1H), 6.66-6.62 (dd, J = 8.4, 2.6, 2.56 Hz, 1H), 6.61-6.56 (dt, J = 5.56, 2.64, 2.6 Hz, 1H), 2.86-2.84 (t, J = 4.68, 4.6 Hz, 4H), 2.53 (broad s, 4H), 2.43-2.36 (quart, J = 7.48, 7.32, 6.88, 6.64 Hz, 4H), 2.16 (s, 3H), 1.78-1.71 (quint, J = 7.56, 7.52, 7.32 Hz, 2H), 1.59-1.52 (quint, J = 7.88, 7.08, 7 Hz, 2H); MS (LC/MS, M+H⁺): 421.30.

Preparation of 5-(4-(naphthalen-1-yl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11f**):

The title compound was prepared according to the procedure for 5-(4-(3,5-dichlorophenyl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (**11d**), except 1-(naphthalen-1-yl)piperazine was substituted for 1-(3,5-dichlorophenyl)piperazine. The reaction mixture 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 20% methanol in ethyl acetate) to provide 5-(4-(naphthalen-1-yl)piperazin-1-yl)-N-(quinolin-2-yl)pentanamide (47% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.87 (broad s, 1H), 8.49-8.47 (d, J = 8.92 Hz, 1H), 8.23-8.18 (m, 2H), 7.86-7.79 (m, 3H) 7.70-7.66 (dt, J= 5.56, 5.52, 1.48, 1.44, 1.4 Hz, 1H), 7.57-7.55 (d, J = 8.2 Hz, 1H), 7.52-7.42 (m, 3H), 7.40-7.28 (t, J = 8.08, 7.52 Hz, 1H), 3.17 (broad s, 4H), 2.74 (broad s, 4H), 2.52-2.49 (t, J = 7.6, 7.16 Hz, 4H), 1.89-1.81 (quint, J = 7.6, 7.52, 7.32 Hz, 2H), 1.70-1.62 (quint, J = 7.92, 7 Hz, 2H); MS (LC/MS, M+H⁺): 439.

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). 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 B_{ns} and B_o 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.

Aqueous solubility (pH 7.4) assay: Compounds were assessed for their solubility at pH 7.4 using the commercially available Millipore MultiScreen™ Solubility filter system (Millipore, Billerica, MA). Analysis was performed by liquid chromatography tandem mass spectrometry (LC/MS/MS).

Cytochrome P450 3A4 inhibition assay: Compounds were assessed for their ability to inhibit human cytochrome P450 3A4 using testosterone as a substrate and LC/MS/MS analysis. Expressed enzymes was used to minimize non-specific binding and membrane partitioning issues.[20]

Microsomal stability assays: Test compounds were assessed for microsomal stability by incubating them at 37 °C in the presence of mouse or human liver microsomes and an NADPH regenerating system as described by Yang et. al.[21] 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 **(10a)** – **(10k)** and **(11a)** – **(11f)**

Entry	Ar	Y	Z	D ₃	D ₂	D ₂ /D ₃	MW	TPSA	cLogP	Sol	RLM
				K_i (nM)		ratio				(μ M)	T _{1/2} (min)
10a	2-OMe-Ph	N	CH	43.4	176	4.0	419	58	3.6	196	10
10b	4-OMe-Ph	N	CH	6896	12999	1.9	419	58	3.6	23	11
10c	2-CF ₃ -Ph	N	CH	94.5	351	3.7	457	49	4.6	41	5
10d	2-OCF ₃ -Ph	N	CH	6.4	43	6.7	473	58	5.0	37	4
10e	3-CF ₃ -Ph	N	CH	18.8	320	17	457	49	4.6	41	8
10f	3-CN-Ph	N	CH	43.4	1875	43.2	414	72	3.4	82	6
10g	2,3-Di-Cl-Ph	N	CH	11.9	138	12.6	457	49	4.9	28	13

10h	3,5-Di-Cl-Ph	N	CH	23.7	291	12.3	457	49	4.9	4	7
10i	2,4-Di-Cl-Ph	N	CH	207.0	1121	5.4	457	49	4.9	2	11
10j	2-Me-5-F-Ph	N	CH	70.6	514	7.3	421	49	4.0	26	6
10k	1-Naphthyl	N	CH	7.8	136	17.4	439	49	4.9	13	13
11a	2-OCF ₃ -Ph	CH	N	2.9	21	7.1	473	58	5.4	12	2
11b	3-CF ₃ -Ph	CH	N	18.6	465	25	457	49	5.1	9	8
11c	2,3-Di-Cl-Ph	CH	N	4.1	94	22.9	457	49	5.3	5	7
11d	3,5-Di-Cl-Ph	CH	N	11.6	578	49.8	457	49	5.3	2	60
11e	2-Me-5-F-Ph	CH	N	13.9	207	14.9	421	49	4.4	42	4
11f	1-Naphthyl	CH	N	10.9	182	16.7	439	49	5.3	13	7

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