Accepted Manuscript

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PII: S0223-5234(17)31093-0

DOI: 10.1016/j.ejmech.2017.12.063

Reference: EJMECH 10041

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 10 November 2017

Revised Date: 16 December 2017

Accepted Date: 16 December 2017

Please cite this article as: Z.-S. Gu, A.-n. Zhou, Y. Xiao, Q.-W. Zhang, J.-Q. Li, Synthesis and antidepressant-like activity of novel aralkyl piperazine derivatives targeting SSRI/5-HT_{1A}/5-HT_7, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2017.12.063.

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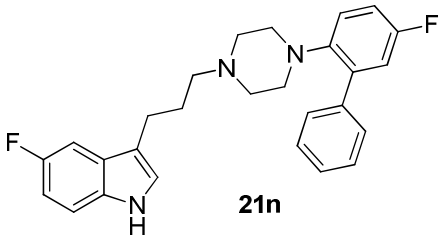
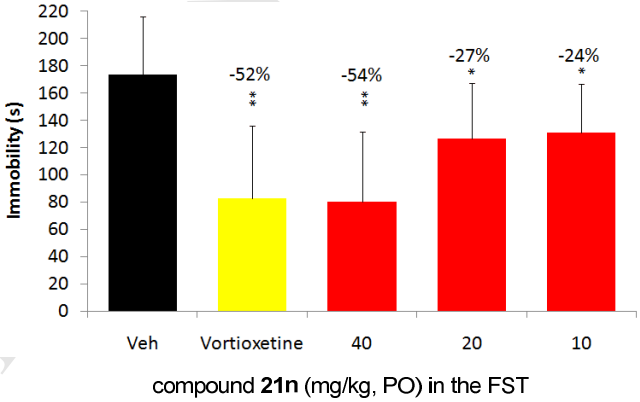
Graphical abstract



IC$_{50}$ (nM)	Ki (nM)	
RUI	5-HT$_{1A}$	5-HT$_7$
25	28	3.3



Synthesis and antidepressant-like activity of novel aralkyl piperazine derivatives targeting SSRI/5-HT_{1A}/5-HT_7

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Abstract

A series of novel aralkyl piperazine derivatives were synthesized, and evaluated for their serotonin reuptake inhibitory and 5-HT_{1A} /5-HT_7 receptors affinities activity. Antidepressant activities *in vivo* of the compounds were screened using the forced swimming test (FST) and tail suspension test (TST). The results indicated that compounds **21k** (RUI, IC_{50} = 31 nM; 5-HT_{1A}, 5-HT_7, k_i = 62, 12 nM) and **21n** (RUI, IC_{50} = 25 nM; 5-HT_{1A}, 5-HT_7, k_i = 28, 3.3 nM) exhibited high affinities for the 5-HT_{1A}/5-HT_7 receptors coupled with potent serotonin reuptake inhibition. Specifically, the most promising compound **21n** possessed a good oral pharmacokinetic properties and an acceptable hERG profile, and showed potent antidepressant-like effect in the FST and TST models.

Key words

Antidepressant, serotonin reuptake inhibitory, 5-HT_{1A} receptor, 5-HT_7 receptor

1. Introduction

Depression is a debilitating disease that is characterized by low mood, slow thought and mental disorder[1]. According to the World Health Organization (WHO), depression, especially major depression disorder, would be the second leading cause of disability worldwide by the year 2020[2]. Over the past six decades, about six major classes of monoamine based drugs have been developed in the therapy of depression[3]. Despite the extensive use of antidepressant drugs, there are still significant unmet needs in the treatment of depression, including a long onset of action, moderate patient response, and numerous adverse effects such as nausea/emesis, weight gain, insomnia and sexual dysfunction[4-5]. Recently, there has been important achievement of antidepressant development, which interacts with dual or multiple targets. The combination of serotonin transporter (SERT) inhibition with various 5-HT receptor subtypes seems to be one of the most valuable approach to antidepressive therapy[6]. Recent clinical research has demonstrated that pindolol could accelerate antidepressants onset time and enhance SSRIs beneficial effects by affecting 5-HT_{1A} receptor [7]. Similarly, the approved antidepressant vilazodone with high affinity and selectivity for 5-HT transporter and 5-HT_{1A} receptor displayed fast antidepressant efficacy with minimal adverse effects[8]. Moreover, the combined administration of low doses of an SSRI with selective 5-HT_7 receptor antagonist (SB-269970) was also found active in behavioral models of depression[9]. Additionally, recent research has indicated that the vortioxetine showed obvious cognitive enhancement effects through blockade of 5-HT_7 receptor [10]. Hence, it stands to reason that agents with dual or multiple binding affinities to SERT/5-HT_{1A}/5-HT_7 may be beneficial as treatment options for depression and other cognitive impairment disorders.

Earlier study by Mewshaw et al. showed that aryloxylethylindolealkylamine derivative **I** displayed high affinities for the SERT and 5-HT_{1A} receptor[11]. Venkatesan et al. reported that the benzofuran derivative **II** exhibited potent binding affinity for the 5-HT_{1A} receptor and SERT [12]. Moreover, our previous research reported that the aryl piperazine benzo[b][1,4]oxazine derivative **III** exhibited high affinities for 5-HT_{1A} receptor coupled with moderate serotonin reuptake inhibitory[13] (Fig. **2**).

Meanwhile, considerable research efforts have also been directed toward the identification of novel compounds targeting 5-HT_{1A} and 5-HT_7 receptor. Leopoldo et al. disclosed that 2-biphenyl piperazine derivatives **IV-VI** showed high affinity for 5-HT_{1A} and 5-HT_7 receptors [14-16] (Fig. **2**). Brasili et al. focused on the development of novel potent 5-HT_{1A} ligands[17], and reported that 1,4-dithiaspiro[4.5]decane derivative **VII** and 1-(2-methoxyphenyl)piperazine derivatives **VIII-X** exhibited high affinity and moderate to good selectivity for 5-HT_{1A}R (Fig. **3**)[18-21].

Our lab has been engaged in multiple receptor-targeting in order to extend the scope and utility of CNS agents. Herein we designed and synthesized a series of novel aralkyl piperazine derivatives by modifying the scaffold of compound **II**. As shown in Fig. **4**, we first explored the effect of different aromatic ring substituents at the N1 position on the affinity for 5-HT_{1A} and 5-HT_7 receptors. Additionally, we examined whether serotonin reuptake inhibition was improved by replacing the benzofuran moiety with indole moiety. From these studies, we have discovered a new series of compounds that exhibited high binding affinities at 5-HT_{1A}/5-HT_7 receptors coupled with potent 5-HT reuptake inhibitory activity, and also demonstrated excellent oral bioavailability and marked antidepressant-like activity in animal behavioral models.

2. Results and discussion

2.1 Chemistry

The synthetic routes of target compounds were outlined in Schemes **1-5**. In scheme **1**, a series of benzofuran-3-yl piperazine derivatives (**6a-d**) were prepared. The starting material benzofuran-3(2H)-one **1** was converted into ethyl ester **2** via the Wittig reaction. Then intermediate **2** was reduced with lithium tetrahydroaluminate into the corresponding alcohol **3**. Activation of alcohol **3** with 4-toluenesulfonyl chloride in the presence of triethylamine at room temperature provided intermediate **4**. The resulting compound **4** was then treated with benzyl piperazine derivatives **5** via SN2 mechanism to yield **6a-d**. The benzofuran-2-yl piperazine derivative **11** was constructed by coupling of 2-iodophenol **7** with but-3-yn-1-ol **8**, and subsequent activation, substitution with 1-([1,1'-biphenyl]-2-yl)piperazine **5c** (Scheme **2**).

According to scheme **3**, the 1H-indole-5-carbonitrile piperazine analog **15** was prepared. The 2-(5-bromo-1H-indol-3-yl)ethanol **12** was treated with CuCN to yield alcohol **13**. The target compound **15** was afforded by bromination of **13** with tetrabromomethane and triphenylphosphine, and substitution with 1-([1,1'-biphenyl]-2-yl)-piperazine **5c**.

The preparation of the desired 5-fluoro-1H-indole piperazine derivatives **21** was depicted in scheme **4**. The alcohols **18a** and **18b** were obtained by the Fischer indole synthesis reaction of 4-Fluorophenylhydrazine hydrochloride **17** with 2,3-dihydrofuran **16a** and 3,4-dihydro-2H-pyran **16b**, respectively. Bromination of compound **18a** with tetrabromomethane and triphenylphosphine provided compound **19a**. Activation of alcohol **18b** with 4-toluenesulfonyl chloride in the presence of triethylamine at room temperature provided compound **19b**. The desired compounds **21a-n** were offered by nucleophilic substitution reaction of intermediates **19a-b** with aryl piperazine derivatives **20**. Finally, the four carbon linker compound **26** was prepared (scheme **5**). The acylation of the aromatic ring **22** with 4-chlorobutanoyl chloride provided compound **23**. Then intermediate **23** was reduced with triethylsilane and trifluoroacetic acid to yield compound **24**. The compound **25** was obtained by substitution of intermediate **24** with aryl piperazine derivative **20f**. The deprotection of intermediate **25** with 4N aqueous NaOH solution gave the desired compound **26**.

The synthesis of commercially unavailable biphenyl piperazine intermediates **20a-i** was shown in scheme **6**. The biphenyl amine compounds **27** were treated with bis(2-chloroethyl)amine hydrochloride **28** via SN2 mechanism to obtain **20a-i**.

2.2 Biology

All synthesized compounds were tested for their inhibition of 5-HT reuptake and binding affinities at 5-HT_{1A}/5-HT_7 receptors *in vitro* assays. The following specific radioligands and tissue sources were used: (1) 5-HT_{1A} receptors, [^{3}H] 8-OH-DPAT, human recombinant (HEK-293 cells); (2) 5-HT_7 receptors, [^{3}H] LSD, human recombinant (CHO cells); (3) Rat serotonin transporter, [^{3}H] serotonin, rat brain synaptosomes. All compounds were initially screened at 10 μM concentration, and

selective compounds (inhibition >90%) were then assayed to obtain their IC_{50} and Ki values. Vortioxetine was used as a reference. The detailed results were summarized in Table **1**.

The role of aromatic ring substitution at the N1 position was first explored. The methoxyl **6a** and methylthio **6b** analogs were selectively inhibitory for 5-HT_{1A} receptor and 5-HT reuptake (inhibition ratio > 90%). Interestingly, the 2-phenyl analog **6c** displayed high potency for 5-HT reuptake inhibition [RUI, IC_{50} = 210 nM] and kept more than 90% inhibition for 5-HT_{1A} and 5-HT_7 receptors, whereas the 3-phenyl analog **6d** showed less than 80% inhibition for 5-HT_7 receptor at the concentration of 10 μM. These results showed the importance of phenyl group at the R1 position in interacting with 5-HT_7 receptor. When the 2-phenyl feature remained, the replacement of the benzofuran-3-yl moiety with the benzofuran-2-yl moiety (compound **11**) dramatically decreased inhibition of 5-HT reuptake [RUI, IC_{50} = 2900 nM]. Meanwhile, the replacement of benzofuran moiety with the 5-carbonitrile-indole moiety (compound **15**) [RUI, IC_{50} = 190 nM] or 5-fluoro-indole moiety (compound **21a**) [RUI, IC_{50} = 110 nM] increased inhibition of 5-HT reuptake. In addition, compound **21a** displayed high affinities for both the 5-HT_{1A} and 5-HT_7 receptors [5-HT_{1A}, k_i = 0.53 nM; 5-HT_7, k_i = 0.26 nM]. The results showed that the 5-fluoro-indole-3-yl could be a preferred substituent (Table **1**).

In the next research, we focused on the modification of biphenyl moiety of compound **21a**. We first introduced different substituents into the pair position of the phenyl group (compounds **21b-e**). As shown in Table **1**, none of these pair substituted compounds exhibited potency for 5-HT reuptake inhibition except 4-fluoro derivative **21b** [RUI, IC_{50} = 590 nM]. Meanwhile, the activity of ortho and meta substituted phenyl compounds was also explored (compounds **21f-h**). Compared with **21a**, the 3-fluorophenyl analog **21f** showed a small decrease in affinity for 5-HT_{1A}/5-HT_7 receptors [5-HT_{1A}, k_i = 5.6 nM; 5-HT_7, k_i = 0.79 nM] and reduction in inhibition of 5-HT reuptake [RUI, IC_{50} = 150 nM]. Similarly, the 3-methylphenyl analog **21g** also exhibited high potency for 5-HT reuptake inhibition [RUI, IC_{50} = 160 nM]. Furthermore, the 2-fluorophenyl analog **21h** displayed a small increase in inhibition

of 5-HT reuptake [RUI, IC_{50} = 96 nM] but a considerable reduction in affinity for 5-HT_{1A}/5-HT_7 receptors [5-HT_{1A}, k_i = 25 nM; 5-HT_7, k_i = 2.3 nM] (**21h** *v* **21a**).

The influence of the chain length on 5-HT reuptake inhibition and 5-HT_{1A} /5-HT_7 receptors affinities was also explored. Surprisingly, the three carbon chain analog **21i** displayed outstanding affinity for 5-HT_{1A}/5-HT_7 receptors [5-HT_{1A}, ki = 13 nM; 5-HT_7, ki = 1.1 nM] and excellent 5-HT reuptake inhibitory activity [IC_{50} = 7.6 nM]. Similarly, 3-fluorophenyl analog **21j** exhibited higher potency for 5-HT reuptake inhibition [RUI, IC_{50} = 42 nM] (**21j** *v* **21f**). However, the compound **26** showed dramatically decreased inhibition of 5-HT reuptake when the linker was elongated to four carbons [RUI, IC_{50} = 120 nM] (**26** *v* **21k**). Those results suggested that the distance of linker chain had a great influence on the 5-HT reuptake activity.

Finally, the effect of R_2 substituent in the central benzene ring of compound **21j** was explored. The 4-fluoro substituted analog **21k** showed a small increase in inhibition of 5-HT reuptake [RUI, IC_{50} = 31 nM] but a big reduction in affinities for the 5-HT_{1A} and 5-HT_7 receptors [5-HT_{1A}, ki = 62 nM; 5-HT_7, ki = 12 nM] (**21k** *vs* **21j**). In contrast, the 3-fluoro **21l** and 5-fluoro **21m** analogs displayed higher potency for the 5-HT_{1A}/5-HT_7 receptors and the 5-HT reuptake inhibition compared to **21k**. As the above mentioned (**21f** *vs* **21a**), the replacement of the 3-fluorophenyl moiety with the phenyl moiety (compound **21n**) at R1 position displayed increased inhibition of 5-HT reuptake [RUI, IC_{50} = 25 nM] and binding affinities for 5-HT_{1A}/5-HT_7 receptors [5-HT_{1A}, ki = 28 nM; 5-HT_7, ki = 3.3 nM] (**21n** *vs* **21k**).

With the above information in hand, the potent compounds **21a-b, 21f, 21h** and **21j-n** were selected for metabolic stability evaluation *in vitro*. The results are shown in Table **2**. The stability values of compounds **21a-b** and **21f** suggested that the presence of an fluorine atom at the meta and pair position of phenyl group could improve metabolic stability. However, the introduction of fluorine atom at the ortho position of phenyl group reduced metabolic stability (**21h** *vs* **21a**). Interestingly, compound **21j** showed increased metabolic stability when the length of the linker extended from two to three carbons (**21j** vs **21f**). Moreover, the metabolic stability influence of the R_2 substituent in the central benzene ring was also explored (**21k-n**).

The 3-fluoro **21l** and 5-fluoro **21m** analogs displayed stabilities equal to **21j**. Interestingly, the 4-fluoro substituted compound **21k** showed significant improvement in metabolic stability (**21k** *vs* **21j**). Similarly, compound **21n** displayed metabolic stability higher than **21j**. Those results suggested that the introduction of a fluoro group into the 4-position of the central benzene ring could improve metabolic stability significantly.

On the basis of in vitro studies, the compounds **21k** and **21n** were selected for further evaluation in the mouse forced swim test (FST). The compounds were administered orally once daily for 7 days at doses of 10, 20 and 40 mg/kg/day (PO), respectively. Vortioxetine was acted as a positive control (40 mg/kg/day, PO). The results are shown in Fig. **5**. Compared with vehicle, the selected compounds **21k** and **21n** reduced immobility times in the FST in a dose-dependent manner that was statistically significant at 20 and 40 mg/kg doses (PO). The positive control, vortioxetine, also produced a statistically significant reduction of immobility time at 40 mg/kg dose.

The most promising compound **21n** was further characterized in the tail suspension test (TST). The results are shown in Fig. **6**. Venlafaxine was used as a reference compound (40 mg/kg/day, PO). The compound **21n** was administered orally once daily for 7 days at doses of 10, 20 and 40 mg/kg/day (PO). Compared to the control group, compound **21n** was dose dependently reduced the immobility time in the TST, which was statistically significant at 20 and 40 mg/kg doses.

Compound **21n** was also tested in the hERG channel binding assay to ascertain if the compound can cause cardiac toxicity. The data showed that compound **21n** displayed acceptable hERG safety profile (IC_{50} =16.66μM).

It was suggested that many 5-HT_{1A}R ligands showed high affinity for α1-adrenoceptors due to their high degree of homology (approximately 45%)[22]. The receptor selectivity of 5-HT_{1A} subtype and a1-adrenoceptor was measured. The data showed that compound **21n** exhibited equal affinity for a1-adrenoceptor (Ki = 28 nM) (Table **3**).

Finally, the pharmacokinetic properties of **21n** were measured in male SD rats

(Table **4**). After administration of 2 mg/kg (PO) **21n** to SD rats, a C_{max} of 86.7 ng/mL was obtained at 2 h. The elimination half-life of **21n** after oral administration was 6.1 h. Compound **21n** also had a potent orally bioavailable (F = 68.5 %) in rats.

3. Conclusion

In summary, a new series of novel aralkyl-piperazine derivatives were investigated, and as our continuing effort to identify novel compounds targeting SSRI/5-HT_{1A}/5-HT_7 for the treatment of depressive disorders. Our SAR studies revealed that the position of aromatic heterocyclic (**6c** vs **11**) and the length of the linker (**21a** vs **21i**) were clearly important for 5-HT inhibitory activity. The data showed that the metabolic stability of the compounds was related to the presence of fluorine atoms in well-defined regions of molecule (**21k** and **21n**). To confirm the potential *in vivo* antidepressant effect of this series of compounds, compounds **21k** and **21n** were selected for FST profiling in mice. The two selected compounds showed a dose-dependent reduction of the immobility time in the FST. The most promising compound **21n** displayed dose dependently reduced the immobility time in TST model. In addition, compound **21n** also had good oral pharmacokinetic properties in rats, and had an acceptable hERG profile. Our data lay a foundation for successful future development of novel aralkyl-piperazine derivatives as next generation of antidepressants.

4. Experimental protocols

4.1 Chemistry

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker NMR AVANCE 600 (600 MHz) or a Varian INOVA-400 (400 MHz) spectrometer with TMS as an internal standard. Chemical shift (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). ESI mass spectra and HRMS were performed on an

Agilent 6210 TOF spectrometer. The progress of all reactions was monitored using TLC on precoated silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60G (Qingdao Haiyang Chemical, China). The chromatograms were viewed under UV light at 254 and/or 265 nm. Uncorrected melting points were determined on an electrothermal melting point apparatus. Solvents and reagents were used without any pretreatment.

*4.1.1 Procedure A. ethyl 2-(benzofuran-3-yl)acetate (**2**)*

A mixture of benzofuran-3(2H)-one (4g, 29.82 mmol) and (Carbethoxymethylene) -triphenylphosphorane (15.58g, 44.73 mmol) in toluene (200 mL) was refluxed for 48h under N_2 atmosphere. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (PE/EtOAc = 50:1, V/V) to give compound **2** as colourless oil (4.56 g, 75%) [23]. MS (ESI) *m/z*: 227.06 $[M+Na]^+$.

*4.1.2 Procedure B. 2-(benzofuran-3-yl)ethanol (**3**)*

To a stirred suspension of $LiAlH_4$ (1.49 g, 39.17 mmol) in dry THF (50 mL) was added slowly a solution of ethyl 2-(benzofuran-3-yl)acetate (**2**) (4 g, 19.58 mmol) in dry THF (20 mL) at 0 ^oC. After the addition, the mixture was stirred at room temperature for 4h and quenched with saturated NH_4Cl solution. The mixture was extracted with DCM and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc = 5:1, V/V) to give compound **3** as yellow oil (2.86 g, 90%) [23]. MS (ESI) *m/z*: 185.11 $[M+Na]^+$.

*4.1.3 Procedure C. 2-(benzofuran-3-yl)ethyl 4-methylbenzenesulfonate (**4**)*

To a stirred solution of p-toluenesulfonyl chloride (4.41 g, 23.13 mmol), triethylamine (3.12 g, 30.83 mmol) and 4-dimethylaminopyridine (188 mg, 1.54 mmol) in dry CH_2Cl_2 (60 mL) was added slowly a solution of benzofuran-ethanol (**3**) (2.5 g, 15.41 mmol) in dry CH_2Cl_2 (25 mL) at 0 ^oC. The reaction mixture was stirred for 1 h at 0 ^oC

and left for overnight at room temperature. The reaction mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc = 5:1, V/V) to give compound **4** as a white solid (3.41 g, 70%) [24]. MS (ESI) *m/z*: 339.07 $[M+Na]^+$.

*4.1.4 Procedure D. General procedure for the preparation of compounds **6a-d***

A mixture of 2-(benzofuran-3-yl)ethyl 4-methylbenzenesulfonate (**4**) (1.58 mmol), phenylpiperazine derivatives (**5**) (1.89 mmol) and K_2CO_3 (3.16 mmol) in CH_3CN (50 mL) was stirred at 80 ^oC for 12 h. The solvent was removed under reduced pressure, then CH_2Cl_2 (50 mL) was added. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1, V/V) to give compound **6**. The compound **6** was dissolved in EA (15 mL), then hydrogen chloride ethyl acetate solution (2N, 1 mL) was added dropwise. The mixture was stirred at r.t. for 12h, then filtered. The residue was washed with EtOAc and EtOH separately，dried in vacuo to give compound **6** hydrochloride.

*4.1.4.1 1-(2-(benzofuran-3-yl)ethyl)-4-(2-methoxyphenyl)piperazine **(6a)** hydrochloride.*

Yield 70 % as white solid. Mp: 228-229 ^oC. MS (ESI) *m/z*: 337.31 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 7.93 (s, 1H), 7.84 (d, J = 7.2 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.40 – 7.28 (m, 2H), 7.08 – 6.90 (m, 4H), 3.81 (s, 3H), 3.68 (d, J = 11.4 Hz, 2H), 3.53 (d, J = 12.3 Hz, 2H), 3.48 – 3.39 (m, 2H), 3.31 – 3.23 (m, 4H), 3.14 (t, J = 11.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 154.59, 151.83, 142.70, 138.62, 127.14, 124.63, 124.00, 122.63, 120.85, 120.01, 118.54, 115.63, 112.09, 111.37, 55.42, 54.16, 50.71, 46.97, 17.85. HRMS calcd for $C_{21}H_{24}N_2O_2$ $[M+H]^+$, 337.1916; found, 337.1918.

*4.1.4.2 1-(2-(benzofuran-3-yl)ethyl)-4-(2-(methylthio)phenyl)piperazine **(6b)** hydrochloride.*

Yield 68 % as a white solid. Mp: 253-254 ^oC. MS (ESI) *m/z*: 353.29 $[M+H]^+$. ^1H NMR

(400 MHz, DMSO) δ 7.93 (s, 1H), 7.84 (d, *J* = 7.1 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.38 – 7.29 (m, 2H), 7.22 – 7.12 (m, 4H), 3.71 (d, *J* = 8.9 Hz, 2H), 3.52 – 3.45 (m, 2H), 3.34 (d, *J* = 9.6 Hz, 2H), 3.27 – 3.19 (m, 6H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 154.61, 147.42, 142.72, 134.30, 127.14, 125.12, 125.00, 124.65, 124.56, 122.64, 120.03, 119.60, 115.61, 111.39, 54.20, 51.30, 47.91, 17.94, 13.52. HRMS calcd for $C_{21}H_{24}N_2OS$ $[M+H]^+$, 353.1688; found, 353.1687.

4.1.4.3 1-([1,1'-biphenyl]-2-yl)-4-(2-(benzofuran-3-yl)ethyl)piperazine ***(6c)*** *hydrochloride.*

Yield 65 % as a white solid. Mp: 237-239 °C. MS (ESI) *m/z*: 383.21 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 7.89 (s, 1H), 7.77 (d, *J* = 7.2 Hz, 1H), 7.66 (d, *J* = 7.1 Hz, 2H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.38 – 7.24 (m, 5H), 7.16 (t, *J* = 8.3 Hz, 2H), 3.51 (d, *J* = 10.9 Hz, 2H), 3.44 – 3.36 (m, 2H), 3.16 – 3.13 (m, 4H), 3.06 – 2.92 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 154.57, 148.25, 142.68, 140.13, 134.03, 131.28, 128.56, 128.26, 127.08, 124.63, 123.51, 122.61, 119.95, 118.57, 115.58, 111.38, 54.11, 50.74, 47.35, 17.83. HRMS calcd for $C_{26}H_{26}N_2O$ $[M+H]^+$, 383.2123; found, 383.2124.

4.1.4.3 1-([1,1'-biphenyl]-3-yl)-4-(2-(benzofuran-3-yl)ethyl)piperazine ***(6d)*** *hydrochloride.*

Yield 65 % as a white solid. Mp: 211-212 °C. MS (ESI) *m/z*: 383.22 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 7.93 (s, 1H), 7.81 (d, *J* = 6.9 Hz, 1H), 7.70 – 7.65 (m, 2H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.40 – 7.28 (m, 4H), 7.26 (s, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 7.04 (dd, *J* = 8.1, 2.2 Hz, 1H), 4.00 (d, *J* = 8.5 Hz, 2H), 3.71 (d, *J* = 6.7 Hz, 2H), 3.52 – 3.43 (m, 2H), 3.31 – 3.18 (m, 6H). ^{13}C NMR (100 MHz, DMSO) δ 154.59, 149.83, 142.69, 141.27, 140.47, 129.70, 128.76, 127.42, 127.14, 126.85, 124.64, 122.63, 120.02, 118.68, 115.67, 115.10, 114.49, 111.38, 54.04, 50.32, 45.49,17.87. HRMS calcd for $C_{26}H_{26}N_2O$ $[M+H]^+$, 383.2123; found, 383.2125.

*4.1.5 Procedure E. 2-(benzofuran-2-yl)ethanol (****9****)*

To a stirred mixture of 2-iodophenol (5.7 g, 25.94 mmol), palladium acetate (292 mg, 1.29 mmol), CuI (247 mg, 1.29 mmol) and triphenylphosphine (340 mg, 1.29 mmol) in dry triethylamine (60 ml) was added 3-butyn-1-ol (2 g, 28.5 mmol) under nitrogen atmosphere. The reaction mixture was then stirred for overnight at room temperature. The mixture was concentrated, diluted with EtOAc (100 mL). The EtOAc layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EA = 3/1, V/V) to give compound **9** as yellow oil (3.7 g, 88%)[24]. MS (ESI) *m/z*: 185.09 $[M+Na]^+$.

*4.1.6 Procedure F. 2-(benzofuran-2-yl)ethyl 4-methylbenzenesulfonate (**10**)*

To a stirred solution of p-toluenesulfonyl chloride (3.53 g, 18.51 mmol), triethylamine (2.5 g, 24.70 mmol) and 4-dimethylaminopyridine (150 mg, 1.23 mmol) in dry CH_2Cl_2 (40 mL) was added slowly a solution of benzofuran-ethanol (**9**) (2 g, 12.33 mmol) in dry CH_2Cl_2 (20 mL) at 0 ^oC. The reaction mixture was stirred for 1 h at 0 ^oC and left for overnight at room temperature. The reaction mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EA= 5:1, V/V) to give compound **10** as a white solid (2.73 g, 70%)[24]. MS (ESI) *m/z*: 339.07 $[M+Na]^+$.

*4.1.7 Procedure G. 1-([1,1'-biphenyl]-2-yl)-4-(2-(benzofuran-2-yl)ethyl)piperazine (**11**)*

A mixture of 2-(benzofuran-2-yl)ethyl 4-methylbenzenesulfonate (**10**) (420 mg, 1.33 mmol), phenylpiperazine derivatives (**5c**) (400 mg, 1.46 mmol) and K_2CO_3 (367 mg, 2.66 mmol) in CH_3CN (30 mL) was Stirred at 80 ^oC for 12 h. The solvent was removed under reduced pressure, then CH_2Cl_2 (50 mL) was added. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1, V/V) to give compound **11**. The compound **11** was dissolved in EtOAc (15 mL), then hydrogen chloride ethyl acetate solution (2N, 1 mL) was added dropwise.

The mixture was stirred at r.t. for 12h, then filtered. The residue was washed with EA and EtOH separately，dried in vacuo to give compound **11** hydrochloride as white solid (378 mg, 68 %). Mp: 204-205 ^{o}C. MS (ESI) *m/z*: 383.24 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 7.65 (d, *J* = 7.2 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.36 – 7.32 (m, 2H), 7.28 – 7.22 (m, 3H), 7.16 – 7.12 (m, 2H), 6.75 (s, 1H), 3.51 – 3.46 (m, 4H), 3.33 – 3.31 (m, 2H), 3.13 (d, *J* = 12.3 Hz, 2H), 3.04 (t, *J* = 12.0 Hz, 2H), 2.96 (dd, *J* = 19.4, 9.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 154.15, 154.06, 148.22, 140.10, 133.98, 131.26, 128.57, 128.53, 128.26, 128.13, 127.05, 123.87, 123.49, 122.85, 120.72, 118.54, 110.75, 103.65, 52.73, 50.84, 47.26, 22.65. HRMS calcd for $C_{26}H_{26}N_2O$ $[M+H]^+$, 383.2123; found, 383.2127.

*4.1.8 Procedure H. 3-(2-hydroxyethyl)-1H-indole-5-carbonitrile (**13**)*

To a solution of 2-(5-bromo-1H-indol-3-yl)ethanol (**12**) (6 g, 24.99 mmol) in anhydrous DMF (200 mL) was added CuCN (22.38 g, 249.9 mmol). The reaction mixture was charged with N_2, then stirred at 130 ^{o}C for 48h. After cooling to room temperature, the mixture was concentrated under reduced pressure, then EtOAc (100 mL) was added. The resulting mixture was filtered, the filtrate was washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EA= 5:1, V/V) to give compound **13** as white solid (1.86 g, 40%). Mp: 125-126 ^{o}C. MS (ESI) *m/z*: 185.06 $[M-H]^-$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.43 – 7.42 (m, 2H), 7.23 (d, *J* = 2.2 Hz, 1H), 3.93 (dd, *J* = 11.4, 5.9 Hz, 2H), 3.03 (t, *J* = 6.3 Hz, 2H).

*4.1.9 Procedure I. 3-(2-bromoethyl)-1H-indole-5-carbonitrile (**14**)*

To a solution of 3-(2-hydroxyethyl)-1H-indole-5-carbonitrile (**13**) (1.5 g, 8.05 mmol) in CH_2Cl_2 (60 mL) was added successively CBr_4 (4 g, 12.08 mmol) and PPh_3 (3.11 g, 11.84 mmol). The mixture was stirred at room temperature for 5h. After the completion of reaction, the solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (PE/EA= 5:1, V/V) to give compound **14** as an white solid (1.2 g, 60%). Mp: 129-130 ^{o}C. MS (ESI) *m/z*:

270.99 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.44 (s, 2H), 7.25 (d, *J* = 2.2 Hz, 1H), 3.64 (t, *J* = 7.2 Hz, 2H), 3.34 (t, *J* = 7.2 Hz, 2H).

*4.1.10 Procedure J. 3-(2-(4-([1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-1H-indole-5-carbonitrile hydrochloride (**15**)*

A mixture of 3-(2-bromoethyl)-1H-indole-5-carbonitrile (**14**) (500 mg, 2.0 mmol), phenylpiperazine derivatives (**5c**) (526 mg, 2.2 mmol) , K_2CO_3 (555 mg, 4.0 mmol) and KI (34 mg, 0.2 mmol) in DMF (40 mL) was Stirred at 100 ^oC for 12 h. The solvent was removed under reduced pressure, then DCM (50 mL) was added. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1, V/V) to give compound **15**. The compound **15** was dissolved in EA (15 mL), then hydrogen chloride ethyl acetate solution (2N, 1 mL) was added dropwise. The mixture was stirred at r.t. for 12h, then filtered. The residue was washed with EtOAc and ethanol separately, dried in vacuo to give compound **15** hydrochloride as white solid (533 mg, 60 %). Mp: 238 - 239 ^oC. MS (ESI) *m/z*: 407.23 $[M+H]^+$. ^1H NMR (600 MHz, DMSO) δ 11.60 (s, 1H), 8.23 (s, 1H), 7.66 (d, *J* = 7.4 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.48 – 7.44 (m, 4H), 7.36 (dd, *J* = 13.0, 6.8 Hz, 2H), 7.26 (d, *J* = 6.5 Hz, 1H), 7.16 (t, *J* = 8.5 Hz, 2H), 3.51 (d, *J* = 11.0 Hz, 2H), 3.33 – 3.31 (m, 2H), 3.22 – 3.12 (m, 4H), 3.05 (t, *J* = 11.9 Hz, 2H), 2.95 (dd, *J* = 20.4, 10.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 148.27, 140.14, 137.95, 134.03, 131.28, 128.56, 128.25, 127.09, 126.53, 126.05, 124.41, 123.89, 123.49, 120.82, 118.57, 112.80, 110.56, 100.55, 55.25, 50.70, 47.37, 19.19. HRMS calcd for $C_{27}H_{26}N_4$ $[M+H]^+$, 407.2236; found, 407.2242.

*4.1.11 Procedure K. General procedure for the preparation of compounds **18a-b***

(4-fluorophenyl)hydrazine hydrochloride (**17**) (16.26 g, 100 mmol) was dissolved in a mixture of N,N-dimethylacetamide (140 mL) and 4% m/m aqueous H_2SO_4 (140 mL) and heated to 100 ^oC. 2,3-dihydrofuran (**16a**) or 3,4-dihydro-2H-pyran (**16b**) (100 mmol) was then added dropwise over 5 min, and the solution was stirred for 3h at 100

^{o}C. After cooling to room temperature, the mixture was extracted with EtOAc (3×100 mL), the combined organic layer was washed with H_2O (3×100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc = 5:1, V/V) to give compound **18**.

*4.1.11.1 2-(5-fluoro-1H-indol-3-yl)ethanol (**18a**)*

Starting with 2,3-dihydrofuran (**16a**), Procedure K was followed to afford compound **18a** as an orange oil (11.64 g, 65 %) [25]. MS (ESI) *m/z*: 178.06 $[M-H]^-$.

*4.1.11.2 3-(5-fluoro-1H-indol-3-yl)propan-1-ol (**18b**)*

Starting with 3,4-dihydro-2H-pyran (**16b**), Procedure K was followed to afford compound **18b** as an orange oil (11.59 g, 60 %) [26]. MS (ESI) *m/z*: 192.08 $[M-H]^-$.

*4.1.12 Procedure L. 3-(2-bromoethyl)-5-fluoro-1H-indole (**19a**)*

To a solution of 2-(5-fluoro-1H-indol-3-yl)ethanol (**18a**) (10 g, 55.8 mmol) in CH_2Cl_2 (200 mL) was added successively CBr_4 (27.76 g, 83.71 mmol) and PPh_3 (21.52 g, 82.03 mmol) at 0 ^{o}C. The mixture was stirred at room temperature for 5h. After the completion of reaction, the solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (PE/EA= 5:1, V/V) to give compound **19a** as a yellow oil (9.46 g, 70%) [27]. MS (ESI) *m/z*: 242.06 $[M+H]^+$.

*4.1.13 Procedure M. 3-(5-fluoro-1H-indol-3-yl)propyl 4-methylbenzenesulfonate (**19b**)*

To a stirred solution of p-toluenesulfonyl chloride (11.84 g, 62.11 mmol), triethylamine (7.86 g, 77.63 mmol) and 4-dimethylaminopyridine (630 mg, 5.17 mmol) in dry CH_2Cl_2 (150 mL) was added slowly a solution of 3-(5-fluoro-1H-indol-3-yl)propan-1-ol (**18b**) (10 g, 51.75 mmol) in dry CH_2Cl_2 (50 mL) at 0 ^{o}C. The reaction mixture was stirred for 1 h at 0 ^{o}C and left for overnight at room temperature. The reaction mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on

silica gel (PE/EA= 5:1, V/V) to give compound **19b** as a pale-yellow solid (11.69 g, 65%) [26]. MS (ESI) *m/z*: 370.09 $[M+Na]^+$.

*4.1.14 Procedure N. General procedure for the preparation of compounds **21a-h***

A mixture of 3-(2-bromoethyl)-5-fluoro-1H-indole (**19a**) (4.13 mmol), phenylpiperazine derivatives (**20**) (4.54 mmol) , K_2CO_3 (8.26 mmol) and KI (0.41 mmol) in DMF (40 mL) was stirred at 100 ^oC for 12 h. The solvent was removed under reduced pressure, then DCM (50 mL) was added. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1, V/V) to give compound **21**. The compound **21** was dissolved in EA (15 mL), then hydrogen chloride ethyl acetate solution (2N, 2 mL) was added dropwise. The mixture was stirred at r.t. for 12h, then filtered. The residue was washed with EtOAc and EtOH separately，dried in vacuo to give compound **21** hydrochloride.

*4.1.14.1 3-(2-(4-([1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-5-fluoro-1H-indole hydrochloride (**21a**)*

Yield 61 % as a white solid. Mp: 259 - 260 ^oC. MS (ESI) *m/z*: 400.25 $[M+H]^+$. ^1H NMR (400 MHz, DMSO) δ 11.09 (s, 1H), 7.66 (d, *J* = 7.3 Hz, 2H), 7.50 – 7.40 (m, 3H), 7.39 – 7.23 (m, 5H), 7.16 (t, *J* = 8.0 Hz, 2H), 6.94 (td, *J* = 9.2, 2.4 Hz, 1H), 3.50 (d, *J* = 10.6 Hz, 2H), 3.32 – 3.28 (m, 2H), 3.18 – 2.90 (m, 8H). ^{13}C NMR (150 MHz, DMSO) δ 158.22 (d, *J* = 229.7 Hz), 148.79 (s), 140.64 (s), 134.53 (s), 133.38 (s), 131.78 (s), 129.07 (s), 129.03 (s), 128.76 (s), 127.58 (s), 127.43 (d, *J* = 9.9 Hz), 125.81 (s), 123.99 (s), 119.07 (s), 112.97 (d, *J* = 9.5 Hz), 109.98 (d, *J* = 3.9 Hz), 109.82 (d, *J* = 26.0 Hz), 103.71 (d, *J* = 23.0 Hz), 55.81 (s), 51.16 (s), 47.86 (s), 19.92 (s). HRMS calcd for $C_{26}H_{26}N_3F$ $[M+H]^+$, 400.2189; found, 400.2195.

*4.1.14.2 5-fluoro-3-(2-(4-(4'-fluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-1H-indole hydrochloride (**21b**)*

Yield 56 % as a white solid. Mp: 258-259 ^oC. MS (ESI) *m/z*: 418.23$[M+H]^+$. ^1H NMR

(400 MHz, DMSO) δ 11.10 (s, 1H), 7.71 (dd, *J* = 8.6, 5.7 Hz, 2H), 7.44 (dd, *J* = 10.0, 2.3 Hz, 1H), 7.39 – 7.33 (m, 2H), 7.31 – 7.23 (m, 4H), 7.16 (t, *J* = 7.4 Hz, 2H), 6.94 (td, *J* = 9.2, 2.5 Hz, 1H), 3.51 (d, *J* = 11.1 Hz, 2H), 3.33 – 3.26 (m, 2H), 3.17 – 3.02 (m, 6H), 2.96 (dd, *J* = 20.2, 10.5 Hz, 2H). ^{13}C NMR (150 MHz, DMSO) δ 161.70 (d, *J* = 242.6 Hz), 157.22 (d, *J* = 230.0 Hz), 148.84 (s), 136.81 (s), 133.71 (s), 133.38 (s), 131.66 (s), 130.87 (d, *J* = 7.7 Hz), 129.16 (s), 127.43 (d, *J* = 9.8 Hz), 125.80 (s), 124.16 (s), 119.31 (s), 115.87 (d, *J* = 21.0 Hz), 112.97 (d, *J* = 9.7 Hz), 109.98 (d, *J* = 4.2 Hz), 109.82 (d, *J* = 26.0 Hz), 103.70 (d, *J* = 23.0 Hz), 55.87 (s), 51.19 (s), 47.94 (s), 19.91 (s). HRMS calcd for $C_{26}H_{25}N_3F_2$ $[M+H]^+$, 418.2095; found, 418.2094.

*4.1.14.3 5-fluoro-3-(2-(4-(4'-methyl-[1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-1H-indole hydrochloride (**21c**)*

Yield 52 % as a white solid. Mp: 246-247 °C. MS (ESI) *m/z*: 414.25$[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.44 (dd, *J* = 10.0, 2.2 Hz, 1H), 7.39 – 7.29 (m, 3H), 7.27 – 7.22 (m, 3H), 7.14 (t, *J* = 7.6 Hz, 2H), 6.94 (td, *J* = 9.2, 2.4 Hz, 1H), 3.51 (d, *J* = 10.5 Hz, 2H), 3.33 – 3.27 (m, 2H), 3.16– 3.09 (m, 4H), 3.05 – 2.92 (m, 4H), 2.35 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 157.22 (d, *J* = 230.0 Hz), 148.78 (s), 137.72 (s), 136.63 (s), 134.44 (s), 133.39 (s), 131.75 (s), 129.67 (s), 128.77 (s), 128.59 (s), 127.44 (d, *J* = 9.8 Hz), 125.80 (s), 123.97 (s), 119.02 (s), 112.97 (d, *J* = 9.6 Hz), 110.01 (d, *J* = 4.2 Hz), 109.81 (d, *J* = 26.0 Hz), 103.71 (d, *J* = 23.0 Hz), 55.81 (s), 51.20 (s), 47.83 (s), 21.32 (s), 19.93 (s). HRMS calcd for $C_{27}H_{28}N_3F$ $[M+H]^+$, 414.2346; found, 414.2343.

*4.1.14.4 5-fluoro-3-(2-(4-(4'-methoxy-[1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-1H-indole hydrochloride (**21d**)*

Yield 53 % as a white solid. Mp: 224 - 225 °C. MS (ESI) *m/z*: 430.23$[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 11.09 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.43 (dd, *J* = 10.0, 2.3 Hz, 1H), 7.36 (dd, *J* = 8.8, 4.6 Hz, 1H), 7.34 – 7.28 (m, 2H), 7.23 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.12 (dd, *J* = 12.4, 4.9 Hz, 2H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.94 (td, *J* = 9.2, 2.4 Hz, 1H), 3.80 (s, 3H), 3.53 (d, *J* = 5.3 Hz, 2H), 3.33 – 3.27 (m, 2H), 3.18 – 3.07

(m, 4H), 3.03 – 2.94 (m, 4H). ^{13}C NMR (150 MHz, DMSO) δ 158.73 (s), 157.22 (d, *J* = 230.0 Hz), 148.72 (s), 134.24 (s), 133.38 (s), 132.76 (s), 131.60 (s), 129.89 (s), 128.54 (s), 127.44 (d, *J* = 9.9 Hz), 125.81 (s), 123.99 (s), 119.03 (s), 114.46 (s), 112.97 (d, *J* = 9.6 Hz), 109.98 (d, *J* = 3.0 Hz), 109.82 (d, *J* = 26.0 Hz), 103.70 (d, *J* = 23.0 Hz), 55.88 (s), 55.50 (s), 51.26 (s), 47.80 (s), 19.92 (s). HRMS calcd for $C_{27}H_{28}N_3FO$ $[M+H]^+$, 430.2295; found, 430.2301.

*4.1.14.5 2'-(4-(2-(5-fluoro-1H-indol-3-yl)ethyl)piperazin-1-yl)-[1,1'-biphenyl]-4-carbonitrile hydrochloride (**21e**)*

Yield 49 % as a white solid. Mp: 274-275 ^{o}C. MS (ESI) *m/z*: 425.23 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 11.10 (s, 1H), 7.89 (q, *J* = 8.3 Hz, 4H), 7.43 (dd, *J* = 14.4, 8.1 Hz, 2H), 7.36 (dd, *J* = 8.7, 4.5 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.23 – 7.19 (m, 2H), 6.94 (t, *J* = 9.0 Hz, 1H), 3.51 (d, *J* = 11.3 Hz, 2H), 3.32 – 3.28 (m, 2H), 3.15 – 3.03 (m, 6H), 2.99 – 2.92 (m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 157.22 (d, *J* = 230.0 Hz), 148.97 (s), 145.50 (s), 133.38 (s), 133.02 (s), 132.99 (s), 131.68 (s), 130.18 (s), 129.90 (s), 127.43 (d, *J* = 9.8 Hz), 125.79 (s), 124.37 (s), 119.54 (d, *J* = 20.3 Hz), 112.97 (d, *J* = 9.6 Hz), 110.11(s), 109.98 (d, *J* = 3.6 Hz), 109.83 (d, *J* = 26.0 Hz), 103.70 (d, *J* = 23.1 Hz), 55.87 (s), 51.07 (s), 48.20 (s), 19.91 (s). HRMS calcd for $C_{27}H_{25}N_4F$ $[M+H]^+$, 425.2142; found, 425.2143.

*4.1.14.6 5-fluoro-3-(2-(4-(3'-fluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-1H-indole hydrochloride (**21f**)*

Yield 54 % as a white solid. Mp: 264-265 ^{o}C. MS (ESI) *m/z*: 418.25 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 11.09 (s, 1H), 7.56 – 7.48 (m, 2H), 7.47 – 7.42 (m, 2H), 7.40 – 7.34 (m, 2H), 7.31 – 7.27 (m, 2H), 7.22 – 7.15 (m, 3H), 6.94 (td, *J* = 9.2, 2.5 Hz, 1H), 3.52 (d, *J* = 11.2 Hz, 2H), 3.32 – 3.28 (m, 2H), 3.16 – 3.02 (m, 6H), 2.99 – 2.90 (m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 162.74 (d, *J* = 241.8 Hz), 157.22 (d, *J* = 230.0 Hz), 148.85 (s), 143.03 (d, *J* = 7.8 Hz), 133.38 (s), 131.82 (s), 130.94 (d, *J* = 8.4 Hz), 129.56 (s), 127.44 (d, *J* = 9.8 Hz), 125.82 (s), 125.02 (s), 124.11 (s), 119.29 (s), 115.50 (d, *J* = 21.6 Hz), 114.36 (d, *J* = 20.6 Hz), 112.97 (d, *J* = 9.6 Hz), 109.97 (d, *J* =

3.8 Hz), 109.81 (d, *J* = 26.1 Hz), 103.70 (d, *J* = 23.0 Hz), 55.82 (s), 51.18 (s), 48.02 (s), 19.92 (s). HRMS calcd for $C_{26}H_{25}N_3F_2$ $[M+H]^+$, 418.2095; found, 418.2096.

*4.1.14.7 5-fluoro-3-(2-(4-(3'-methyl-[1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-1H-indole hydrochloride (**21g**)*

Yield 48 % as a white solid. Mp: 262-263 °C. MS (ESI) *m/z*: 414.22 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 11.08 (s, 1H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.44 – 7.40 (m, 2H), 7.38 – 7.29 (m, 4H), 7.24 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.17 – 7.12 (m, 3H), 6.94 (td, *J* = 9.2, 2.5 Hz, 1H), 3.52 (d, *J* = 11.0 Hz, 2H), 3.33 – 3.29 (m, 2H), 3.18 – 3.06 (m, 4H), 3.03 – 2.88 (m, 4H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 156.71 (d, *J* = 229.9 Hz), 148.24 (s), 140.10 (s), 137.67 (s), 134.02 (s), 132.86 (s), 131.26 (s), 128.81 (s), 128.39 (d, *J* = 5.2 Hz), 127.71 (s), 126.93 (d, *J* = 9.9 Hz), 125.31 (s), 123.38 (s), 118.47 (s), 112.46 (d, *J* = 9.7 Hz), 109.46 (d, *J* = 4.7 Hz), 109.30 (d, *J* = 26.0 Hz), 103.20 (d, *J* = 23.0 Hz), 55.33 (s), 50.74 (s), 47.28 (s), 21.10 (s), 19.38 (s). HRMS calcd for $C_{27}H_{28}N_3F$ $[M+H]^+$, 414.2346; found, 414.2351.

*4.1.14.8 5-fluoro-3-(2-(4-(2'-fluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)ethyl)-1H-indole hydrochloride (**21h**)*

Yield 57 % as a white solid. Mp: 184-185 °C. MS (ESI) *m/z*: 418.24 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 11.09 (s, 1H), 7.57 – 7.50 (m, 1H), 7.48 – 7.39 (m, 3H), 7.38 – 7.15 (m, 7H), 6.93 (td, *J* = 9.2, 2.4 Hz, 1H), 3.49 (d, *J* = 11.6 Hz, 2H), 3.30 – 3.24 (m, 2H), 3.15 – 2.99 (m, 6H), 2.77 (dd, *J* = 18.5, 8.4 Hz, 2H). ^{13}C NMR (150 MHz, DMSO) δ 159.30 (d, *J* = 244.0 Hz), 157.21 (d, *J* = 229.8 Hz), 149.76 (s), 133.35 (s), 132.03 (s), 131.85 (s), 130.10 (s), 129.93 (d, *J* = 7.7 Hz), 129.77 (s), 127.77 (d, *J* = 15.0 Hz), 127.41 (d, *J* = 9.6 Hz), 125.82 (s), 124.99 (s), 124.05 (s), 119.88 (s), 116.35 (d, *J* = 22.2 Hz), 112.95 (d, *J* = 9.8 Hz), 109.92 (d, *J* = 6.6 Hz), 109.81 (d, *J* = 26.6 Hz), 103.70 (d, *J* = 23.0 Hz), 55.77 (s), 51.41 (s), 48.23 (s), 19.91 (s). HRMS calcd for $C_{26}H_{25}N_3F_2$ $[M+H]^+$, 418.2095; found, 418.2091.

*4.1.15 Procedure O. General procedure for the preparation of compounds **21i-n***

A mixture of 3-(5-fluoro-1H-indol-3-yl)propyl 4-methylbenzenesulfonate (**19b**) (2.87 mmol), phenylpiperazine derivatives (**20**) (3.16 mmol) and K_2CO_3 (5.75 mmol) in CH_3CN (40 mL) was stirred at 80 ^oC for 12 h. The solvent was removed under reduced pressure, then CH_2Cl_2 (50 mL) was added. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1, V/V) to give compound **21**. The compound **21** was dissolved in EA (15 mL), then hydrogen chloride ethyl acetate solution (2N, 2 mL) was added dropwise. The mixture was stirred at r.t. for 12h, then filtered. The residue was washed with EA and EtOH separately，dried in vacuo to give compound **21** hydrochloride.

*4.1.15.1 3-(3-(4-([1,1'-biphenyl]-2-yl)piperazin-1-yl)propyl)-5-fluoro-1H-indole hydrochloride (**21i**)*

Yield 69 % as a white solid. Mp: 174-175 ^oC. MS (ESI) *m/z*: 414.25 $[M+H]^+$. ^1H NMR (600 MHz, DMSO) δ 10.99 (s, 1H), 7.64 (d, *J* = 7.3 Hz, 2H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.36 – 7.28 (m, 4H), 7.27 – 7.22 (m, 2H), 7.14 (t, *J* = 7.4 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.91 (td, *J* = 9.2, 2.4 Hz, 1H), 3.48 – 3.34 (m, 4H), 3.10 – 3.05 (m, 4H), 3.00 (t, *J* = 11.7 Hz, 2H), 2.84 (dd, *J* = 20.7, 9.4 Hz, 2H), 2.69 (t, *J* = 7.3 Hz, 2H), 2.06 – 2.00 (m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 157.12 (d, *J* = 229.4 Hz), 148.75 (s), 140.62 (s), 134.49 (s), 133.45 (s), 131.75 (s), 129.04 (s), 129.01 (s), 128.75 (s), 127.64 (d, *J* = 9.9 Hz), 127.56 (s), 125.17 (s), 123.95 (s), 119.01 (s), 113.60 (d, *J* = 4.4 Hz), 112.78 (d, *J* = 9.8 Hz), 109.51 (d, *J* = 26.0 Hz), 103.51 (d, *J* = 22.8 Hz), 55.88 (s), 51.31 (s), 47.76 (s), 24.11 (s), 22.29 (s). HRMS calcd for $C_{27}H_{28}N_3F$ $[M+H]^+$, 414.2346; found, 414.2350.

*4.1.15.2 5-fluoro-3-(3-(4-(3'-fluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)propyl)-1H-indole hydrochloride (**21j**)*

Yield 67 % as a white solid. Mp: 232-233 ^oC. MS (ESI) *m/z*: 432.25 $[M+H]^+$. ^1H NMR (600 MHz, DMSO) δ 10.99 (s, 1H), 7.52 – 7.45 (m, 2H), 7.42 (d, *J* = 10.3 Hz, 1H), 7.39 – 7.24 (m, 5H), 7.20 – 7.12 (m, 3H), 6.91 (td, *J* = 9.2, 2.3 Hz, 1H), 3.41 (d,

J = 11.5 Hz, 2H), 3.13 – 3.06 (m, 4H), 3.02 (t, *J* = 11.9 Hz, 2H), 2.85 (dd, *J* = 20.4, 9.5 Hz, 2H), 2.69 (t, *J* = 7.4 Hz, 2H), 2.03 (dt, *J* = 15.4, 7.6 Hz, 2H). ^{13}C NMR (150 MHz, DMSO) δ 162.72 (d, *J* = 241.8 Hz), 157.12 (d, *J* = 229.5 Hz), 148.81 (s), 143.00 (d, *J* = 7.7 Hz), 133.46 (s), 133.29 (s), 131.79 (s), 130.92 (d, *J* = 8.4 Hz), 129.54 (s), 127.64 (d, *J* = 9.6 Hz), 125.16 (s), 125.00 (s), 124.06 (s), 119.22 (s), 115.47 (d, *J* = 21.6 Hz), 114.34 (d, *J* = 20.7 Hz), 113.62 (d, *J* = 4.4 Hz), 112.78 (d, *J* = 9.6 Hz), 109.50 (d, *J* = 26.0 Hz), 103.51 (d, *J* = 22.8 Hz), 55.87 (s), 51.32 (s), 47.89 (s), 24.12 (s), 22.28 (s). HRMS calcd for $C_{27}H_{27}N_3F_2$ $[M+H]^+$, 432.2251; found, 432.2248.

*4.1.15.3 3-(3-(4-(3',5-difluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)propyl)-5-fluoro-1H-indole hydrochloride (**21k**)*

Yield 67 % as a white solid, the purity was 98.1% by HPLC analysis. Mp: 243-244 °C. MS (ESI) *m/z*: 450.40 $[M+H]^+$. ^{1}H NMR (600 MHz, DMSO) δ 10.98 (s, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.51 – 7.43 (m, 2H), 7.33 (dd, *J* = 8.7, 4.6 Hz, 1H), 7.30 (dd, *J* = 10.0, 2.2 Hz, 1H), 7.26 (s, 1H), 7.23 – 7.13 (m, 4H), 6.91 (td, *J* = 9.2, 2.4 Hz, 1H), 3.40 (d, *J* = 11.2 Hz, 2H), 3.13 – 3.07 (m, 2H), 3.04 – 2.97 (m, 4H), 2.83 (q, *J* = 14.0 Hz, 2H), 2.69 (t, *J* = 7.3 Hz, 2H), 2.03 (dt, *J* = 14.9, 7.4 Hz, 2H). ^{13}C NMR (150 MHz, DMSO) δ 162.66 (d, *J* = 242.0 Hz), 158.99 (d, *J* = 239.0 Hz), 157.11 (d, *J* = 229.5 Hz), 145.33 (s), 141.51 (d, *J* = 7.8 Hz), 135.44 (d, *J* = 6.6 Hz), 133.45 (s), 130.95 (d, *J* = 8.3 Hz), 127.64 (d, *J* = 9.6 Hz), 125.15 (s), 121.23 (d, *J* = 8.1 Hz), 118.21 (d, *J* = 22.8 Hz), 115.71 (d, *J* = 21.5 Hz), 115.57 (d, *J* = 20.7 Hz), 114.87 (d, *J* = 20.7 Hz), 113.61 (d, *J* = 4.4 Hz), 112.78 (d, *J* = 9.6 Hz), 109.50 (d, *J* = 26.0 Hz), 103.50 (d, *J* = 22.7 Hz), 55.84 (s), 51.34 (s), 48.19 (s), 24.13 (s), 22.27 (s). HRMS calcd for $C_{27}H_{26}N_3F_3$ $[M+H]^+$, 450.2157; found, 450.2158.

*4.1.15.4 3-(3-(4-(3',6-difluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)propyl)-5-fluoro-1H-indole hydrochloride (**21l**)*

Yield 61 % as a white solid. Mp: 197-199 °C. MS (ESI) *m/z*: 450.24 $[M+H]^+$.

^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 7.56 – 7.47 (m, 1H), 7.43 – 7.19 (m,

7H), 7.01 (dd, *J* = 18.4, 8.8 Hz, 2H), 6.91 (td, *J* = 9.2, 2.5 Hz, 1H), 3.38 – 3.34 (m, 2H), 3.11 – 2.97 (m, 6H), 2.76 – 2.65 (m, 4H), 2.06 – 1.95 (m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 162.51 (d, *J* = 242.0 Hz), 160.05 (d, *J* = 242.0 Hz), 157.11 (d, *J* = 229.5 Hz), 150.80 (d, *J* = 4.7 Hz), 135.47 (d, *J* = 8.0 Hz), 133.44 (s), 130.83 (d, *J* = 8.3 Hz), 130.54 (d, *J* = 10.4 Hz), 127.63 (d, *J* = 9.6 Hz), 126.64 (s), 125.15 (s), 121.34 (d, *J* = 15.2 Hz), 117.22 (d, *J* = 21.8 Hz), 115.43 (s), 115.01 (d, *J* = 20.8 Hz), 113.61 (d, *J* = 4.4 Hz), 112.77 (d, *J* = 9.6 Hz), 110.89 (d, *J* = 23.1 Hz), 109.49 (d, *J* = 26.0 Hz), 103.50 (d, *J* = 22.7 Hz), 55.83 (s), 51.23 (s), 47.80 (s), 24.10 (s), 22.26 (s). HRMS calcd for $C_{27}H_{26}N_3F_3$ $[M+H]^+$, 450.2157; found, 450.2158.

*4.1.15.5 3-(3-(4-(3',4-difluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)propyl)-5-fluoro-1H-indole hydrochloride (**21m**)*

Yield 64 % as a white solid. Mp: 233-234 °C. MS (ESI) *m/z*: 450.20 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 7.52 – 7.44 (m, 2H), 7.42 – 7.23 (m, 5H), 7.21– 7.15 (m, 1H), 7.01 – 6.87 (m, 3H), 3.40 (d, *J* = 12.4 Hz, 2H), 3.15 – 3.06 (m, 4H), 3.04 – 2.81 (m, 4H), 2.69 (t, *J* = 7.3 Hz, 2H), 2.08 – 1.94 (m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 162.84 (d, *J* = 243.9 Hz), 162.78 (d, *J* = 242.0 Hz), 157.11 (d, *J* = 229.5 Hz), 150.64 (d, *J* = 8.6 Hz), 142.22 (d, *J* = 7.8 Hz), 133.45 (s), 133.37 (s), 131.08 (d, *J* = 8.1 Hz), 129.20 (s), 127.63 (d, *J* = 9.8 Hz), 125.16 (s), 124.90 (s), 115.41 (d, *J* = 21.8 Hz), 114.45 (d, *J* = 20.7 Hz), 113.60 (d, *J* = 4.2 Hz), 112.77 (d, *J* = 9.5 Hz), 110.03 (d, *J* = 20.7 Hz), 109.50 (d, *J* = 26.1 Hz), 106.63 (d, *J* = 23.4 Hz), 103.50 (d, *J* = 22.7 Hz), 55.84 (s), 51.04 (s), 47.60 (s), 24.12 (s), 22.27 (s). HRMS calcd for $C_{27}H_{26}N_3F_3$ $[M+H]^+$, 450.2157; found, 450.2157.

*4.1.15.6 5-fluoro-3-(3-(4-(5-fluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)propyl)-1H-indole hydrochloride (**21n**)*

Yield 68 % as a white solid, the purity was 98.3% by HPLC analysis. Mp: 215-216 °C. MS (ESI) *m/z*: 432.24 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 7.68 – 7.64 (m, 2H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.40 – 7.25 (m, 4H), 7.20 – 7.08 (m, 3H), 6.91 (td, *J* = 9.2, 2.5 Hz, 1H), 3.37 (d, *J* = 12.1 Hz, 2H), 3.12 – 3.05 (m, 2H), 3.02 – 2.97

(m, 4H), 2.89 – 2.77 (m, 2H), 2.69 (t, *J* = 7.4 Hz, 2H), 2.08 – 1.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 158.48 (d, *J* = 238.7 Hz), 156.60 (d, *J* = 229.4 Hz), 144.75 (s), 138.70 (s), 136.16 (d, *J* = 7.5 Hz), 132.94 (s), 128.57 (s), 128.34 (s), 127.57 (s), 127.12 (d, *J* = 9.7 Hz), 124.66 (s), 120.44 (d, *J* = 8.5 Hz), 117.57 (d, *J* = 22.7 Hz), 114.48 (d, *J* = 21.5 Hz), 113.08 (d, *J* = 4.6 Hz), 112.27 (d, *J* = 9.7 Hz), 109.00 (d, *J* = 25.9 Hz), 103.00 (d, *J* = 22.7 Hz), 55.34 (s), 50.79 (s), 47.54 (s), 23.60 (s), 21.77 (s). HRMS calcd for $C_{27}H_{27}N_3F_2$ $[M+H]^+$, 432.2251; found, 432.2249.

*4.1.16 Procedure P. 4-chloro-1-(5-fluoro-1-tosyl-1H-indol-3-yl)butan-1-one **23***

To an ice-cold solution of 5-fluoro-1-tosyl-1H-indole **22** (3 g, 10.36 mmol) in CH_2Cl_2 (45 mL) was added $AlCl_3$ (1.52 g, 11.40 mmol), followed by the addition of 4-chlorobutanoyl chloride (1.6 g, 11.40 mmol) in CH_2Cl_2 (15 mL). After the addition, the mixture was stirred at r.t. for 3h. Then reaction mixture was poured slowly into ice-water, then stirred for 15 min. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc = 5/1, V/V) to give compound **23**. Yield (2.65 g, 65 %) as a white solid. Mp: 127-129 ^{o}C. MS (ESI) *m/z*: 394.07 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.00 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.88 (dd, *J* = 9.1, 4.3 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.11 (td, *J* = 9.0, 2.6 Hz, 1H), 3.68 (t, *J* = 6.2 Hz, 2H), 3.10 (t, *J* = 7.0 Hz, 2H), 2.39 (s, 3H), 2.31 – 2.18 (m, 2H).

*4.1.17 Procedure Q. 3-(4-chlorobutyl)-5-fluoro-1-tosyl-1H-indole **24***

To a stirred solution of 4-chloro-1-(5-fluoro-1-tosyl-1H-indol-3-yl)butan-1-one **23** (2.5g, 6.34 mmol) in trifluoroacetic acid (45 mL) was added dropwise $HSiEt_3$ (2.2 g, 19.04 mmol) at 0 ^{o}C. After the addition, the mixture was stirred at 0 ^{o}C for 30 min, then heated to 50 ^{o}C for 3 h. The reaction mixture was concentrated under reduced pressure, then water (40 mL) was added. The resulting mixture was stirred at r.t. for 30 min, much yellow solid precipitated. The mixture was filtered, the residue was washed with water, dried in vacuo to give an pale-yellow solid. The solid was

recrystallized from EtOAc to give the desired product. Yield (1.39g, 58 %) as a white solid. Mp: 104 - 105 ^{o}C. MS (ESI) *m/z*: 402.05 $[M+Na]^{+}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 9.0, 4.4 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.35 (s, 1H), 7.21 (d, *J* = 8.1 Hz, 2H), 7.10 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.03 (td, *J* = 9.0, 2.5 Hz, 1H), 3.59 – 3.50 (m, 2H), 2.64 (dd, *J* = 6.9, 6.2 Hz, 2H), 2.34 (s, 3H), 1.83 – 1.79 (m, 4H).

4.1.18 Procedure R. 3-(4-(4-(3',5-difluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)butyl)-5-fluoro-1-tosyl-1H-indole ***25***

A mixture of 3-(4-chlorobutyl)-5-fluoro-1-tosyl-1H-indole **24** (1g, 2.63 mmol), phenylpiperazine derivatives **20f** (0.79 g, 2.89 mmol) , K_2CO_3 (0.73 g, 5.26 mmol) and KI (44 mg, 0.26 mmol) in DMF (30 mL) was stirred at 100 ^{o}C for 12 h. The solvent was removed under reduced pressure, then DCM (50 mL) was added. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1, V/V) to give compound **25** as a yellow solid. Yield (1.21 g, 75 %). Mp: 64 - 65 ^{o}C. MS (ESI) *m/z*: 618.23 $[M+H]^{+}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 9.0, 4.4 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.43 – 7.31 (m, 4H), 7.19 (d, *J* = 8.1 Hz, 2H), 7.10 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.04 – 6.96 (m, 5H), 2.80 (t, *J* = 4.7 Hz, 4H), 2.59 (t, *J* = 7.4 Hz, 2H), 2.37 – 2.29 (m, 7 H), 1.69 – 1.62 (m, 4H), 1.54 – 1.47 (m, 2H).

4.1.19 Procedure S. 3-(4-(4-(3',5-difluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)butyl)-5-fluoro-1H-indole hydrochloride ***26***

A mixture of 3-(4-(4-(3',5-difluoro-[1,1'-biphenyl]-2-yl)piperazin-1-yl)butyl)-5-fluoro-1-tosyl-1H-indole **25** (1.2 g, 1.94 mmol), 4N NaOH solution (4.8 mL) in the co-solvent of EtOH (30 mL) and THF (5 mL) was stirred at 80 ^{o}C for 12h. After cooling to room temperature, the solvent was evaporated, then the residue was diluted with CH_2Cl_2 (60 mL). The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 100/1, V/V) to give compound **26**. The

compound **26** was dissolved in EA (30 mL), then hydrogen chloride ethyl acetate solution (2N, 2 mL) was added dropwise. The mixture was stirred at r.t. for 12h, then filtered. The residue was washed with EtOAc and EtOH separately，dried in vacuo to give compound **26** hydrochloride as white solid. Yield (0.56 g, 58 %). Mp: 249-250 ^{o}C. MS (ESI) *m/z*: 464.23 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO) δ 7.57 – 7.43 (m, 3H), 7.32 (dd, *J* = 8.8, 4.6 Hz, 1H), 7.28 – 7.14 (m, 6H), 6.89 (td, *J* = 9.2, 2.5 Hz, 1H), 3.37 (d, *J* = 12.0 Hz, 2H), 3.15 – 2.94 (m, 6H), 2.89 – 2.74 (m, 2H), 2.67 (t, *J* = 7.1 Hz, 2H), 1.76 – 1.56 (m, 4H). ^{13}C NMR (150 MHz, DMSO) δ 162.66 (d, *J* = 242.0 Hz), 158.99 (d, *J* = 239.0 Hz), 157.06 (d, *J* = 229.4 Hz), 145.34 (s), 141.51 (d, *J* = 7.8 Hz), 135.49 (d, *J* = 7.2 Hz), 133.43 (s), 130.94 (d, *J* = 8.3 Hz), 127.71 (d, *J* = 9.6 Hz), 125.13 (s), 125.02 (s), 121.27 (d, *J* = 8.1 Hz), 118.20 (d, *J* = 23.0 Hz), 115.72 (d, *J* = 22.2 Hz), 115.58 (d, *J* = 21.6 Hz), 114.86 (d, *J* = 20.7 Hz), 114.65 (d, *J* = 4.4 Hz), 112.72 (d, *J* = 9.6 Hz), 109.35 (d, *J* = 25.8 Hz), 103.40 (d, *J* = 22.7 Hz), 55.71 (s), 51.28 (s), 48.16 (s), 27.36 (s), 24.52 (s), 23.29 (s). HRMS calcd for $C_{28}H_{28}N_3F_3$ $[M+H]^+$, 464.2314; found, 464.2318.

*4.1.20 Procedure T. General procedure for the preparation of compounds **20a-h***

A mixture of biphenyl amine **27** (90 mmol), Bis(2-chloroethyl)amine hydrochloride **28** (90 mmol), K_2CO_3 (90 mmol) and KI (90 mmol) in xylene (450 mL) was stirred at 140 ^{o}C for 48 h. After cooling to room temperature, the solvent was evaporated, then DCM (150 mL) was added. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH = 20/1, V/V) to give compound **20**.

*4.1.20.1 1-(2'-fluoro-[1,1'-biphenyl]-2-yl)piperazine (**20a**)*

Yield (22 %) as a yellow solid. Mp: 63-64 ^{o}C. MS (ESI) *m/z*: 257.23 $[M+H]^+$.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (td, J = 7.5, 1.8 Hz, 1H), 7.36 – 7.31 (m, 1H), 7.31 – 7.23 (m, 2H), 7.19 – 7.13 (m, 1H), 7.13 – 7.06 (m, 3H), 2.80 – 2.77 (m, 4H), 2.71 – 2.68 (m, 4H).

*4.1.20.2 1-(3'-fluoro-[1,1'-biphenyl]-2-yl)piperazine (**20b**)*

Yield (38%) as a white solid. Mp: 223-224^{o}C. MS (ESI) *m/z*: 257.18 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.30 (m, 3H), 7.28 – 7.26 (m, 1H), 7.24 – 7.23 (m, 1H), 7.15 (t, *J* = 7.1 Hz, 1H), 7.07 – 6.98 (m, 2H), 3.13 – 3.06 (m, 8H).

*4.1.20.3 1-(3'-methyl-[1,1'-biphenyl]-2-yl)piperazine (**20c**)*

Yield (41 %) as a pale-yellow solid. Mp: 172-174 ^{o}C. MS (ESI) *m/z*: 253.21 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.7 Hz, 1H), 7.31 – 7.28 (m, 2H), 7.28 – 7.27 (m, 1H), 7.25 – 7.23 (m, 1H), 7.16 – 7.09 (m, 2H), 7.01 (d, *J* = 7.4 Hz, 1H), 3.15 – 3.10 (m, 4H), 3.07 – 3.04 (m, 4H), 2.39 (s, 3H).

*4.1.20.4 1-(4'-methyl-[1,1'-biphenyl]-2-yl)piperazine (**20d**)*

Yield (39%) as a white solid. Mp: 253 - 254 ^{o}C. MS (ESI) *m/z*: 253.19 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.0 Hz, 2H), 7.29 (dd, J = 7.7, 1.5 Hz, 1H), 7.24 (dd, J = 7.6, 1.6 Hz, 1H), 7.21 (d, J = 7.9 Hz, 2H), 7.13 (td, J = 7.5, 0.8 Hz, 1H), 7.01 (d, J = 7.9 Hz, 1H), 3.13 – 3.10 (m, 4H), 3.07 – 3.05 (m, 4H), 2.38 (s, 3H).

*4.1.20.5 2'-(piperazin-1-yl)-[1,1'-biphenyl]-4-carbonitrile (**20e**)*

Yield (33%) as a white solid. Mp: 259-260 ^{o}C. MS (ESI) *m/z*: 264.14 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.66 (m, 4H), 7.38 (td, *J* = 8.1, 2.0 Hz, 1H), 7.26 – 7.17 (m, 2H), 7.10 (d, *J* = 7.9 Hz, 1H), 3.16 – 3.04 (m, 8H).

*4.1.20.6 1-(3',5-difluoro-[1,1'-biphenyl]-2-yl)piperazine (**20f**)*

Yield (36%) as a white solid. Mp: 253 - 254 ^{o}C. MS (ESI) *m/z*: 275.14 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.36 (m, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.22 (d, *J* = 9.8 Hz, 1H), 7.08 – 6.96 (m, 4H), 3.09 (s, 8H).

*4.1.20.7 1-(3',6-difluoro-[1,1'-biphenyl]-2-yl)piperazine (**20g**)*

Yield (27%) as a pale-yellow solid. Mp: 181 - 182 ^{o}C. MS (ESI) *m/z*: 275.22 $[M+H]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 14.0, 7.9 Hz, 1H), 7.33 – 7.27 (m, 1H),

7.22 (d, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 9.7 Hz, 1H), 7.06 (td, *J* = 8.4, 2.3 Hz, 1H), 6.93 (t, *J* = 8.7 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 3.18 – 3.15 (m, 4H), 3.07 – 3.05 (m, 4H).

*4.1.20.8 1-(3',4-difluoro-[1,1'-biphenyl]-2-yl)piperazine (**20h**)*

Yield (23%) as a yellow solid. Mp: 104-105 ^{o}C. MS (ESI) *m/z*: 275.17 $[M+H]^{+}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.35 (m, 1H), 7.29 (s, 1H), 7.22 – 7.18 (m, 2H), 7.03 (td, *J* = 8.4, 1.9 Hz, 1H), 6.86 (td, *J* = 8.2, 2.4 Hz, 1H), 6.76 (dd, *J* = 10.5, 2.4 Hz, 1H), 3.17 – 3.10 (m, 8H).

*4.1.20.91-(5-fluoro-[1,1'-biphenyl]-2-yl)piperazine (**20i**)*

Yield (38%) as a white solid. Mp: 254 - 255 ^{o}C. MS (ESI) *m/z*: 257.14 $[M+H]^{+}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.48 (m, 2H), 7.44 – 7.38 (m, 2H), 7.36 – 7.30 (m, 1H), 6.99 (d, *J* = 7.5 Hz, 3H), 3.06 (s, 8H).

4.2 Biology evaluation

4.2.1 In vitro binding assays

4.2.1.1 5-HT reuptake

The synaptosomes (150 µg) are incubated for 15 min at 37°C with 0.1 µCi [^{3}H]serotonin in the absence (control) or presence of the test compound or the reference compound in a buffer containing 106.2 mM NaCl, 4.5 mM KCl, 2.25 mM $MgSO_4$, 1.08 mM NaH_2PO_4, 22.5 mM $NaHCO_3$, 9.9 mM glucose, 9 µM EGTA and 45 µM ascorbic acid (pH 7.4). Basal control activity is determined by incubating the same mixture for 15 min at 4°C in the presence of 10 µM imipramine to block the uptake. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) and rinsed twice with ice-cold incubation buffer using a 96-sample cell harvester (Unifilter, Packard) to eliminate free [^{3}H]serotonin. The filters are dried and the retained radioactivity is measured in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard).The results are expressed as a percent inhibition of the control uptake of [^{3}H]serotonin. The standard inhibitory reference compound is imipramine, which is tested in each

experiment at several concentrations to obtain an inhibition curve from which its IC_{50} value is calculated[28].

4.2.1.2 5-HT_{1A} receptor

Cell membrane homogenates (36 µg protein) are incubated for 60 min at 22°C with 0.3 nM [^{3}H]8-OH-DPAT in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgSO_4$, 0.5 mM EDTA and 2 µg/ml aprotinine. Nonspecific binding is determined in the presence of 10 µM 8-OH-DPAT. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding. The standard reference compound is 8-OH-DPAT, which is tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated[29].

4.2.1.3 5-HT_7 receptor

Cell membrane homogenates (24 µg protein) are incubated for 120 min at 22°C with 4 nM [^{3}H] LSD in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 10 mM $MgSO_4$ and 0.5 mM EDTA. Nonspecific binding is determined in the presence of 10 µM 5-HT. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding. The standard reference compound is 5-HT, which is tested in each experiment at several concentrations to

obtain a competition curve from which its IC_{50} is calculated[30].

4.2.1.4 α1-Adrenoceptor

Membrane homogenates of cerebral cortex (160 µg protein) are incubated for 60 min at 22°C with 0.25 nM [^{3}H]prazosin in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA and 0.1% BSA. Nonspecific binding is determined in the presence of 0.5 µM prazosin. Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard). The results are expressed as a percent inhibition of the control radioligand specific binding. The standard reference compound is prazosin, which is tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated[31].

4.2.2 Metabolic Stability test

Solutions preparation: The stock solutions of test article and positive control were prepared at a concentration of 10 mM using DMSO as diluents. All stock solutions were then diluted to working concentrations at 0.25 mM with 70% acetonitrile. The cofactor used in this study was NADPH regenerating system, that was composed of 6.5 mM NADP, 16.5 mM G-6-P, 3 U/mL G-6-P D. The quench reagent was consisted of acetonitrile containing tolbutamide and propanolol (serve as internal standard). The buffer used in this study was 100 mM, phosphate buffer with 3.3 mM $MgCl_2$. Incubation mixtures containing 0.5 mg/mL liver microsomal protein and 1 µM test article/positive control in 100 mM potassium phosphate buffer.

Assay procedure: The 0-minute samples were prepared by addition of an 80 µL aliquot of each incubation mixture to 300 µL quench reagent to precipitate proteins. The samples were vortexed, and then a 20 µL aliquot of the NADPH regenerating

system was added in. The reaction was initiated by addition of 80 µL of the NADPH regenerating system to 320 µL of each incubation mixture. The final incubation conditions achieved in 400 µL are: 0.5 mg/mL microsomal protein, 1 µM test article/positive control, 1.3 mM NADP, 3.3 mM glucose 6 phosphate, 0.6 U/mL glucose 6 phosphate dehydrogenase. The mixtures were incubated in a 37°C water bath with gentle shaking. A 100 µL aliquot of each mixture was removed at 10, 30, 90 minutes to a clean 96-well plate which contains 300 µL quench reagent to precipitate proteins, and centrifuged (4000 ×g, 10 min). 80 µL of supernatant are taken into 96-well assay plates pre-added with 160 µL ultrapure water, and then analyzed by LC-MS/MS[32].

4.2.3 Measurement of antidepressant efficacy in mouse forced swim test (FST).

To evaluate the antidepressant efficacy of the compounds, depressive-like states of the mice in forced swim test were measured according to the methods described by Porsolt et al.[33] Mice were placed in an inescapable transparent cylindrical tanks (30 cm height x 20 cm diameters) filled with water (25 ± 1^{o}C , 15 cm depth) and their escape related mobility behavior is measured. Mice were administered to vehicle, vortioxetine (40 mg/kg/day) as a positive control, and test compounds (10, 20 and 40 mg/kg/day) for 7 days (PO).The mice were tested 1h after the last dose. The test length for mice is six minutes. Only the last four minutes of the test are analyzed. During the behavioral analysis, the time that each mouse spends mobile is measured. The total amount of mobility time is then subtracted from the 240 seconds of test time and is then stated as the immobility time. Data was analyzed by one way analysis of variance (ANOVA). *:$p<0.05$, **:$p<0.01$, denote a significant difference compared to vehicle group, Dunnett's post hoc test. Data are expressed as mean ± SD.

4.2.4Measurement of antidepressants activity in mouse tail suspension test (TST)

The method, which detects antidepressant activity, the procedure was performed essentially as described by Steru et al. [34] Mice were individually suspended 75 cm above the tabletop with an adhesive tape placed 1 cm from the tip of the tail.

Antidepressants decrease the duration of immobility. The behavior of the animal was recorded for 5 min. Ten-twelve mice were tested in each group. The test was performed blind. Compounds were typically evaluated at 3 doses (10-40 mg/kg/day), administered orally once-daily for 7 days: 1h before the test after the last dose, and compared with a vehicle control group. Venlafaxine (40 mg/kg/day), administered under the same experimental conditions, was used as the positive reference substance. Data were analyzed by one way analysis of variance (ANOVA) followed by posthoc comparisons where appropriate. An effect was considered significant if $p < 0.05$.

4.2.5 hERG test

CHO-K1 (Chinese Hamster Ovary) cells stably transfected with human hERG cDNA were used. The cells were harvested by trypsinization and maintained in Serum Free Medium at room temperature before recording. The test solutions were prepared in the Extracellular Solution on the day of patch clamp assay. The assay can tolerate up to 1% DMSO. After whole cell configuration was achieved, the cell was held at -80 mV. A -50 mV pulse was delivered for 50 ms to measure the leaking current, which was subtracted from the tail current on-line. Then the cell was depolarized to +20 mV for 5 s, followed by a 5 s pulse to -50 mV to reveal the hERG tail current. This paradigm was delivered once every 15 s to monitor the current amplitude. The Extracellular Solution (control) was applied first and the cell was stabilized in the solution for 2 min. Then the test compound was applied from low to high concentrations sequentially on the same cell. The cells were incubated with each test concentration for 2 min. Cisapride was tested concurrently at multiple concentrations to obtain an IC_{50} value. The percent inhibition of the hERG channel is calculated by comparing the tail current amplitude before and after application of the compound (the current difference is normalized to control)[35].

4.2.6 Pharmacokinetic study

Male SD rats (B&K Universal Group Ltd, China) were used. For intravenous administration, prepared dosing solution was injected via the femoral vein. The rats

were fasted overnight before drug administration and until 6 h after dosing. For the po experiment, rats (three in each group) were given a single dose of 2 mg/kg, and heparinized samples of blood were collected at 15, 30 min, 1, 2, 4, 6, 8, and 9 h postdose. For the iv experiment, rats (three in each group) were given a single 0.5 mg/kg dose, and blood samples were collected at 5, 15, 30 min, 1, 2, 4, 6 and 8 h postdose. Plasma was harvested after centrifugation and stored frozen at -40 until analyzed. The concentrations of compounds in plasma were determined by LC/MS/MS (API3200). The results are shown as the maximum plasma concentration (C_{max}), the time to reach peak plasma concentration (T_{max}), terminal half-life ($t_{1/2}$), and the area under the plasma concentration-time curve from zero to time infinity ($AUC_{0\text{-}inf}$).

Acknowledgments

The authors gratefully acknowledge the financial support from the National Science and Technology Major Project (Grant No. 2017ZX09101004-002-009), the National Natural Science Foundation of China (Grant No. 81703358), Shanghai S&T Innovation Action Plan (Grant Nos. 16431903400, 14431901500, 17431903900) . We thank M.S. Feng-Hua Gu (Department of pharmacology, Shanghai Institute of Pharmaceutical Industry, China) for *in vivo* studies.

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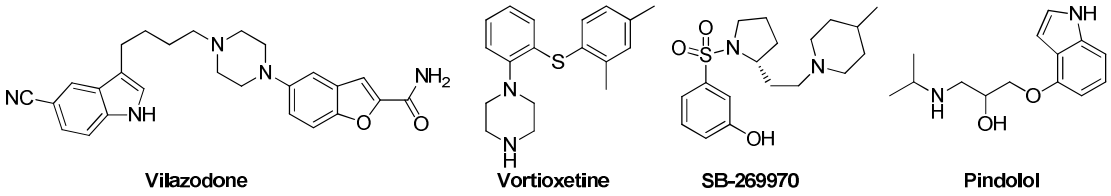


Fig. 1. Chemical structures of representative antidepressant compounds

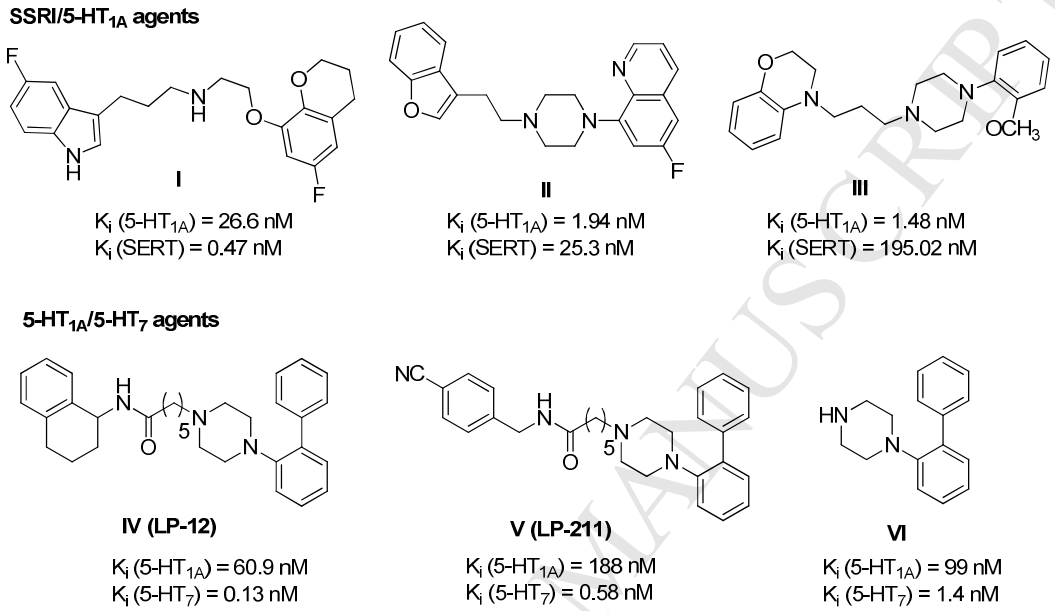


Fig. 2. Structures of SSRI/5-HT_{1A} agents and 5-HT_{1A}/5-HT_7 agents

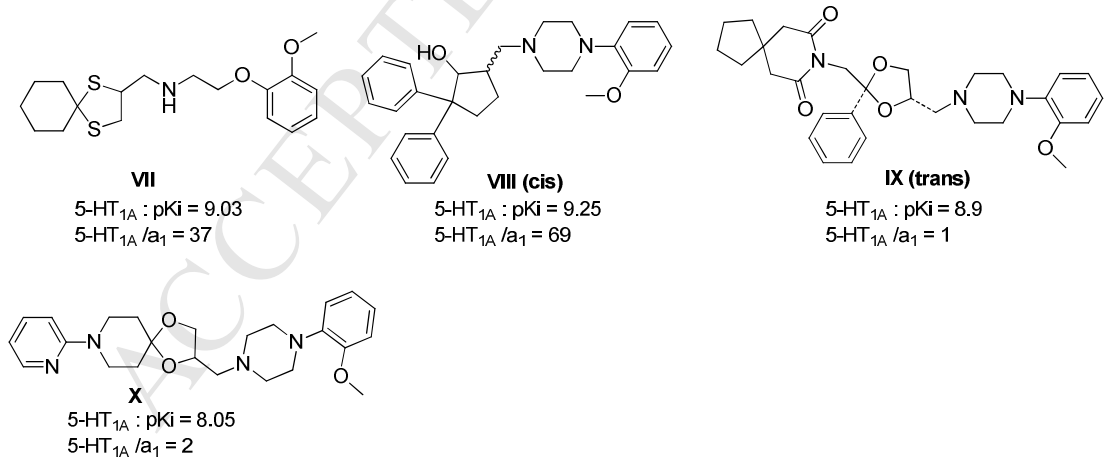


Fig. 3. Structures of 5-HT_{1A}R/α1-adrenoceptor ligands

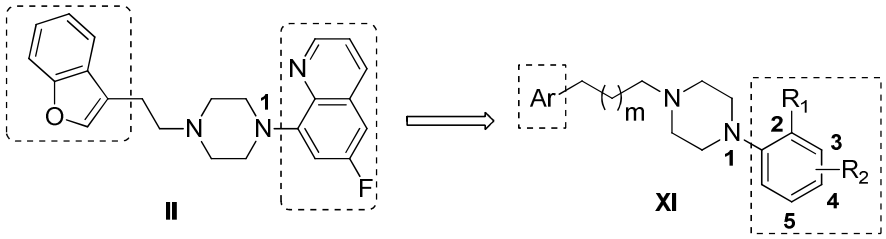
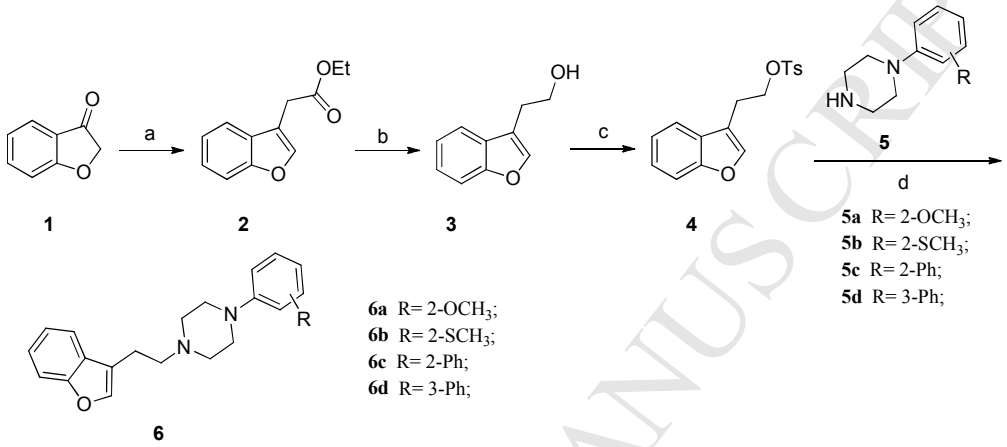
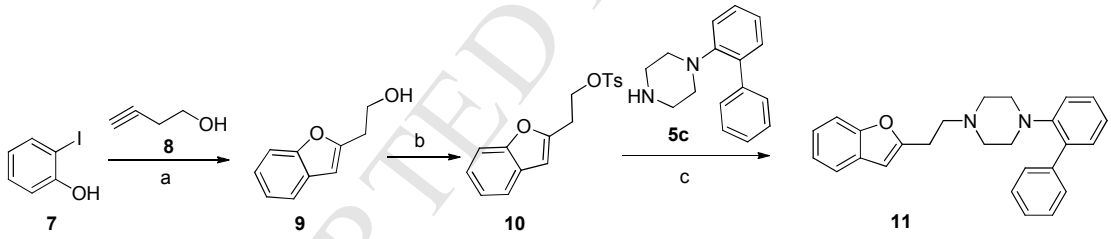


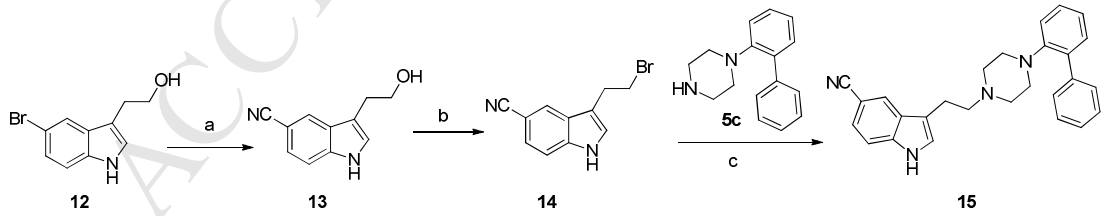
Fig. 4. Rational design of novel SSRI/5-HT_{1A}/5-HT_7 multimodal activity compounds



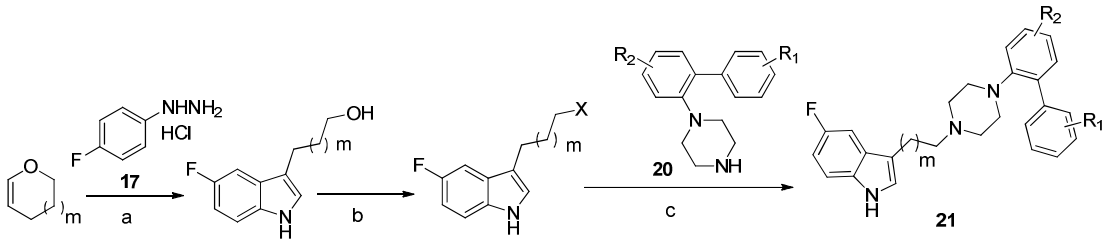
Scheme 1. Reagents and conditions: (a) (Carbethoxymethylene)triphenylphosphorane, Toluene, reflux, N_2 atmosphere; (b) $LiAlH_4$, THF, 0 $^oC \rightarrow$r.t.; (c) TsCl, Et_3N, DMAP, DCM, r.t.; (d) K_2CO_3, CH_3CN, 80 ^oC.



Scheme 2. Reagents and conditions: (a) CuI, $Pd(OAc)_2$, PPh_3, Et_3N, 30 ^oC; (b) TsCl, Et_3N, DMAP, DCM, r.t.; (c) K_2CO_3, CH_3CN, 80 ^oC.



Scheme 3. Reagents and conditions: (a) CuCN, DMF, 130 ^oC, N_2 atmosphere; (b) CBr_4, PPh_3, DCM, r.t.; (c) K_2CO_3, KI, DMF, 100 ^oC.



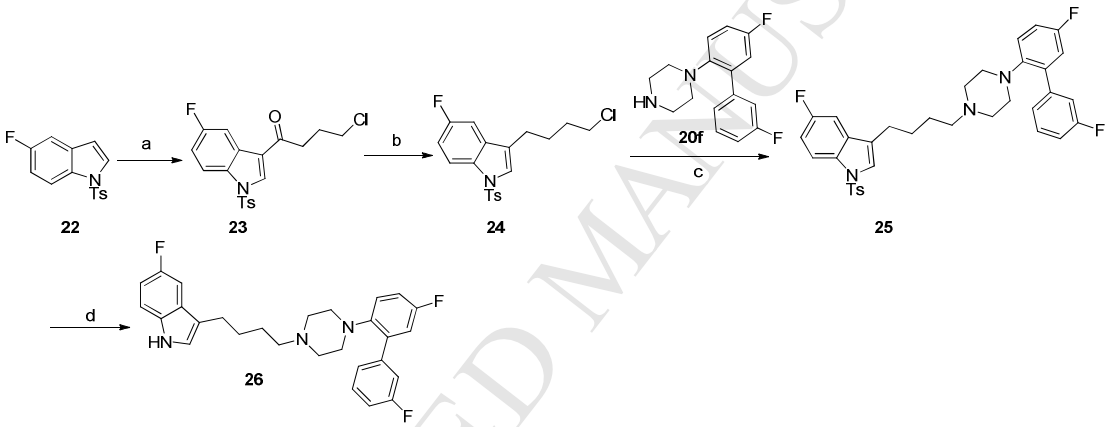
16a m=0
16b m=1

18a m= 0
18b m= 1

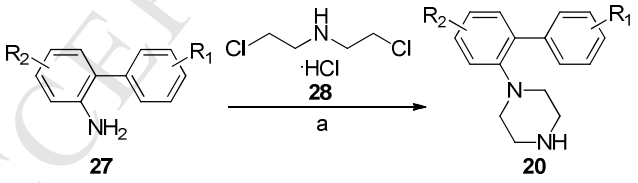
19a m= 0, X= Br
19b m= 1, X= OTs

21a m= 0, R_1= H, R_2= H; **21b** m= 0, R_1= 4-F, R_2= H; **21c** m= 0, R_1= 4-CH_3, R_2= H;
21d m= 0, R_1= 4-OCH_3, R_2= H; **21e** m= 0, R_1= 4-CN, R_2= H; **21f** m= 0, R_1= 3-F, R_2= H;
21g m= 0, R_1= 3-CH_3, R_2= H; **21h** m= 0, R_1= 2-F, R_2= H; **21i** m= 1, R_1= H, R_2= H;
21j m= 1, R_1= 3-F, R_2= H; **21k** m= 1, R_1= 3-F, R_2= 4-F; **21l** m= 1, R_1= 3-F, R_2= 3-F;
21m m= 1, R_1= 3-F, R_2= 5-F; **21n** m= 1, R_1=H, R_2= 4-F;

Scheme 4. Reagents and conditions: (a) H_2SO_4 (4%), DMAC, 100 °C; (b) for **19a**: CBr_4, PPh_3, DCM, r.t.; for **19b**: TsCl, Et_3N, DMAP, DCM, r.t.; (c) for **21a-h**: K_2CO_3, KI, DMF, 100 °C; for **21i-n**: K_2CO_3, CH_3CN, 80 °C.



Scheme 5. Reagents and conditions: (a) 4-chlorobutanoyl chloride, $AlCl_3$, DCM, 0 °C→r.t.; (b) $HSiEt_3$, TFA, 50 °C; (c) K_2CO_3, KI, DMF, 100 °C; (d) 4N NaOH, EtOH/THF, 80 °C;

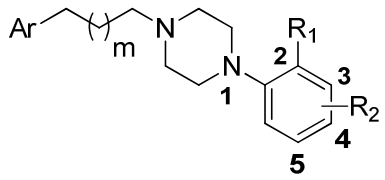


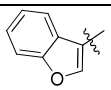
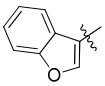
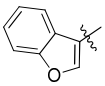
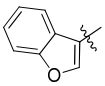
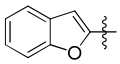
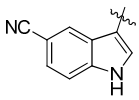
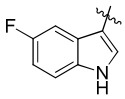
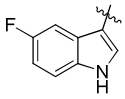
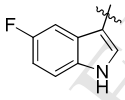
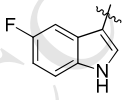
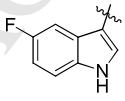
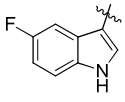
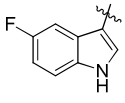
20a R_1 = 2-F, R_2 = H; **20b** R_1 = 3-F, R_2 = H; **20c** R_1 = 3-CH_3, R_2 = H;
20d R_1 = 4-CH_3, R_2 = H; **20e** R_1 = 4-CN, R_2 = H; **20f** R_1 = 3-F, R_2 = 4-F;
20g R_1 = 3-F, R_2 = 3-F; **20h** R_1 = 3-F, R_2 = 5-F; **20i** R_1 = H, R_2 = 4-F;

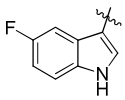
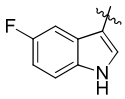
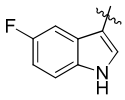
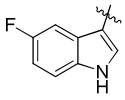
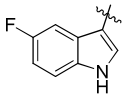
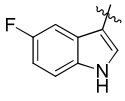
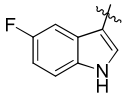
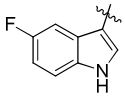
Scheme 6. Reagents and conditions: (a) K_2CO_3, KI, xylene, 140 °C.

Table 1

5-HT_{1A} and 5-HT_7 Receptor Binding and 5-HT Reuptake Inhibition of target compounds



Compd.	Ar	R_1	R_2	m	RUI (IC_{50}, nM)	5-HT_{1A} (K_i, nM)	5-HT_7 (K_i, nM)
6a		OCH_3	H	0	110.3%[a]	98.5%[a]	85.6%[a]
6b		SCH_3	H	0	112.3%[a]	99.7%[a]	86.8%[a]
6c		Ph	H	0	210[b]	99.5%[a]	102.3%[a]
6d		H	3-Ph	0	99.4%[a]	97.5%[a]	71.9%[a]
11		Ph	H	0	2900[b]	97.7%[a]	96.4%[a]
15		Ph	H	0	190[b]	97.6%[a]	93.7%[a]
21a		Ph	H	0	110[b]	0.53[b]	0.26[b]
21b		4-F-Ph	H	0	590[b]	101.2%[a]	94.9%[a]
21c		4-CH_3-Ph	H	0	1700[b]	100.9%[a]	99.5%[a]
21d		4-OCH_3-Ph	H	0	82.8%[a]	99.2%[a]	94.7%[a]
21e		4-CN-Ph	H	0	84.2%[a]	99.5%[a]	97.3%[a]
21f		3-F-Ph	H	0	150[b]	5.6[b]	0.79[b]
21g		3-CH_3-Ph	H	0	160[b]	98.1%[a]	97.3%[a]

21h		2-F-Ph	H	0	96 [b]	25 [b]	2.3 [b]
21i		Ph	H	1	7.6 [b]	13 [b]	1.1 [b]
21j		3-F-Ph	H	1	42 [b]	8.1 [b]	1.1 [b]
21k		3-F-Ph	4-F	1	31 [b]	62 [b]	12 [b]
21l		3-F-Ph	3-F	1	10 [b]	18 [b]	0.55 [b]
21m		3-F-Ph	5-F	1	9.4 [b]	15 [b]	2.6 [b]
21n		Ph	4-F	1	25 [b]	28 [b]	3.3 [b]
26		3-F-Ph	4-F	2	120 [b]	96.0% [a]	97.5% [a]
Vortioxetine					2.9 [b]	9.5 [b]	26 [b]

[a] Percent inhibition measured at a concentration of 10 μM.

[b] IC_{50} and K_i values were obtained from 8 concentrations of the compound, each in duplicate. (Binding assays were conducted by Eurofins Cerep SA, Celle L'Evescault, France).

Table 2

Rat Liver Microsomal Metabolic Stability Assay

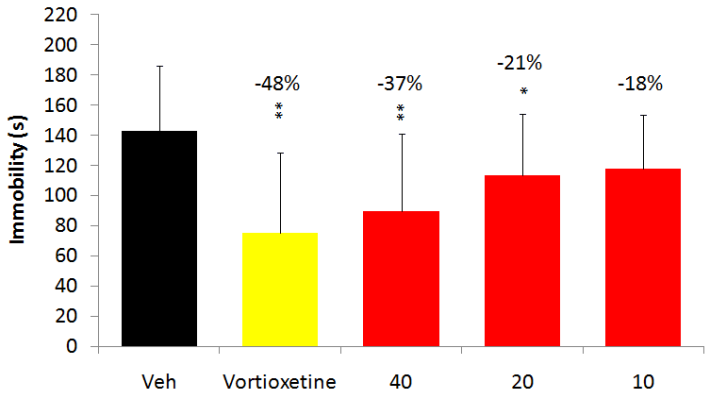
Compd.	$t_{1/2}$ (min)	CL (μL/min/mg)
21a	6.1	225.6
21b	9.6	143.7
21f	9.0	154.7
21h	4.0	343.1
21j	27.7	50.0
21k	56.8	24.4
21l	26.9	51.5
21m	28.8	48.1
21n	46.5	29.8

Vortioxetine	9	153.2
Omeprazole	8.5	162.6

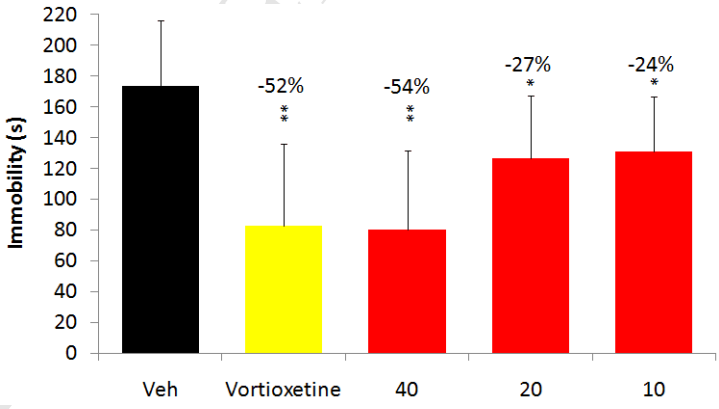
Table 3

Binding Affinity for α1-Adrenoceptor for Selected Compound

Compd.	α_1 (K_i, nM)	5-HT_{1A}/α_1
21n	28	1
Prazosin	0.15	



compound **21k** (mg/kg, PO)



compound **21n** (mg/kg, PO)

Fig. 5. Effect of treatment of mice with compounds **21k** and **21n** at graded doses on the immobility time in the forced swim test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

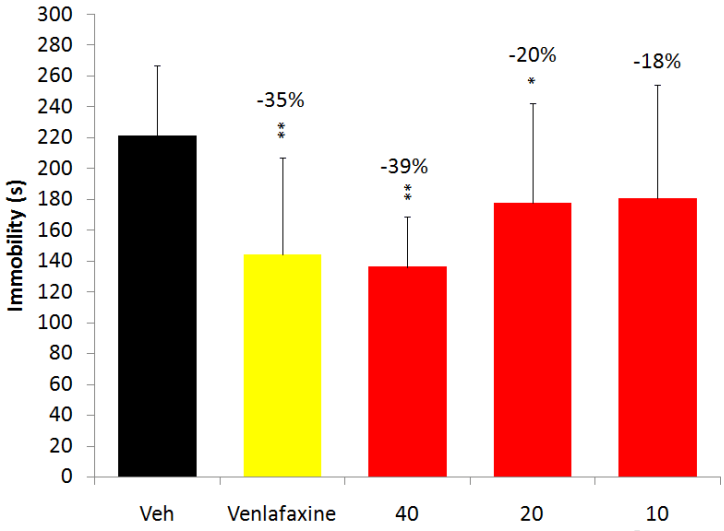


Fig. 6. Effect of treatment of mice with compound **21n** at graded doses on the immobility time in the tail suspension test. Results are represented as mean ± SEM. with n = 10 in each group. Values are significant at *P < 0.05, **P < 0.01 when compared with vehicle group.

Table 4

Pharmacokinetic parameters of compound **21n**.

Dose (mg/kg)	$AUC_{(0-t)}$ (ngh/mL)	$AUC_{(0-\infty)}$ (ngh/mL)	$MRT_{(0-t)}$ (h)	$V_{z/F}$ (L/kg)	$CL_{z/F}$ (L/h/kg)	$T_{1/2Z}$ (h)	T_{max} (h)	C_{max} (ng/mL)	F (%)
2 (po)	536.1	898.2	4.3	17.1	2.4	6.1	1.7	86.7	68.5%
0.5 (iv)	286.7	327.8	1.9	7.4	1.5	3.3	0.1	342.4	

Highlights

- A series of aralkyl piperazine derivatives have been synthesized.
- All compounds were evaluated for SSRI/5-HT_{1A}/5-HT_7 activities *in vitro.*
- The potent compounds were screened using the forced swimming test and the tail suspension test *in vivo*.
- Compound **21n** displayed a good pharmacokinetic properties and potent antidepressant-like profiles *in vitro* and *in vivo*.