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Synthesis and biological evaluation of 2-(4-fluorophenoxy)-2-phenyl-ethyl piperazines as serotonin-selective reuptake inhibitors with a potentially improved adverse reaction profile

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Abstract—Three new 2-(4-fluorophenoxy)-2-phenyl-ethyl piperazines, 1-(3-chlorophenyl)-4-[2-(4-fluorophenoxy)-2-phenylethyl]-piperazine **7**, 1-[2-(4-fluorophenoxy)-2-phenylethyl]-4-(2-methoxyphenyl)-piperazine **8**, and 1-[2-(4-fluorophenoxy)-2-phenylethyl]-4-(3-trifluoromethylphenyl)-piperazine **9**, modeled after the potent antidepressant fluoxetine and coupled with several functionalized piperazines, have been prepared by chemical synthesis as selective serotonin reuptake inhibitors (SSRIs) with a potentially improved adverse reaction profile. Typical SSRIs, although very effective in the treatment of depression, still face the troublesome side effect of sexual dysfunction. A number of pharmacological agents-notably, drugs in the piperazine class-have been used to reverse SSRI-induced sexual dysfunction, and evidence for developing an improved SSRI by coupling a fluoxetine congener with the pharmacophore of a reversal agent holds promise. Preliminary data indicates that the hydrochloride (HCl) salts **10**, **11**, and **12** each exhibit single-site binding at the site of the serotonin reuptake transporter (SERT). However, each of the three compounds are much less potent than typical SSRIs, showing micromolar (μM) affinity for the SERT with IC_{50} values of 1.45 μM, 3.27 μM, and 9.56 μM, respectively. Further biological evaluation of compounds **10**, **11**, and **12** is needed before definitive conclusions can be made with regard to each compound's potential for use as an SSRI-type candidate which is devoid of sexual side effects. Nevertheless, the initial findings are quite encouraging, thus lending credence to the idea of hybridizing an SSRI congener with that of the pharmacophore of an agent known to reverse or treat SSRI-induced sexual dysfunction.

1. Introduction

In the late-1980s, the serotonin-selective reuptake inhibitor (SSRI) fluoxetine became the mainstay of treatment for clinical depression-replacing the more toxic tricyclic antidepressants (TCAs).[1] SSRIs have a more favorable adverse reaction profile in comparison to the TCAs and are much easier to tolerate. The structures of some of the typically prescribed SSRIs are depicted in Figure 1. However, SSRIs are not innocuous drugs. In spite of the progress in the treatment of depression with the advent of the SSRIs, some troublesome side effects still remain-most notably, sexual dysfunction. As prescribing of SSRIs increased, the side effect of sexual dysfunction emerged, and clinicians searched for methods to treat or reverse SSRI-induced sexual dysfunction. A number of pharmacological agents-notably, drugs in the piperazine class-have been used to reverse SSRI-induced sexual dysfunction, and evidence for developing an improved SSRI by coupling a fluoxetine congener with the pharmacophore of a reversal agent holds promise.

It is widely believed that a number of monoamine neurotransmitters (Fig. 2) have a role in the etiology of depression and, consequently, its treatment. The most notable of these neurotransmitters include norepinephrine (NE) and serotonin (5-HT).[2] It is well known that inhibiting the reuptake of NE or 5-HT (or both) has elicited a favorable response in patients being treated for depression. Without question, however, inhibition of 5-HT reuptake has been the most widely studied, and treatment of depression has focused on inhibiting the reuptake mechanism of the neurotransmitter.[3] Indeed, it was this premise which led to the hypothesis that altering 5-HT function could lead to an effective mechanism in the treatment of depression.[4]

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The basis for the role of serotonin in the etiology of depression was hypothesized by Lapin and Oxenkrug in 1969.[5] Before that time, most hypotheses relied on the premise that biogenic amines including NE, 5-HT and dopamine (DA) were being produced in insufficient quantities in the limbic system of afflicted patients. Adding to the confusion is the fact that medications which alter the action or release of each of these neurotransmitters have elicited favorable responses in clinically depressed patients. Examples like bupropion and venlafaxine (Fig. 3), both administered as racemates, have mechanisms of action which are not confined to one neurotransmitter system.[6]

Research in the treatment of depression remains focused on 5-HT because, to date, a good number of selective 5-HT reuptake blockers tested in clinical trials have been effective in treating major depression. The general mechanism of action is hypothesized as enhanced 5-HT neurotransmission due to the increased availability of 5-HT in the synapse of these neurons.[7] SSRIs generally have a 50- to 100-fold or greater selectivity for the inhibition of serotonin uptake in vitro when compared to their ability to inhibit NE or DA uptake.[8]

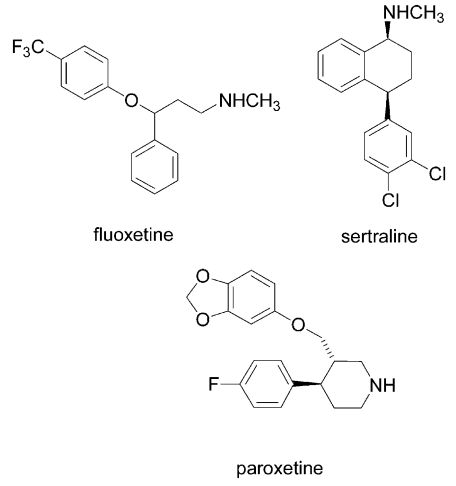


Figure 1. Representative serotonin-selective reuptake inhibitors.

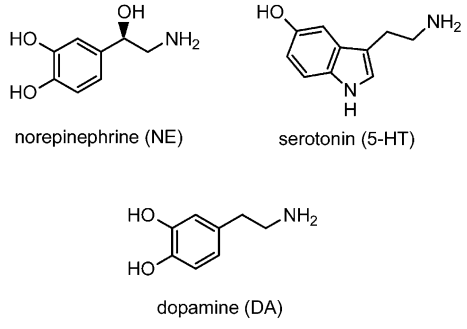


Figure 2. Structures of endogenous monoamine neurotransmitters.

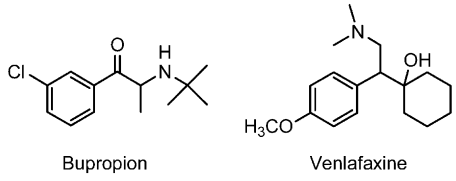


Figure 3. Antidepressant compounds not confined to one neurotransmitter system.

Sexual dysfunction is a common problem in affective disorders such as major depression. It is important to note, however, that the drugs used in the treatment of depression can cause sexual dysfunction as well. Antidepressants such as the TCAs, monoamine oxidase inhibitors, and the SSRIs have all been reported to cause sexual side effects.[9] The inhibitory effects of the SSRIs on sexual function are well documented, and the frequency of occurrence varies widely. Some sources list ranges varying from 1% up to 75%[10] while the majority of sources estimate the occurrences in the 20–40% range.[11] By switching to a less serotonergic drug, it is surmised that the non-selective effects of 5-HT can be reduced within the synapse of the 5-HT neuron. Although the connection between serotonergic activity and sexual function is not straightforward, it is believed that the increased central serotonergic activity of SSRIs (at the receptor subtypes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, 5-HT_{2A} and 5-HT_3) plays a role in the emergence of sexual dysfunction.[10] It follows, therefore, that reducing the activity of SSRIs at these receptors could allow the suppression or inhibition of the sexual response to be corrected. Strategies which have been tried in treating or reversing sexual dysfunction include: titration of an antidepressant dose, scheduling of a drug holiday during treatment, and administering a second drug (along with an SSRI, for example) to counteract the SSRI's sexual side effects.[11]

The drugs which have been administered along with a SSRI include a broad range of medicinal agents (structures depicted in Fig. 4). Drugs such as: amantadine (normally used in the treatment of Parkinsonism), cyproheptadine, buspirone, trazodone, nefazodone, methylphenidate, mianserin, yohimbine, and even sildenafil citrate. Bupropion has also been of benefit and is depicted in Figure 3.

The success of each of these agents varies widely, but the best studied agents include bupropion and buspirone.[13] Buspirone, which has a pyrimidinyl-piperazine group, is generally used in the treatment of generalized anxiety disorder and has been used with some success in treating patients with SSRI-induced sexual dysfunction.[12] It is interesting to note that other piperazine-containing groups (specifically, functionalized phenylpiperazines) have likewise been used with success in treating sexual dysfunction. These agents include: sildenafil citrate, trazodone, and nefazodone.

Based on the information that buspirone, trazodone, and nefazodone have all been used successfully in treating SSRI-induced sexual dysfunction, it is proposed that coupling the piperazinyl moiety to a structural homologue of an SSRI may have utility in synthesizing an SSRI candidate with an adverse reaction profile devoid of sexual side effects. The strategy, therefore, is to covalently couple a structural homologue of the SSRI antidepressant, fluoxetine, with that of the piperazinyl-

containing portion of each of the following compounds: buspirone, trazodone, and nefazodone (Fig. 4).

2. Results and discussion

In the attempt to create a novel serotonin reuptake inhibitor with an improved side effect profile, three new 2-(4-fluorophenoxy)-2-phenyl-ethyl piperazines, fluoxetine structural homologues with three different piperazine side chains, have been synthesized and biologically evaluated for their potential as antidepressants.

It was decided that a racemic mixture of 2-amino-1-phenylethanol **1** would serve as a good source of starting material. The primary amine in compound **1** was protected with di-*tert*-butyl-dicarbonate (*t*-boc anhydride) **2**, to afford protected alcohol **3**. This was subsequently converted to 4-fluorophenyl ether **4** using Mitsunobu conditions, a combination of triphenylphosphine (PPh_3) and diethylazodicarboxylate (DEAD). The protected ether was deprotected with 4N HCl in 1,4-dioxane, giving free amine **5**. Bromide **6** was afforded by treating free amine **5** with a combination of $TiBr_4$ and *tert*-butyl nitrite (Scheme 1).

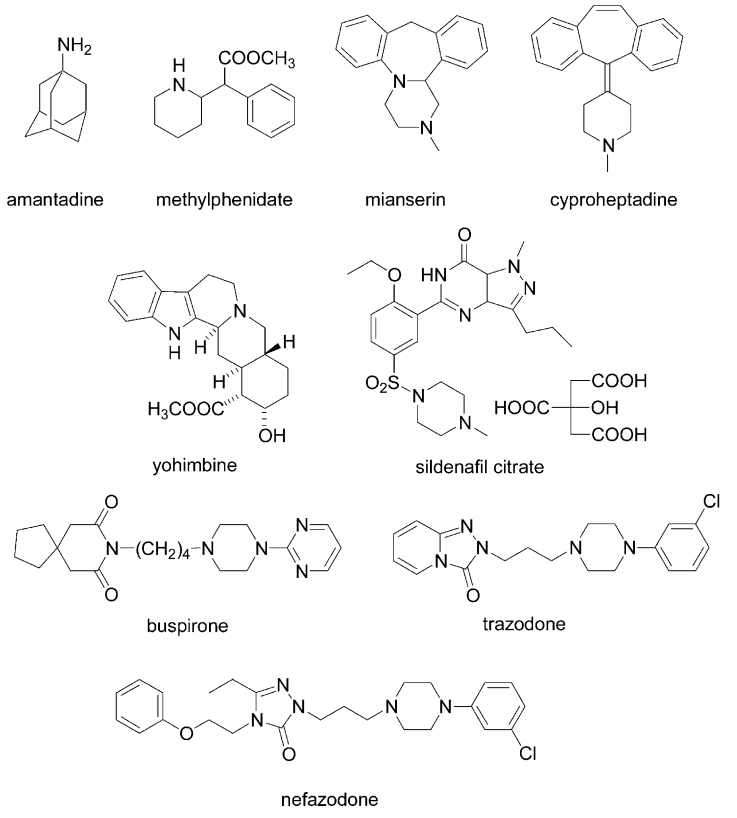
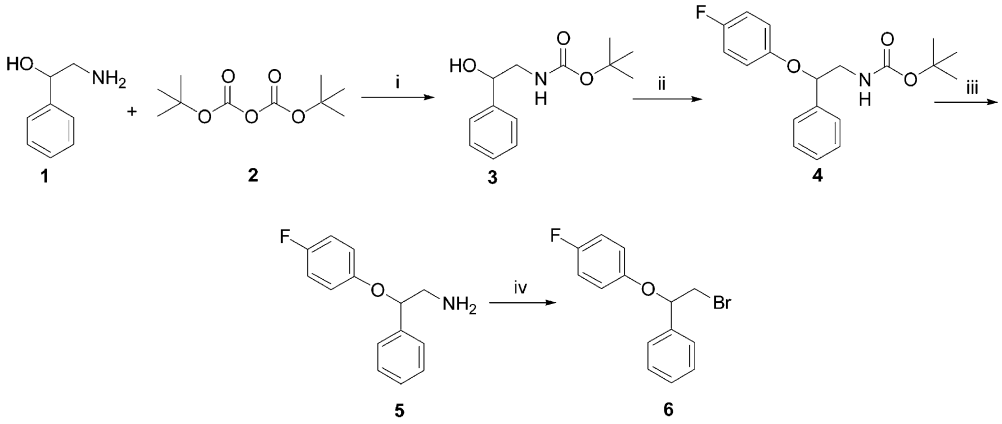
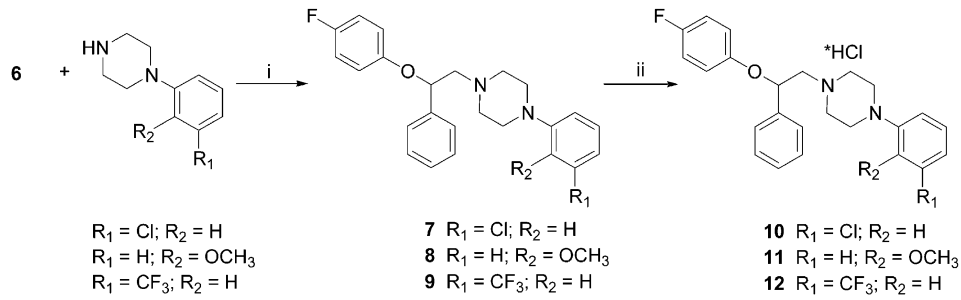


Figure 4. Drugs administered with an SSRI in a effort to reverse/treat sexual dysfunction.



Scheme 1. Synthesis of 2-(4-fluorophenoxy)-2-phenyl-ethyl bromide. Reagents and conditions: (i) DMF, 15 min at 50 °C, 8 h at rt 78%; (ii) DMF, 4-fluorophenol, PPh_3, DEAD dropwise at 0 °C, 8 h at rt, 43%; (iii) 4 N HCl in Dioxane, 1.5 h at rt, 64%; (iv) DMF, $TiBr_4$, *t*-butyl nitrite, 1 h at rt, 33%.



Scheme 2. Synthesis of 2-(4-fluorophenoxy)-2-phenyl-ethyl piperazines and their corresponding hydrochloride salts. Reagents and conditions: (i) K_2CO_3, DMF, reflux 16–18 h, 18–39%; (ii) 2 M HCl in anh. ether, 6–68%.

Bromide intermediate **6**, synthesized as described in Scheme 1, was subsequently used as starting material in the next set of reactions. 1-(3-chlorophenyl)-piperazine and m-trifluoromethylphenyl-piperazine were commercially available in their free base form, while 1-(2-methoxyphenyl)-piperazine was available as the hydrochloride salt. In this latter case, the free base form of the compound was generated using a strong base (e.g.; NaOH) in aqueous solution followed by extraction with ethyl ether. Compounds **7**, **8**, and **9** were obtained by a nucleophilic substitution reaction between the bromide in compound **6** and the secondary amino group of each piperazine-containing compound. The corresponding hydrochloride salts (HCl), **10**, **11**, and **12**, were obtained by treatment of the free base form of each with 2M HCl in diethyl ether. These compounds were used as such in IC_{50} testing of 5-HT reuptake activity (Scheme 2).

The specific uptake of [^{3}H]5-HT by human platelets was typically >90% specific as defined by 10 μM fluoxetine. Dose-response curves for inhibition of [^{3}H]5-HT uptake by the test compounds are presented in Figures 5, 6 and 7. The inhibition curves all had slope coefficients that did not differ from unity, indicating that the compounds all interacted with a single site on the SERT. Compounds **10** and **11** were the most potent inhibitors of [^{3}H]5-HT accumulation, with IC_{50} values of 1.45±0.08 μM and 3.27±0.23 μM, respectively. These values did not differ from each other at the $p<0.05$ level of significance (one-way ANOVA followed by a Student–Newman-Keuls *t*-test). Compound **12** was the least potent compound in the series with an IC_{50} value of 9.56±2.4 μM; this was only one-sixth the potency of compound **10** and three-fold less potent than compound **11** ($p<0.05$). It is apparent that the new compounds are significantly less potent than fluoxetine, which exhibits nanomolar (nM) affinity for the SERT,[17] but it is not possible at this stage to decide whether the decreased potency is due to electrostatic, stereochemical, or steric considerations or some combination of these. For example, the phenylpiperazine moiety of the new compounds adds considerable bulk to the side-chain amine of fluoxetine and also changes its electronic character with the addition of new lone pair electrons and an electron-rich aromatic ring. It is possible that the electron-withdrawing, hydrophobic trifluoromethyl group present on the phenyl ring of **12** is responsible for the attenuated serotonin uptake inhibition activity of this compound when compared to compounds **10** and **11**, but the methoxy substitution on compound **11** also represents a positional isomer and this may influence binding through a steric mechanism. Interestingly, tests conducted by Hyttel in 1982 indicated that trazodone, mianserin, and bupropion had IC_{50} values for serotonin uptake of 0.58 μM, 1.2 μM, and 19.0 μM, respectively.[13] This finding is then encouraging given that trazodone, mianserin, and bupropion have all

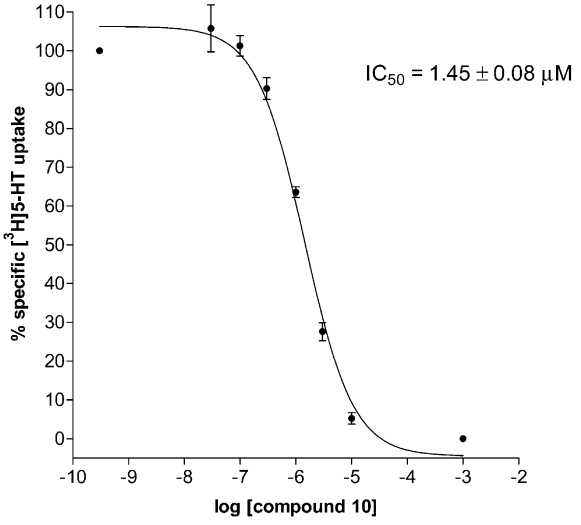


Figure 5. Inhibition of [^{3}H]5-HT uptake into human platelets by compound **10**.

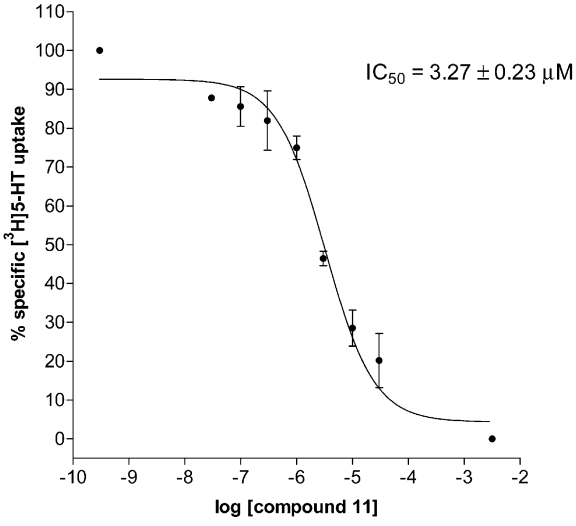


Figure 6. Inhibition of [^{3}H]5-HT uptake into human platelets by compound **11**.

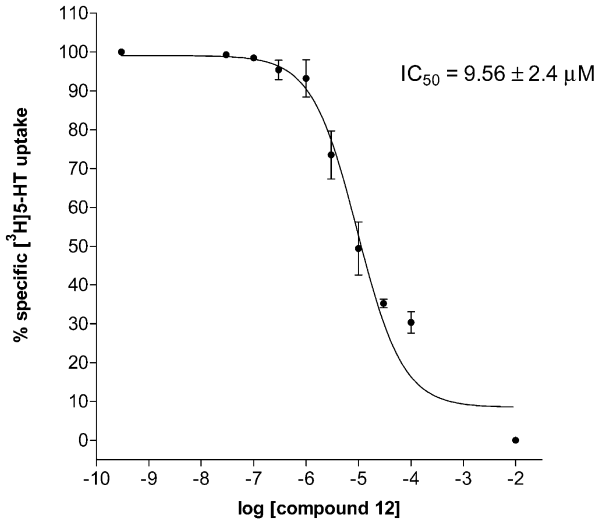


Figure 7. Inhibition of [^{3}H]5-HT uptake into human platelets by compound **12**.

been used with success in reversing or treating SSRI-induced sexual dysfunction.

3. Conclusions

We have prepared three new fluoxetine analogues, 2-(4-fluorophenoxy)-2-phenyl-ethyl piperazines, which demonstrate single-site binding at the site of the serotonin reuptake transporter (SERT). However, each of the three compounds are much less potent than typical SSRIs, such as parent compound fluoxetine, which exhibits nanomolar (nM) affinity for the SERT. Compounds **10**, **11**, and **12**, on the other hand, have micromolar (μM) affinity for the SERT with IC_{50} values of 1.45 μM, 3.27 μM, and 9.56 μM, respectively. It is surmised that the electron-withdrawing, hydrophobic trifluoromethyl group present on the phenyl ring of **12** is responsible for the reduced serotonin reuptake activity of this compound when compared with that of compounds **10** and **11**. Further biological evaluation of compounds **10**, **11**, and **12** is needed before definitive conclusions can be made with regard to each compound's potential for use as an SSRI-type candidate which is devoid of sexual side effects.

4. Experimental

4.1. General methods

Chemicals used in synthesis were obtained from the Aldrich Chemical Company, Fisher Scientific and ACROS Chemicals. Tetrahydrofuran (THF), dried with potassium and benzophenone, and methylene chloride, dried with calcium hydride, were freshly distilled immediately prior to use. Dimethylformamide (DMF) was purchased as an anhydrous commercial product in a SureSeal® container from the Aldrich Chemical Company. Silica gel plates for analytical thin layer chromatography (TLC) were obtained from EM Science. Column chromatography was performed using silica gel (300–400 mesh) obtained from Merck EM Science. ^{1}H and ^{13}C NMR were recorded in deuterated chloroform ($CDCl_3$) using an AMX 360 MHz (90 MHz for ^{13}C) and/or DPX Avance 300 MHz (75 MHz for ^{13}C) Bruker NMR spectrometers. Peaks are listed as broad (b), singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m), with the coupling constant (*J*) expressed in hertz (Hz). High resolution mass spectra were recorded using a VG/Fisons High Resolution GC/Mass Spectrometer using perfluorokerosene (PFK) as the calibration standard. All elemental analyses were conducted by Atlantic Microlab, Inc. in Norcross, Georgia. Melting points were determined in open capillary tubes using a Thomas-Hoover electronically heated melting point apparatus and are uncorrected.

4.1.1. (2-Hydroxy-2-phenylethyl)-carbamic acid *tert*-butyl ester (3). To a well-stirred solution of 2-amino-1-phenylethanol **1** (5.05 g, 36.8 mmol) in anhydrous DMF (50 mL) under N_2 at 25 °C was added 1.5 molar equivalents of di-*tert*-butyl dicarbonate (*t*-boc anhydride) (13.24 g, 60.7 mmol). After dissolution of the *t*-boc anhydride, the mixture was brought to 50 °C for approximately 15 min and then allowed to cool to 25 °C. After stirring overnight (8–12 h), ethyl acetate (EtOAc) was added to the reaction mixture. Water was then added, and the organic layer was washed three times with water and subsequently dried over anhydrous magnesium sulfate ($MgSO_4$). The mixture was then filtered and the solvent removed under reduced pressure. The resulting residue was suspended in a minimal amount of EtOAc and heated to 55–60 °C until completely dissolved. The mixture was allowed to cool overnight, remaining undisturbed, and afforded the desired product **3** (6.73 g, 28.4 mmol, 78%) as translucent, slightly yellow crystals. ^{1}H NMR ($CDCl_3$): δ 7.31 (5H, m), 4.97 (1H, bs), 4.82 (1H, dd, $J = 3.9$, 3.7 Hz), 3.47 (1H, ddd, $J = 14.0$, 6.5, 3.2 Hz), 3.27 (1H, dd, $J = 7.8$, 5.4 Hz), 3.22 (1H, dd, $J = 8.6$, 6.1 Hz), 1.71 (1H, bs), 1.44 (9H, s). ^{13}C- NMR ($CDCl_3$): δ 157.0 (faint), 142.2, 128.9, 128.9, 128.2, 126.3, 126.3, 80.3, 74.4, 48.8, 28.8, 28.8, 28.8. Melting point: 123–124 °C.

4.1.2. [2-(4-Fluorophenoxy)-2-phenylethyl]-carbamic acid *tert*-butyl ester (4). To a well-stirred solution of **3** (6.73 g, 28.4mmol) in anhydrous THF (40 mL) under N_2 at 25 °C was added 2 molar equivalents (equiv) of 4-fluorophenol (6.55 g, 58.4 mmol). After complete dissolution of the 4-fluorophenol, 1.5 equiv of triphenylphosphine (PPh_3) (11.68 g, 44.5 mmol) was added to the mixture and allowed to completely dissolve. The reaction vessel was then cooled in an ice bath to 0 °C. After 20 min, 1.5 equiv of diethylazodicarboxylate (DEAD) (7.96 g, 45.7 mmol) was slowly added dropwise to the stirring mixture. The reaction vessel was subsequently allowed to return to room temperature (25 °C). After stirring overnight (8–12 h), the triphenylphosphine salts formed during the reaction were removed by filtration and the solvent evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash column chromatography (silica gel, 5:95 EtOAc/hexanes) which afforded the desired intermediate **4** (4.04 g, 12.2 mmol, 43%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.34

(1H, d, $J=5.4$ Hz), 7.33 (1H, d, $J=8.0$ Hz), 7.31 (1H, d, $J=4.8$ Hz), 7.30 (1H, d, $J=7.1$ Hz), 7.27 (1H, d, $J=4.2$ Hz), 6.89 (1H, d, $J=10.6$ Hz), 6.85 (1H, d, $J=9.0$ Hz), 6.78 (1H, d, $J=9.2$ Hz), 6.76 (1H, d, $J=9.2$ Hz), 5.16 (1H, dd, $J=8.5$, 3.4 Hz), 5.02 (1H, bs), 3.63 (1H, ddd, $J=14.4$, 8.0, 3.7 Hz), 3.39 (1H, ddd, $J=13.3$, 8.3, 4.6 Hz), 1.43 (9H, s). ^{13}C NMR ($CDCl_3$): δ 158.7, 156.0, 155.8, 153.8, 138.5, 128.7, 128.1, 128.1, 126.1, 126.1, 117.0, 116.9, 115.9, 115.6, 80.0, 68.1, 47.2, 28.4, 28.4, 28.4. ^{19}F-NMR ($CDCl_3$): δ −123.8. Melting point: 98–102 °C.

4.1.3. 2-(4-Fluorophenoxy)-2-phenyl-ethylamine (5). A 30 mL volume of 4 N HCl in dioxane was prepared by adding dropwise 10 mL of 12 N (i.e.; concentrated) hydrochloric acid to 20 mL of well-stirred amount of 1,4-dioxane. The *t*-boc protected 4-fluorophenyl ether intermediate **4** (4.04 g, 12.2 mmol) was dissolved in a minimal amount of 1,4-dioxane. The 4 N HCl was then added dropwise to the reaction vessel at 25 °C over approximately a 10 min period. After 90 min, a saturated solution of sodium bicarbonate ($NaHCO_3$) was added to the reaction mixture. The aqueous layer was washed with three separate 30 mL portions of CH_2Cl_2. The combined organic layers were then dried over anhydrous $MgSO_4$ and filtered. Purification was performed by flash column chromatography (on silica gel) starting with 100% EtOAc until any remaining starting material had eluted; then the polarity of the eluent was increased using 30:70 methanol/EtOAc. Analytical thin-layer chromatography showed that the desired product had an R_f value of approximately 0.4 when developed in 30:70 methanol/EtOAc. Fractions containing the desired product were combined and the solvent was removed under reduced pressure. The resultant residue after solvent evaporation was re-suspended in CH_2Cl_2 and filtered. The solvent from the filtrate was evaporated under reduced pressure and afforded the desired free amine **5** (1.80 g, 7.8 mmol, 64%) as a slightly yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.28 (5H, m), 6.86 (1H, d, $J=9.3$ Hz), 6.83 (1H, d, $J=8.0$ Hz), 6.79 (1H, d, $J=9.3$ Hz), 6.78 (1H, d, $J=9.4$ Hz), 5.06 (1H, dd, $J=7.1$, 4.4 Hz), 3.14 (1H, d, $J=6.8$ Hz), 3.12 (1H, d, $J=7.7$ Hz), 2.35 (2H, bs). ^{13}C NMR ($CDCl_3$): δ 158.6, 156.0, 154.0, 139.0, 128.7, 128.0, 126.1, 126.1, 117.0, 116.9, 115.8, 115.6, 82.3, 49.0. ^{19}F-NMR ($CDCl_3$): δ −124.0.

4.1.4. 2-(4-Fluorophenoxy)-2-phenyl-ethyl bromide (6). To a rapidly stirred solution of 1.1 equiv of $TiBr_4$ (3.15 g, 8.67 mmol) in 40 mL of DMF at 25 °C under N_2 was added dropwise 1.1 equiv of *t*-butyl nitrite (1.2 mL of 90% t-butyl nitrite, d = 0.86, 8.6 mmol) dissolved in approx. 5 mL of DMF. Upon addition of the t-butyl nitrite, a color change from reddish orange to orange-yellow was observed. The primary amine starting material **5** (1.80 g, 7.8 mmol) was dissolved in approximately 5 mL of DMF and added dropwise to the reaction flask over a 20-min period. Gas evolution was observed during addition of the amine starting material and was complete within approximately 5 min following its complete addition. After complete gas evolution, the reaction mixture was added to 150 mL of 20% aqueous hydrochloric acid (20% aq HCl) and extracted with four 30 mL portions of ethyl ether. The combined organic layers were subsequently dried over anhydrous $MgSO_4$. The solvent was then removed under reduced pressure. Analytical thin-layer chromatography (TLC) indicated that the desired product had an R_f value of approx. 0.85 when developed in 30:70 EtOAc/hexanes. Purification was performed using flash column chromatography (silica gel, 100% hexanes) to isolate the desired bromide product **6** (0.74 g, 2.6 mmol, 33%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.37 (5H, m), 6.89 (1H, d, $J=8.0$ Hz), 6.87 (1H, d, $J=8.0$ Hz), 6.83 (1H, d, $J=4.5$ Hz), 6.81 (1H, d, $J=4.6$ Hz), 5.22 (1H, dd, $J=8.4$, 4.0 Hz), 3.72 (1H, dd, $J=10.9$, 8.4 Hz), 3.61 (1H, dd, $J=10.9$, 4.0 Hz). ^{13}C NMR ($CDCl_3$): δ 159.6, 156.5, 154.3, 154.3, 138.9, 129.3, 129.2, 126.8, 118.0, 117.9, 116.4, 116.1, 81.7, 36.4. ^{19}F NMR ($CDCl_3$): δ −123.2.

4.1.5. 1-(3-Chlorophenyl)-4-[2-(4-fluorophenoxy)-2-phenyl-ethyl]-piperazine (7). To a well-stirred solution of approx. 1.1 equiv of 1-(3-chlorophenyl)-piperazine (0.26 g, 1.3 mmol) in 20 mL DMF at 25 °C under N_2 and fitted with a reflux condenser was added a minimum of 1.25 equiv of anhydrous K_2CO_3 (0.27 g, 1.9 mmol). The starting material **6** (0.24 g, 0.83 mmol) was dissolved in a small amount of DMF (approx. 5 mL) and added to the reaction mixture. After stirring for 10 min, the reaction mixture was then brought to reflux temperature and allowed to reflux under an inert atmosphere for 16 h. Upon completion of the reaction, water was added and the reaction mixture was extracted with four 30 mL portions of CH_2Cl_2. The organic layers were combined and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was adsorbed onto silica gel and purified via flash column chromatography. Analytical thin layer chromatography (TLC) indicated the desired product had an R_f value of approx. 0.75 when developed in 30:70 EtOAc/hexanes. Purification via flash column chromatography (on silica gel) was performed with 500 mL of hexanes until any remaining starting material had completely eluted; then the polarity of the eluent mixture was increased to 10:90 EtOAc/hexanes to afford the desired product **7** (0.24 g, 0.57 mmol, 69%) as an opalescent oil. ^{1}H NMR ($CDCl_3$): δ 7.31 (5H, m), 7.15 (1H, dd, $J=8.1$, 8.0 Hz), 6.82 (7H, m), 5.27 (1H, dd, $J=8.4$, 3.2 Hz), 3.19 (4H, dd, $J=5.1$, 5.0 Hz), 3.04 (1H, dd, $J=13.8$, 8.5 Hz), 2.82 (2H, ddd, $J=9.4$, 8.1, 5.0 Hz), 2.73 (3H, ddd, $J=6.9$, 6.9, 3.1 Hz). ^{13}C NMR ($CDCl_3$): δ 154.3, 152.7, 147 (faint), 140.6, 135.3, 130.4, 129.1, 129.1, 128.3, 126.5, 126.5, 119.7, 117.6, 117.5, 116.3, 116.1, 116.0, 114.3, 80.1, 65.7, 53.8, 53.8, 49.2, 49.2. ^{19}F NMR ($CDCl_3$): δ −124.0. HRMS (EI) M^+ calculated for $C_{24}H_{24}ClFN_2O$: 410.91, found 410.16. Elemental analysis (%) calculated for $C_{24}H_{24}ClFN_2O$: C, 70.15; H, 5.89; N, 6.82; F, 4.62; Cl, 8.63. Found: C, 70.43; H, 6.02; N, 6.75; F, n/a; Cl, n/a.

4.1.6. 1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-(2-methoxyphenyl)-piperazine (8). *a. Formation of the free base form of 1-(2-methoxyphenyl)-piperazine.*

The free base form of 1-(2-methoxyphenyl)-piperazine was formed by dissolving a liberal amount of the

corresponding hydrochloride salt in deionized water and adding an excess of 2 M aqueous NaOH. After 10 min, the aqueous mixture was extracted with four 30 mL portions of ethyl ether. The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The secondary amine (free base form) of 1-(2-methoxyphenyl)-piperazine was isolated as an off-white solid and confirmed via NMR spectroscopy. ^{1}H NMR ($CDCl_3$): δ 7.07 (1H, ddd, J = 9.1, 8.0, 4.5 Hz), 6.94 (1H, d, J = 8.6 Hz), 6.93 (1H, d, J = 4.1 Hz), 6.89 (1H, d, J = 7.7 Hz), 3.87 (3H, s), 3.38 (4H, d, J = 15.3 Hz), 3.38 (2H, d, J = 4.0 Hz), 3.14 (1H, bs), 2.85 (1H, bs), 1.83 (1H, s). ^{13}C NMR ($CDCl_3$): δ 152.2, 141.7, 122.7, 120.9, 118.1, 111.1, 55.2, 52.0, 52.0, 46.3, 46.3 Melting point: 180–183 °C (dec.).

b. Formation the coupled product **8**

To a well-stirred solution of approx. 1.6 equiv of 1-(2-methoxyphenyl)-piperazine (0.34 g, 1.78 mmol) in 20 mL DMF at 25 °C under N_2 and fitted with a reflux condenser was added a minimum of 1.25 equiv of anhydrous K_2CO_3 (0.34 g, 2.45 mmol). The starting material **6** (0.245 g, 0.83 mmol) was dissolved in a small amount of DMF (approx. 5 mL) and added to the reaction mixture. After stirring for 10 min, the reaction mixture was then brought to reflux temperature and allowed to reflux under an inert atmosphere for 16 h. Upon completion of the reaction, water was added and the reaction mixture was extracted with four 30 mL portions of CH_2Cl_2. The organic layers were combined and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was adsorbed onto silica gel and purified via flash column chromatography. Analytical thin layer chromatography (TLC) indicated the desired product had an R_f value of approx. 0.7 when developed in 30:70 EtOAc/hexanes. The flash column chromatography (on silica gel) was performed with 500 mL of hexanes until any remaining starting material had completely eluted; then the polarity of the eluent mixture was increased to 10:90 EtOAc/hexanes to afford the desired product **8** (0.114 g, 0.28 mmol, 34%) as an opalescent oil. ^{1}H NMR ($CDCl_3$): δ 7.32 (5H, m), 6.9 (8H, m), 5.30 (1H, dd, J = 8.5, 3.1 Hz), 3.86 (3H, s), 3.1 (5H, dd, J = 13.7, 8.5 Hz), 2.8 (5H, m). ^{13}C NMR ($CDCl_3$): δ 152.6, 152.6, 141.7, 140.9, 129.1, 129.1, 129.1, 128.2, 126.5, 126.5, 123.3, 121.4, 118.6, 117.7, 117.6, 116.2, 116.0, 111.5, 79.9, 66.0, 55.7, 55.7, 54.2, 51.1, 51.1. ^{19}F NMR ($CDCl_3$): δ-124.2. HRMS (EI) M^+ calculated for $C_{25}H_{27}FN_2O_2$: 406.49; found 406.21. Elemental analysis (%) calculated for $C_{25}H_{27}FN_2O_2$: C, 73.87; H, 6.69; F, 4.67; N, 6.89. Found: C, 73.84; H, 6.95; F, n/a; N, 6.92.

4.1.7. 1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-(3-trifluoromethylphenyl)-piperazine (9). To a well-stirred solution of approx. 1.1 equiv of m-trifluoromethylphenyl-piperazine (0.44 g, 1.9 mmol) in 20 mL DMF at 25 °C under N_2 and fitted with a reflux condenser was added a minimum of 1.25 equiv of anhydrous K_2CO_3 (0.33 g, 2.4 mmol). The starting material **6** (0.41 g, 1.4 mmol) was dissolved in a small amount of DMF (approx. 5 mL) and added to the reaction mixture. After stirring for 10 min, the reaction mixture was then brought to reflux temperature and allowed to reflux under an inert atmosphere for 16 h. Upon completion of the reaction, water was added and the reaction mixture was extracted with four 30 mL portions of CH_2Cl_2. The organic layers were combined and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was adsorbed onto silica gel and purified via flash column chromatography. Analytical thin layer chromatography (TLC) indicated the desired product had an R_f value of approx. 0.75–0.8 when developed in 30:70 EtOAc/hexanes. Purification via flash column chromatography (on silica gel) was performed with 500 mL of hexanes until any remaining starting material had completely eluted; then the polarity of the eluent mixture was increased to 10:90 EtOAc/hexanes to afford the desired product **9** (0.11 g, 0.25 mmol, 18%) as an opalescent oil. ^{1}H NMR ($CDCl_3$): δ 7.32 (6H, m), 7.09 (1H, d, J = 7.6 Hz), 7.07 (1H, d, J = 7.8 Hz), 7.05 (1H, d, J = 7.0 Hz), 6.88 (1H, d, J = 9.3 Hz), 6.85 (1H, d, J = 8.2 Hz), 6.80 (1H, d, J = 9.3 Hz), 6.79 (1H, d, J = 9.3 Hz), 5.28 (1H, dd, J = 8.4, 3.3 Hz), 3.24 (4H, dd, J = 5.0, 5.0 Hz), 3.05 (1H, dd, J = 13.9, 8.5 Hz), 2.84 (2H, ddd, J = 10.8, 5.2, 5.2 Hz), 2.74 (3H, m). ^{13}C NMR ($CDCl_3$): δ 152 (faint), 151 (faint), 145 (faint), 140 (faint), 129.5, 129.5, 128.7, 128.7, 127.9, 126.1, 126.1, 118.6, 117.2, 117.1, 115.8, 115.8, 115.6, 112, 112, 79.7, 65.2, 53.4, 53.4, 48.7, 48.7. ^{19}F NMR ($CDCl_3$): δ −63.1, −123.5. HRMS (EI) M^+ calculated for $C_{25}H_{24}F_4N_2O$: 444.46, found 444.18. Elemental analysis (%) calculated for $C_{25}H_{24}F_4N_2O$: C, 67.56; H, 5.44; F, 17.10; N, 6.30. Found: C, 68.20; H, 5.82; F, 15.22; N, 5.90.

4.1.8. Formation of the hydrochloride salt (10) from piperazinyl-containing base (7). To a well-stirred solution of **7** (0.235 g, 0.57 mmol) in 30mL of anhydrous THF was added 10 equiv of 2M HCl in anhydrous diethyl ether (3 mL, 6.0 mmol) at 25 °C under N_2. The mixture was allowed to stir for approx. 12 to 16 h. Upon completion of the reaction, the reaction mixture was filtered and the filtered residue was washed with freshly distilled CH_2Cl_2. The solute was then allowed to dry and was subsequently re-dissolved in approx. 5 mL of anhydrous EtOH. The resulting solution was refluxed briefly for 5–10 min and the solvent then was removed under reduced pressure. The remaining solid was placed under vacuum until all residual solvent was removed affording the desired hydrochloride salt **10** as a greenish crystalline solid (0.17 g, 0.39mmol, 68%). Confirmation of the HCl salt **10** was made by treating a small amount of the final product with strong base, extracting with ethyl ether, removing the organic solvent under reduced pressure, and confirming via NMR using $CDCl_3$. NMR data corresponded with those results previously reported for the free base form **7**. TLC was also performed (30:70 EtOAc/hexanes) and the R_f value of approx. 0.75 matched that of **7**. Reverse phase TLC was performed on the HCl salt **10** and R_f values of 0.3 in 100% CH_3CN and 0.8 in 2:98H_2O/CH_3CN were recorded, respectively.

4.1.9. Formation of the hydrochloride salt (11) from piperazinyl-containing base (8). To a well-stirred solution of **8** (0.11 4 g, 0.28 mmol) in 30 mL of anhydrous THF was

added 10 eq. of 2 M HCl in anhydrous diethyl ether (1.6 mL, 3.2 mmol) at 25 °C under N_2. The mixture was allowed to stir for approx. 12 to 16 h. Upon completion of the reaction, the reaction mixture was filtered and the filtered residue was washed with freshly distilled CH_2Cl_2. The solute was then allowed to dry and was subsequently re-dissolved in approx. 5 mL of anhydrous EtOH. The resulting solution was refluxed briefly for 5–10 min and the solvent was then removed under reduced pressure. The remaining solid was placed under vacuum until all residual solvent was removed affording the desired hydrochloride salt **11** as a white crystalline solid (0.0112 g, 0.025 mmol, 9%), albeit in low yield. Confirmation of the HCl salt **11** was made by treating a small amount of the final product with strong base, extracting with ethyl ether, removing the organic solvent under reduced pressure, and confirming via NMR using $CDCl_3$. NMR data corresponded with those results previously reported for the free base form **8**. TLC was also performed (30:70 EtOAc/hexanes) and the R_f value of approx. 0.7 matched that of **8**. Reverse phase TLC was performed on the HCl salt **11** and R_f values of 0.25 in 100% CH_3CN and 0.75 in 2:98H_2O/CH_3CN were recorded, respectively.

4.1.10. Formation of the hydrochloride salt (12) from piperazinyl-containing base (9). To a well-stirred solution of **9** (0.11 g, 0.25 mmol) in 30 mL of anhydrous THF was added 10 equiv of 2 M HCl in anhydrous diethyl ether (1.4 mL, 2.8 mmol) at 25 °C under N_2. The mixture was allowed to stir for approx. 12 to 16 h. Upon completion of the reaction, the reaction mixture was filtered and the filtered residue was washed with freshly distilled CH_2Cl_2. The solute was then allowed to dry and was subsequently re-dissolved in approx. 5 mL of anhydrous EtOH. The resulting solution was refluxed briefly for 5–10 min and the solvent then was removed under reduced pressure. The remaining solid was placed under vacuum until all residual solvent was removed affording the desired hydrochloride salt **12** (0.0496 g, 0.103 mmol, 42%). Confirmation of the HCl salt **12** was made by treating a small amount of the final product with strong base, extracting with ethyl ether, removing the organic solvent under reduced pressure, and confirming via NMR using $CDCl_3$. NMR data corresponded with those previously reported for the free base form **9**. TLC was also performed (30:70 EtOAc/hexanes) and the R_f value of approx. 0.75–0.8 matched that of **9**. Reverse phase TLC was performed on the HCl salt **12** and R_f values of 0.35 in 100% CH_3CN and 0.85 in 2:98H_2O/CH_3CN were recorded, respectively.

4.2. [^{3}H] 5-HT uptake assay

Outdated human platelets were obtained from the blood bank at Pitt County Memorial Hospital, Greenville, NC. Platelets from 5–10 donors were pooled, 10% dimethylsulfoxide was added, and aliquots were stored frozen at −80 °C until use. For assays, 5 mL of platelets were thawed and suspended in 20 mL ice-cold Krebs-Ringer-HEPES (KRH) buffer as previously described.[14] The KRH buffer contained the following ingredients: 124.0 mM NaCl, 2.9 mM KCl, 1.3 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 5.2 mM D-glucose, 25.0 mM HEPES, 0.1 mM sodium ascorbate, 0.1 mM pargyline. The buffer was adjusted to pH 7.4 with 5 M NaOH. The ability of platelets to accumulate [^{3}H]serotonin was measured in the absence and presence of test drugs as follows: a 490 μL aliquot of the platelet suspension was added to glass tubes containing 5 μL test drug (various concentrations, dissolved in DMSO), 5 μL DMSO (for total determinations), or 5 μL fluoxetine hydrochloride dissolved in DMSO (for nonspecific determinations; final concentration, 10 μM). The assay tubes were preincubated in a 37 °C shaking water bath for 5 min. The tubes were then returned to the ice bath and chilled for 10 min. [^{3}H]Serotonin was added (5 μL of stock solution; final concentration, 10 nM), giving a total incubation volume of 500 μL. All tubes were returned to the 37 °C shaking water bath for 5 min to initiate neurotransmitter uptake. Uptake was terminated by chilling the test tubes in the ice bath. After adding 3 mL ice-cold KRH, each assay tube was immediately vacuum filtered through glass fiber filters (Whatman GF/B) pretreated with 0.1% polyethyleneimine. Filters were washed with 2×3 mL ice-cold KRH, allowed to dry briefly under vacuum, then placed in liquid scintillation vials. Scintillation cocktail (8 mL) was added and the vials were sealed, vortexed, and allowed to stand overnight. Radioactivity was measured using liquid scintillation spectroscopy (Packard Tri-Carb 1600 CA). Specific uptake was defined as uptake at 37 °C minus uptake in the presence of 10 μM fluoxetine. Under these conditions, specific [^{3}H]serotonin uptake was typically 90% of total uptake. The IC_{50} value for each test drug was determined from displacement curves using at least 6 drug concentrations, each run in triplicate. Data were transformed from dpm to% specific uptake and fitted to a four-parameter logistic curve using commercial computer software, from which the IC_{50} values are obtained.

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