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Structure-Activity Relationships for a Novel Series of Citalopram (1-(3-(dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) Analogues at Monoamine Transporters

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Abstract

(±)-Citalopram (**1**, 1-(3-(dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile), and its eutomer, escitalopram (S(+)-**1**) are selective serotonin reuptake inhibitors (SSRIs) that are used clinically to treat anxiety and depression. To further explore structure-activity relationships at the serotonin transporter (SERT), a series of (±)-4- and 5-substituted citalopram analogues were designed, synthesized and evaluated for binding at the SERT, dopamine transporter (DAT) and norepinephrine transporter (NET) in native rodent tissue. Many of these analogues showed high SERT binding affinities (K_i = 1–40 nM) and selectivities over both NET and DAT. Selected enantiomeric pairs of analogues were synthesized and both retained enantioselectivity as with S- and R-**1**, wherein S > R at the SERT. In addition, the enantiomeric pairs of **1** and **5** were tested for binding at the homologous bacterial Leucine transporter (LeuT), wherein low affinities and the absence of enantioselectivity suggested distinctive binding sites for these compounds at SERT as compared to LeuT. These novel ligands will provide molecular tools to elucidate drug-protein interactions at the SERT and to relate those to behavioral actions, *in vivo*.

Introduction

The serotonin transporter (SERT) is a monoamine transporter that along with the norepinephrine transporter (NET) and the dopamine transporter (DAT) belongs to the neurotransmitter: sodium symporter (NSS) family. The SERT acts as a serotonergic signal terminator in the brain, by transporting excess serotonin from the synapse into the presynaptic cell terminal.1 Selective serotonin reuptake inhibitors (SSRIs) are used clinically to treat anxiety and depression. Citalopram ((±)-**1**) is a clinically used antidepressant that binds with high affinity (K_i = 1.94 nM) and selectivity to the SERT relative to other monoamine transporters.2 Despite the clinical success of the SSRIs,

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Supporting Information Available

Elemental analysis results are available free of charge via the Internet at http://pubs.acs.org.

elucidation of the specific drug-protein interactions that lead to clinical efficacy remain unknown.

The 3-dimensional (3-D) structure of proteins in the NSS family (also called SLC6) was unknown until 2005 when the leucine transporter (LeuT) was cocrystallized along with the substrate leucine and two sodium ions.3 LeuT is a bacterial NSS homolog with 12 transmembrane segments with both N and C- termini located intracellularly.3 Using the crystal structure of LeuT as a model, drug-protein interactions between substrates and inhibitors at the monoamine transporters have been studied.4–20 Homology models have predicted critical residues for binding the SSRIs and tricyclic antidepressants, guiding site-directed mutagenesis and pharmacological studies to elucidate further drug-protein interactions and relate these to the behavioral actions of these drugs.5–8, 11, 13, 15, 17, 18, 21–25

Several reports have been published describing citalopram (**1**) and its eutomer, S(+)-**1**, which has ~30 fold higher binding affinity at SERT than its R(−)-enantiomer.26, 27 Site-directed mutagenesis studies on the SERT have also revealed considerable differences in binding and uptake profiles between S- and R-**1**.28 Additional studies suggest that R-**1** may attenuate the effects of S-**1**, which has been hypothesized to involve an allosteric binding domain 22, 29, 30 and is supported by a recent clinical study.31 It has been suggested that differing conformational changes induced by S- and R-**1** at the SERT may effect downstream signaling proteins differently and these in turn may be related to antidepressant actions, *in vivo*.28

Only limited structure-activity relationships (SAR) studies have been reported on the citalopram pharmacophore, especially with enantiomeric pairs.32, 33 In the first report published in 1977 by Bigler, et al., compound **1** and a large series of analogues with substitutions in the isobenzofuranyl and pendant phenyl rings were reported. Substitutions including halogen, nitrile, methoxy and trifluoromethyl groups resulted in a narrow range of high potencies for inhibition of [^{14}C]5-HT uptake.32 More recently emphasis on the dimethylamino group as playing a principal role for the selectivity at SERT over NET was reported.33 However, enantioselectivity of **1** and several analogues was only recently reported wherein site-specific mutagenesis was used to guide computational protein-ligand docking studies, which predicted detailed binding domain interactions at the SERT.24 In the present study the synthesis of (±)-4- and 5-substituted analogues of **1** and their evaluation for binding at SERT, NET, and DAT is reported. In addition, the S- and R-enantiomers of two of these analogues (**5** and **9**) were also prepared and evaluated for enantioselective binding at all three monoamine transporters. The enantioselective binding of R- and S-fluoxetine to the homologous bacterial transporter LeuT recently appeared.8 In this report, S- and R-**1** as well as the enantiomers of **5** were evaluated for competition of [^{3}H]Leucine in LeuT.

Chemistry

All analogues were synthesized by the strategy shown in Scheme 1, starting from either the commercially available 5-bromophthalide or 6-bromophthalide (**3**), which was synthesized from commercially available 3-bromo-2-methylbenzoic acid using a two-step procedure.34 The second step of this procedure used chromyl chloride to oxidize the methyl group of 3-bromo-2-methylbenzoate (**2**) and form the isobenzofuran ring in a one-pot reaction. The reaction yielded the expected compound **3** (57% yield) with another side product **4** as reported.

The fluorophenyl group and the dimethylamino moiety were introduced by a double Grignard reaction using a modification of a previously described procedure,35 to give diol

intermediates (not shown). In this double Grignard reaction, a commercially available THF solution of 4-fluorophenylmagnesium bromide was used as the first Grignard reagent, while (3-(dimethylamino)propyl)magnesium chloride was freshly made and used as the second Grignard reagent. To make the magnesium chloride reagent, dibromoethane was used as an initiator (See details in Experimental Methods section). By treating with HCl in ethanol, the ring closed compounds **5** and **6** were obtained. Suzuki coupling of the two benzofurans **5** and **6** gave a set of 4- and 5- substituted analogues **7–20** shown in Scheme 1. In addition, compound **21** was synthesized by halogen exchange using CuI and KI, at 150 °C.

These novel isobenzofuran analogues **5–21** were assessed for SERT, NET and DAT binding affinities, which will be discussed in detail in the SAR section. The resulting SAR and our interest in further investigating SERT tolerance of analogues with extended steric bulk lead us to choose the Br-analogue **5** and the vinyl compound **9**, for further synthesis of enantiomeric pairs, as shown in Scheme 2. Compounds **1**, S-**1** and R-**1** were also synthesized for comparison by a similar procedure also shown in Scheme 2.

Chiral resolution of the diols **22** and **23** was successfully performed by(+)-di-p-toluoyl-D-tartaric acid or (−)-di-p-toluoyl-L-tartaric acid. The ring closure reactions under the condition of triethylamine and methanethiosulfonyl chloride gave compounds S(+)-**1**, R(−)-**1**, S(+)-**5**, and R(−)-**5**. Compounds S(+)-**9**, and R(−)-**9** were obtained by Suzuki coupling of S(+)-**5**, and R(−)-**5**, with trans-phenylvinylboronic acid, respectively.

All the compounds were purified by flash column chromatography, analytically characterized as the free bases, and then converted to the oxalate salts for biological testing, unless otherwise described in the experimental methods.

Biological Results

All the compounds were tested in radioligand competition binding assays for SERT, NET, and DAT, using [^{3}H]citalopram, [^{3}H]nisoxetine and [^{3}H]WIN 35,428 in rat brain stem, frontal cortex, and caudate-putamen, respectively. The K_i values are displayed in Table 1 and Table 2. Experimental details of these assays have been previously published.36

Structure-Activity Relationships

SAR results showed most modifications at the 4- and 5-positions of the dihydroisobenzofuran ring were well tolerated at the SERT and none of the compounds demonstrated high binding affinity at NET or DAT (Table 1). The 5-Br analogue **5**, was as active (K_i = 1.04 nM) and at least as selective (K_i ratio > 10,000) for SERT over NET as **1** (K_i = 1.94 nM, ratio = 3070), and was chosen for chiral resolution (Scheme 2) to give the enantiomerically pure compounds S(+)-**5** and R(−)-**5**. The 5-I-analogue **21** also demonstrated high binding affinity (K_i = 1.42 nM) and selectivity (ratio >10,000 over NET) for SERT. A ~1000-fold selectivity for SERT over DAT was also observed for these racemic compounds, in which the 5-Br compound **5** was more selective than its iodo analogue, **21**. Likewise the 4-Br-analogue **6** showed high binding affinity (K_i = 3.87 nM) with selectivity (K_i) ratios for SERT of 1590 over NET and 56 over DAT.

The additional extended aryl substitutions at the 4- and 5-positions were typically well tolerated (e.g., **7** to **20**) at SERT. All the 5-(3'-substituted-phenyl) analogues showed moderate to high binding affinities at SERT (K_i range 1–60 nM), while di-substitution of the phenyl ring (e.g., **12** (K_i = 143 nM) *v*. **16** (K_i = 61.2 nM) and **13** (K_i = 108 nM) *v*. **15** (K_i = 21.8)) showed lower affinities, suggesting steric limitations at the 5-position. The same trend was observed for NET and DAT affinities with **12** and **16**.

Compound **9**, which has the phenylvinyl substituent, showed higher binding affinity at SERT (K_i = 9.32 nM) compared to its saturated analogue **14** (K_i = 38.1 nM). This suggested that the more rigid structure of the trans-styryl moiety was favored. Further modification, as in compound **17**, which introduced a chloro group in the vinylphenyl ring, retained SERT affinity (K_i = 33.6 nM) as well as selectivity (ratio = 747 over NET, 60 over DAT). As we are interested in identifying positions on the citalopram pharmacophore where sterically bulky groups might be extended, the results with compound **9** suggested it was the best candidate for chiral resolution, in order to potentially optimize SERT binding affinity with an enantiopure compound. Thus synthesis of the enantiomers S(+)-**9** and R (−)-**9** was undertaken. (Scheme 2).

The enantiomers of **5** and **9** (Table 2), showed the expected chiral selectivity at SERT, with the S(+)-enantiomers being more active than the R(−)-enantiomers in all cases. Specifically, S(+)-**5** (K_i = 0.92) showed ~ 26 fold higher binding affinity at SERT than its enantiomer R(−)-**5** (K_i = 23.6). Compound S(+)-**9** (K_i = 10.6) showed ~ 11 fold higher binding affinity at SERT than its enantiomer R(−)-**9** (K_i = 113).

The enantiomers of **1** and **9**, showed the opposite enantioselectivity at NET compared to SERT, in which the R(−)-compounds showed higher binding affinities over the S(+)-enantiomers; while the enantiomers of **5** showed the same enantioselectivity at SERT and NET, although in all cases the enantioselectivity was low (< 2–3-fold).

When the 3D structures of S- and R-**5** were superimposed, an interesting overlap in structures was observed (Fig. 2.) We reasoned that the enantioselectivity at SERT for the citalopram analogues might be due to the subtle differences in the binding of the two aryl ring systems. Moreover, this overlap corresponds to a recently described study using a different experimental approach in which Kolodso et al,24 studied several sets of enantiomers of citalopram and close analogues in 15 SERT mutants to confirm that the 180° rotation of the dihydroisobenzofuran ring at the chiral center explains different binding modes of the citalopram enantiomers at SERT. This study and complimentary investigations19, 20, 25 provide 3-D models of S-citalopram binding in the S1 substrate site of SERT.

As the crystal structures of SERT and DAT have yet to be elucidated, 3D molecular models are based on high-resolution crystal structures of LeuT.3, 14, 23 In addition, LeuT has been used directly for binding and functional studies using selected SSRI's. Selected SSRIs and tricyclic antidepressants have also been shown to bind in the extracellular vestibule of LeuT, although these compounds have very low affinity for LeuT relative to SERT.3, 8, 14, 18, 23 The extracellular vestibule also appears to comprise a second substrate site (S2) in LeuT that is essential for transport.6, 11, 39 It has recently been shown that cocaine analogs bind to the primary substrate site (S1) in DAT,16 and it seems likely that the high affinity site for inhibition in SERT that is responsible for the behavioral actions of the SSRIs is also the S1 site.5, 24, 25, 28 However, it has been argued that the S2 site in SERT, by analogy with LeuT, is also a target for inhibitor binding.8

The potential halogen interchangeability in compound **5** as compared to the parent compound **1** (Br *v.* F, see Fig. 3) coupled with the recent report on the R- and S-enantiomers of the SSRI fluoxetine at LeuT8 led us to investigate the binding affinities of S- and R-**1** and the Br-analogues S-**5** and R-**5** for competition with [^{3}H]Leucine in LeuT. As fluoxetine and **1** share significant structural overlap, we reasoned that the pendant F-phenyl substituent on our analogues might access the described halogen binding pocket in an enantioselective manner and further, that by substituting the 5-CN group of **1** with a Br group, the interchangeability of the halogens might render this compound less enantioselective.

Interestingly, both sets of enantiomers showed low binding affinities for LeuT similar to R-fluoxetine, but in contrast, the analogues of **1** showed no enantioselectivity (Table 3). It is possible that 1) **1** and fluoxetine might bind differently to LeuT such that the analogues of **1** do not access the halogen binding pocket, although it seems more likely that 2) the 5-CN group of **1** can interact at this binding site equivalently to the halogenated CF_3 group of fluoxetine and therefore the interchangeability of the F with the CN would in essence, cancel enantioselectivity (Fig. 3). This interpretation would broaden characterization of the halogen binding pocket in LeuT to be an electron withdrawing group pocket and not simply limited to halogens. Hence, our findings support previous studies on SERT24, 25 and also differentiate this binding mode at SERT from that of LeuT, for these compounds.

In summary, a series of 1-(3-(dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile analogues was synthesized many of which showed moderate to high SERT binding affinities (K_i range 1–40 nM) and selectivities over NET and DAT comparable to the parent ligand, **1**. In addition, the novel chiral compounds of **5** and **9** showed the same enantioselectivity at SERT with S > R. The S(+)- and R(−)-enantiomers of **1** and **5** demonstrated low affinity binding (IC_{50} = 0.4–1.7 mM) to LeuT, comparable to the values recently reported for R-fluoxetine (R-**24**.)8 However, in contrast to the fluoxetine enantiomers, no enantioselectivity was demonstrated with the enantiomers of **1** and **5**, at LeuT, despite their having halogenated aryl ring(s) that might be expected to interact with the halogenated binding pocket described.8 Further investigation into the SAR of the S2 *v*. S1 site in SERT will likely show further divergence between these two sites and ultimately provide another set of molecular tools with which to study the SERT-drug interactions that lead to the behavioral actions of the SSRIs.

Experimental Methods

Reaction conditions and yields were not optimized, and spectroscopic data refer to the free base unless otherwise described for each compound. Flash chromatography was performed using silica gel (EMD Chemicals, Inc.; 230–400 mess, 60 Å). ^1H and ^{13}C NMR spectra were acquired using a Varian Mercury Plus 400 spectrometer. Chemical shifts are reported in parts-per-million (ppm) and referenced according to deuterated solvent for ^1H spectra ($CDCl_3$, 7.26; $(CD_3)_2SO$, 2.50; CD_3OD, 3.31), ^{13}C spectra ($CDCl_3$, 77.2; $(CD_3)_2SO$, 39.5; CD_3OD, 49.0), ^{19}F spectra ($CFCl_3$, 0). Infrared spectra were recorded as a KBr thin film using a Perkin-Elmer Spectrum RZ I FT-IR spectrometer or recorded as powder using an Avatar 370 FT-IR thermo Nicolet spectrometer. Gas chromatography-mass spectrometry (GC/MS) data were acquired using an Agilent Technologies (Santa Clara, CA) 6890N GC equipped with an HP-5MS column (cross-linked 5% PH ME siloxane, 30 m × 0.25 mm i.d. × 0.25 μM film thickness) and a 5973 mass-selective ion detector in electron-impact mode. Ultrapure grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port and transfer line temperatures were 250 and 280 °C, respectively, and the oven temperature gradient used was as follows: the initial temperature (100°C) was held for 3 min and then increased to 295 at 15 °C/min over 13 min, and finally maintained at 295 °C for 10 min. Combustion analysis was performed by Atlantic Microlab, Inc. (Norcross, GA) and agrees within 0.4% of calculated values. Melting point determinations were conducted using a Thomas-Hoover melting point apparatus and are uncorrected. Anhydrous solvents were purchased from Aldrich or J. T. Baker and were used without further purification, except for tetrahydrofuran, which was freshly distilled from sodium-benzophenone ketyl. All other chemicals and reagents were purchased from Aldrich Chemical Co., Combi-Blocks, TCI, America., Matrix Scientific; Lancaster Synthesis, Inc. (Alfa Aesar) and AK Scientific, Inc. The final products were converted into oxalate salts, typically by treating the free base with 1:1 molar ratio of oxalic acid in acetone followed by precipitation from a combination of organic solvents. On the basis of NMR, GC-MS, and combustion data, all final compounds

are > 95% pure; Chiral purity was determined either by HPLC or by NMR with a chemical shift reagent as described.

General Procedure A for double Grignard reaction

To a cooled (0°C) suspension of substituted phthalide (40 mmol) in dry THF (60 mL) was added a solution of 4-fluorophenylmagnesium bromide in THF (1.0 M, 44 mL, 44 mmol) dropwise over 30 min. The reaction was allowed to warm to room temperature and stirred for 3 hr, then cooled to 0 °C. The second freshly made Grignard reagent (3-(Dimethylamino)propyl)magnesium chloride (~50 mmol, 0.8 M, 60 mL) was added dropwise over 30 min. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was treated with aq NH_4Cl solution (sat.), and extracted with ether several times. The organic layer was washed with sat. $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated to give the crude diol product.

General Procedure B for coupling of heteroaryl bromides with boronic acid

To a suspension of boronic acid (1–1.5 eq), heteroaryl bromide (1 eq), and Na_2CO_3 in a mixture of solvents DME/H_2O (3/1, 4 mL for 1 mmol scale reaction) was added $Pd(PPh_3)_4$ (5 mol%) under Argon. The mixture was warmed to 80 °C overnight. The solvent was then removed under reduced pressure, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to a crude product, which was then purified by flash column chromatography to give the pure product.

General Procedure C for resolution

To a solution of the purified racemic product in 2-propanol was added a solution of (+)-di-*p*-toluoyl-*D*-tartaric acid or (−)-di-*p*-toluoyl-*L*-tartaric acid monohydrate (4:1) in a small volume of 2-propanol. The salt precipitated and was collected by filtration, dried and converted to the free base in aq NaOH (2 M). The mixture was extracted with chloroform, dried over $MgSO_4$, and concentrated. The resolution was repeated three times to give the enantiomerically pure free base.

General Procedure D for ring closure reaction

To a solution of diol (1 eq) in dichloromethane at 0 °C, was added mesyl chloride (1.5 eq) and triethylamine (4 eq) dropwise. The reaction mixture was stirred on ice for 3 hr, and then extracted from dichloromethane. The organic layer was washed with aq $NaHCO_3$ (sat.) and brine, dried over $MgSO_4$, concentrated and purified by flash column chromatography to give pure product.

3-(5-Cyano-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine, citalopram (1)

Compound **1** was prepared by following the general procedure D using **22** (10 g, 30 mmol), eluting with chloroform/MeOH (10:1, 5:1)to give the product (5.4 g) in 55% yield; GC-MS (EI) *m/z* 324 (M^+). The oxalate salt was precipitated from 2-propanol; mp 156–157 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.79 (s, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.57-7.54 (m, 2H), 7.14 (dd, *J* = 9.2, 8.4 Hz, 2H), 5.18 (dd, *J* = 13.2, 14.0 Hz, 1H), 5.14 (dd, *J* = 13.2, 14.0 Hz, 1H), 2.91 (t, *J* = 8.0 Hz, 2H), 2.58 (s, 6H), 2.18 (t, *J* = 8.0 Hz, 2H), 1.45 (m, 1H), 1.38 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 149.5, 140.7, 140.6, 132.8, 127.7, 127.6, 126.5, 123.8, 119.5, 116.0, 115.8, 111.3, 91.0, 71.8, 57.5, 43.2, 37.7, 20.2; IR (powder) 1158, 1234, 2226 cm^{-1}; Anal. ($C_{20}H_{21}FN_2O \cdot C_2H_2O_4$) C, H, N.

(*S*)-(+)-3-(5-Cyano-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine, escitalopram (S-1)

Compound **S-1** was prepared by following the general procedure D using **S-22** (1.63 g, 4.77 mmol), eluting with chloroform/MeOH (10:1, 5:1) to give product (0.5 g) in 46% yield. The oxalate salt was precipitated from acetone/EtOAc; mp 144–146 °C; Anal. ($C_{20}H_{21}FN_2O•C_2H_2O_4$) C, H, N; HPLC (Shim-pack HRC-CN 4.6 × 250 mm, 5.0 μM, with 12 mM β-CD in aqueous buffer (10% CAN, 1% TEA, with AcOH to adjust pH4.0))40 area ratio of the peaks with retention time 44.38 : 46.04 = 100 : 0 (*ee* > 99%); $[\alpha]_D27 = 11.69 \pm 0.06$ (c = 2, MeOH).

(*R*)-(−)-3-(5-Cyano-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (R-1)

Compound **R-1** was prepared by following the general procedure D using **R-22** (1 g, 2.91 mmol), eluting with chloroform/MeOH (10:1, 5:1) to give product (0.48 g) in 51% yield. GC-MS (EI) *m*/*z* 324 (M^+). The oxalate salt was precipitated from acetone/EtOAc; mp 148–149 °C; Anal. ($C_{20}H_{21}FN_2O·C_2H_2O_4·1/4\ H_2O$) C, H, N; HPLC (Shim-pack HRC-CN 4.6 × 250 mm, 5.0 μM, with 12 mM β-CD in aqueous buffer (10% CAN, 1% TEA, with AcOH to adjust pH4.0))40 area ratio of the peaks with retention time 44.38 : 46.04 = 0 : 100 (*ee* > 99%); $[\alpha]_D24 = -11.69 \pm 0.05$ (c = 2, MeOH).

Methyl 3-bromo-2-methylbenzoate (2).34

Concentrated H_2SO_4 (0.5 mL) was added to a stirred solution of 3-bromo-2-methylbenzoic acid (2.15 g, 10 mmol) in MeOH (20 mL) at room temperature. The resulting mixture was stirred at reflux for 12 h. Methanol was partially removed under reduced pressure, and ether (150 mL) was added to dilute the residue. The solution was washed with aq $NaHCO_3$, water, brine and dried over Na_2SO_4. The resulting organic layer was concentrated to give an oil that later solidified as a brown solid. Yield: 2.22 g (97%); mp 30–31 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.69 (m, 2H), 7.09 (t, *J* = 8 Hz, 1H), 3.90 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 138.6, 135.8, 132.6, 129.1, 127.0, 126.7, 52.3, 20.6; GC-MS (EI) m/z 228, 230 (M+).

6-Bromo-phthalide (3)

To a solution of Methyl 3-bromo-2-methylbenzoate (**2**)34 (1.02 g, 4.4 mmol) in CCl_4 (6 mL) was added a solution of chromyl chloride (0.72 mL, 8.9 mmol) in CCl_4 (6 mL) dropwise over a period of 1 h, at 0 °C. The reaction mixture was stirred at room temperature for 2 h and slowly heated to reflux for 20 h. The reaction mixture was cooled to room temperature and then quenched by pouring into an ice-cold aq Na_2SO_3 solution (sat., 6 mL). The mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated to give the crude product, which was purified by flash column chromatography eluting with hexane/EtOAc (6:1) to give **3** (0.54 g) in 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.6 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 5.24 (s, 2H); GC-MS (EI) m/z 212, 214 (M^+). Side product **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 4.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 5.20 (s, 2H), 3.96 (s, 3H); GC-MS (EI) m/z 264 (M^+).

3-(5-bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (5)

Compound **5** was prepared by following the general procedure A using 5-bromophthalidite to give the crude diol (6 g, 15 mmol), followed by treating with aq HCl (sat. 37%, 10 mL) in EtOH (15 mL) for 10–15 min. The reaction mixture was concentrated under vacuum and purified by flash column chromatography eluting with chloroform/MeOH (30:1). ^{1}H NMR

(400 MHz, $CDCl_3$) δ 7.44-7.39 (m, 3H), 7.34 (s, 1H), 7.13 (d, *J* = 8 Hz, 1H), 6.98 (dd, *J* = 8.8, 8.8 Hz, 2H), 5.11 (d, *J* = 4.8 Hz, 2H), 2.22 (dd, *J* = 7.6, 6.8 Hz, 2H), 2.13 (s, 6H), 2.12 (m, 2H), 1.46 (m, 1H), 1.33 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.0, 143.8, 142.0, 141.4, 131.2, 127.6, 125.3, 124.6, 121.5, 115.9, 115.7, 90.6, 71.7, 57.4, 37.8, 20.0; ^{19}F NMR (376 MHz, DMSO-d_6) δ −116.5. The oxalate precipitated from acetone; mp 142–144 °C; IR (powder) 1164, 1216 cm^{-1}; GC-MS (EI) *m/z* 377, 379 (M^+); Anal. ($C_{19}H_{21}BrFNO\cdot C_2H_2O_4$) C, H, N.

(*S*)-(+)-3-(5-bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (S-5)

Compound **S-5** was prepared by following the general procedure D using **S-23** (2.65 g, 6.6 mmol) to give product (1.14 g) in 45% yield. GC-MS (EI) *m/z* 377, 379 (M^+). The oxalate salt precipitated from acetone; mp 141–143 °C; Anal. ($C_{19}H_{21}BrFNO\cdot C_2H_2O_4\cdot H_2O$) C, H, N. $[\alpha]_D25 = 2.73 \pm 0.17$ (c = 1, MeOH) (lit. 2.2)35

(*R*)-(−)-3-(5-bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (R-5)

Compound **R-5** was prepared by following the general procedure D using **R-23** (4.77 g, 12 mmol) to give product (3.15 g) in 70% yield; GC-MS (EI) *m/z* 377, 379 (M^+). The oxalate salt precipitated from acetone; mp 154–155 °C; Anal. ($C_{19}H_{21}BrFNO\cdot C_2H_2O_4$) C, H, N; $[\alpha]_D26 = -2.80 \pm 0.40$ (c = 1, MeOH). (lit. −2)35

3-(4-Bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (6)

Compound **6** was prepared by following the general procedure A using 6-bromophthalidite **3** (0.54 g, 2.5 mmol) to give the crude diol. The crude product (GC-MS (EI) m/z 377, 379 (M^+)) was treated with aq HCl (sat. 37%, 10 mL) in EtOH (15 mL) for 10–15 min. The reaction mixture was concentrated under vacuum and purified by flash column chromatography eluting with chloroform/MeOH (10:1, 5:1) to give the product as a syrup (0.88 g) in 90% yield; GC-MS (EI) *m/z* 377, 379 (M^+). The oxalate precipitated from acetone; mp 172–173 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.57-7.46 (m, 4H), 7.26 (t, *J* = 8.0 Hz, 1H), 7.12 (dd, *J* = 8.0, 8.8 Hz, 2H), 5.15 (dd, *J* = 13.2, 10 Hz, 1H), 5.11 (dd, *J* = 12.8, 9.6 Hz, 1H), 2.96 (t, *J* = 8.0 Hz, 2H), 2.62 (s, 6H), 2.17 (t, *J* = 7.6 Hz, 2H), 1.48 (m, 1H), 1.41 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 146.5, 141.3, 139.2, 131.3, 131.0, 127.6, 127.5, 121.2, 115.9, 115.7, 92.4, 73.1, 57.2, 42.9, 37.9, 19.9; ^{19}F NMR (376 MHz, DMSO-d_6) δ −116.2; IR (powder) 1174, 1225 cm^{-1}; Anal. ($C_{19}H_{21}BrFNO\cdot C_2H_2O_4$) C, H, N.

3-(1-(4-Fluorophenyl)-5-phenyl-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (7)

Compound **7** was prepared by following the general procedure B using **5** (0.3 g, 0.8 mmol) and phenyl boronic acid (0.1 g, 0.82 mmol), eluting with chloroform/MeOH (10:1) to give the product (0.22 g) in 75% yield; GC-MS (EI) *m/z* 375 (M^+). The oxalate was precipitated from Acetone/EtOAc; mp 112–115 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 7.60-7.52 (m, 7H), 7.41 (dd, *J* = 7.2, 8.0 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.12 (dd, *J* = 8.8, 8.8 Hz, 2H), 5.16 (dd, *J* = 12.8, 12.0 Hz, 1H), 5.13 (dd, *J* = 12.8, 12.0 Hz, 1H), 2.54 (m, 2H), 2.31 (s, 6H), 2.14 (m, 2H), 1.35 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 141.3, 129.6, 127.4, 121.6, 119.7, 117.2, 112.9, 110.6, 105.8, 105.4, 90.52, 71.7, 58.0, 44.4, 21.4; IR (powder) 1159, 1220 cm^{-1}; Anal. ($C_{25}H_{26}FNO\cdot 1/2C_2H_2O_4\cdot 3/8\ H_2O$) C, H, N.

3-(5-(3-Amino-phenyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (8)

Compound **8** was prepared by following the general procedure B using **5** (0.3 g, 0.8 mmol) and 3-amino-phenyl boronic acid (0.12 g, 0.79 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give product (0.29 g) in 95% yield; GC-MS (EI) *m/z* 390 (M^+). The oxalate was precipitated from Acetone; mp 115–117 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (br, 1H), 8.04 (br, 1H), 7.61-7.43 (m, 5H), 7.15 (dd, *J* = 8.4, 7.6 Hz, 2H), 7.06 (t, *J* = 8.0 Hz, 1H), 6.76 (s, 1H), 6.71 (d, *J* = 6.4 Hz, 1H), 6.52 (d, *J* = 7.6 Hz, 1H), 5.19 (dd, *J* = 12.8, 12.8 Hz, 1H), 5.15 (dd, *J* = 12.8, 12.8 Hz, 1H), 2.96 (m, 2H), 2.63 (s, 6H), 2.18 (m, 2H), 1.47 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 160.6, 155.5, 149.8, 141.5, 141.3, 130.1, 127.6, 127.5, 126.9, 122.7, 120.0, 115.8, 115.6, 115.1, 112.8, 104.9, 90.6, 72.2, 57.7, 43.0, 20.2; ^{19}F NMR (376 MHz, DMSO-d_6) δ −117.2; IR (powder) 1158, 1228 cm^{-1}; Anal. ($C_{25}H_{27}FN_2O\cdot C_2H_2O_4\cdot 1/2\ H_2O$) C, H, N.

(E)-3-(1-(4-Fluorophenyl)-5-styryl-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (9)

Compound **9** was prepared by following the general procedure B using **5** (0.2 g, 0.53 mmol) and 2-phenylvinyl boronic acid (0.08 g, 0.79 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give the product (0.2 g) in 95% yield; GC-MS (EI) *m/z* 401(M^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (m, 10H), 6.97 (dd, *J* = 8.8 Hz, 2H), 6.35 (m, 2H), 5.11 (d, *J* = 9.2 Hz, 2H), 2.30 (m, 4H), 2.18 (s, 6H), 1.49 (m, 1H), 1.38 (m, 1H). The oxalate was precipitated from Acetone; mp 118–120 °C; ^{1}H NMR (400 MHz, MeOD) δ 7.86-7.48 (m, 6H), 7.40 (d, *J* = 8 Hz, 1H), 7.33 (dd, *J* = 7.6, 8 Hz, 2H), 7.23 (m, 1H), 7.18 (s, 2H), 7.06, (dd, *J* = 9.2, 8.4 Hz, 2H), 5.19 (dd, *J* = 12, 11.2 Hz, 1H), 5.17 (dd, *J* = 11.2, 12.4 Hz, 1H), 3.09 (t, *J* = 8 Hz, 2H), 2.75 (s, 6H), 2.27 (m, 2H), 1.71 (m, 1H), 1.69 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 143.8, 141.8, 139.9, 137.6, 136.5, 131.8, 129.6, 129.4, 128.6, 127.6, 127.5, 127.2, 119.6, 117.2, 115.8, 115.6, 110.0, 90.6, 77.9, 57.5, 43.1, 36.0, 20.2; ^{19}F NMR (376 MHz, DMSO-d_6) δ −117.0; IR (powder) 1169, 1230, 1718 cm^{-1}; Anal. ($C_{27}H_{28}FNO\cdot C_2H_2O_4\cdot 1/2\ H_2O$) C, H, N.

(*S*)-(+)-(E)-3-(1-(4-Fluorophenyl)-5-styryl-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (S-9)

Compound **S-9** was prepared by following the general procedure B using **S-5** (0.4 g, 1.1 mmol) and trans-phenylvinylboronic acid (0.15 g, 1 mmol) to give the product (0.2 g) in 49% yield; GC-MS (EI) *m/z* 401 (M^+). The oxalate salt precipitated from acetone; mp 138–139 °C; Anal. ($C_{27}H_{28}FNO\cdot C_2H_2O_4\cdot H_2O$) C, H, N; $[\alpha]_D25$ = 11.0 ± 0.00 (c = 0.83, MeOH).

(*R*)-(+)-(E)-3-(1-(4-Fluorophenyl)-5-styryl-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (R-9)

Compound **R-9** was prepared by following the general procedure B using **R-5** (0.4 g, 1.1 mmol) and trans-phenylvinylboronic acid (0.15 g, 1 mmol) to give product (0.13 g) in 30% yield; GC-MS (EI) *m/z* 401 (M^+). The oxalate salt precipitated from acetone; mp 114–117 °C; Anal. ($C_{27}H_{28}FNO\cdot C_2H_2O_4\cdot H_2O$) C, H, N; $[\alpha]_D25$ = −11.2 ± 0.32 (c = 0.82, MeOH).

3-(5-(3'-Cyano-phenyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (10)

Compound **10** was prepared by following the general procedure B using **5** (0.6 g, 1.6 mmol) and 3-cyanophenyl boronic acid (0.23 g, 1.6 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give the product (0.54 g) in 84% yield; GC-MS (EI) *m/z* 400 (M^+). The oxalate salt was precipitated from 2-propanol. mp 157–159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.98 (m, 1H), 7.80 (m, 1H), 7.65-7.59 (m, 6 H), 7.15 (m, 2H), 5.19 (dd, *J* = 6.6, 6.8

Hz, 1H), 5.18 (dd, *J* = 6.6, 6.8 Hz, 1H), 2.96 (m, 2H), 2.61 (s, 6H), 2.20 (m, 2H), 1.49 (m, 1H), 1.47 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 163.1, 160.7, 144.6, 141.7, 140.3, 138.6, 132.3, 131.8, 131.0, 130.9, 127.6, 127.5, 123.1, 120.8, 119.5, 115.9, 115.6, 112.8, 90.7, 72.1, 57.4, 42.9, 38.0, 20.1; ^{19}F NMR (376 MHz, DMSO-d_6) δ −116.7; IR (powder) 1164, 1225, 2226 cm^{-1}; Anal. ($C_{26}H_{25}FN_2O\cdot C_2H_2O_4\cdot 1/4\ H_2O$) C, H, N.

3-(1-(4-Fluorophenyl)-5-(3'-methoxyphenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (11)

Compound **11** was prepared by following the general procedure B using **5** (0.6 g, 1.6 mmol) and 3-methoxyphenyl boronic acid (0.27 g, 1.8 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give product (0.52 g) in 86% yield. The oxalate salt precipitated from acetone. GC-MS (EI) *m/z* 405(M$^+$). Recrystallization from methanol gave purer compound. mp 147–149 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (m, 5H), 7.34 (t, *J* = 8 Hz, 1H), 7.18-7.13 (m, 4H), 6.90 (d, *J* = 7.2 Hz, 1H), 5.20 (dd, *J* = 12.8, 12.4 Hz, 1H), 5.17 (dd, *J* = 12.8, 12.8 Hz, 1H), 3.79 (s, 3H), 2.97 (t, *J* = 8.0 Hz, 2H), 2.63 (s, 6H), 2.20 (t, *J* = 7.6 Hz, 2H), 1.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.4, 143.8, 142.1, 140.7, 140.1, 130.7, 127.6, 127.5, 127.3, 122.9, 120.5, 119.8, 115.8, 115.6, 113.8, 113.0, 90.6, 72.2, 57.5, 55.8, 43.0, 38.0, 20.1; IR (powder) 1181, 1222 cm^{-1}; Anal. ($C_{26}H_{28}FNO_2\cdot C_2H_2O_4\cdot 1/2\ H_2O$) C, H, N.

3-(5-(3,4-Dichlorophenyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (12)

Compound **12** was prepared by following the general procedure B using **5** (0.5 g, 1.3 mmol) and 3, 4-dichlorophenyl boronic acid (0.29 g, 1.5 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give the product (0.52 g) in 90% yield. The oxalate salt was precipitated from acetone. mp 151–152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.69-7.56 (m, 7H), 7.14 (dd, *J* = 9.2, 8.4 Hz, 2 H), 5.17 (ddd, *J* = 12.4, 11.6, 12 Hz, 2H), 2.93 (m, 2H), 2.60 (s, 6H), 2.25 (m, 2H), 1.47 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.6, 141.1, 140.3, 138.0, 131.7, 129.3, 127.7, 127.6, 127.5, 123.1, 120.7, 115.9, 115.6, 90.7, 72.1, 57.6, 43.1, 20.2; IR (powder) 1125, 1154 cm^{-1}; GC-MS (EI) *m/z* 443 (M$^+$). Recrystallization from methanol gave pure **12**. Anal. ($C_{25}H_{24}Cl_2FNO\cdot C_2H_2O_4$) C, H, N.

3-(5-(3,5-Difluorophenyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine oxalate (13)

Compound **13** was prepared by following the general procedure B using **5** (0.66 g, 1.75 mmol) and 3, 5-difluorophenyl boronic acid (0.32 g, 2 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give product (0.7 g) in 97% yield. The oxalate salt precipitated from acetone; mp 145–147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67-7.57 (m, 5H), 7.40 (d, *J* = 7.6 Hz, 2H), 7.19 (m, 3 H), 5.20 (dd, *J* = 12.8, 12.4 Hz, 1H), 5.17 (dd, *J* =12.8, 13.6 Hz, 1H), 2.96 (t, *J* = 8 Hz, 2H), 2.62 (s, 6H), 2.20 (t, *J* = 7.2 Hz, 2H), 1.50 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.3, 160.7, 144.9, 144.2, 140.2, 138.1, 127.6, 127.4, 123.1, 120.8, 115.9, 115.7, 113.0, 110.7, 110.5, 90.7, 57.4, 46.8, 43.0, 20.1; ^{19}F NMR (376 MHz, DMSO-d_6) δ −110.3, −116.9; IR (powder) 1158, 1222, 1345 cm^{-1}; GC-MS (EI) *m/z* 411 (M$^+$). Recrystallization from methanol gave pure **13**. Anal. ($C_{25}H_{24}F_3NO\cdot C_2H_2O_4\cdot 1/4\ H_2O$) C, H, N.

3-(1-(4-Fluorophenyl)-5-phenethyl-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (14)

A suspension of **9** (140 mg, 0.35 mmol) and a catalytic amount of Pd/C in MeOH was hydrogenated under 45 psi at RT for 5 hrs. The reaction mixture was filtered and the filtrate was concentrated to give the oxalate salt (80 mg) in 55% yield; mp 109–111 °C; ^{1}H NMR

(400 MHz, DMSO-d_6) δ 7.51 (m, 2H), 7.35 (d, *J* = 8 Hz, 1H), 7.25-7.08 (m, 9H), 5.08 (dd, *J* = 12.4, 12.4 Hz, 1H), 5.05 (dd, *J* = 12.4, 12.8 Hz, 1H), 3.97 (t, *J* = 7.6 Hz, 2H), 2.81 (m, 2H), 2.58 (s, 6H), 2.45 (m, 2H), 2.11 (m, 2H), 1.13 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 142.1, 142.0, 139.3, 129.0, 128.9, 128.5, 127.5, 127.4, 126.5, 122.2, 121.8, 115.7, 115.5, 90.5, 72.1, 57.4, 42.9, 38.2, 37.8, 37.6, 20.0; ^{19}F NMR (376 MHz, DMSO-d_6) δ −116.99, IR (powder) 1159, 1210 cm^{-1}; GC-MS (EI) *m/z* 403 (M^+) Anal. ($C_{27}H_{30}FNO\cdot C_2H_2O_4\cdot 1/3\ H_2O$) C, H, N.

3-(5-(3-Fluorophenyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (15)

Compound **15** was prepared by following the general procedure B using **5** (0.85 g, 2.2 mmol) and 3-fluorophenylboronic acid (0.49 g, 2.3 mmol), eluting with chloroform/MeOH (30:1) to give product (0.67 g) in 67% yield. GC-MS (EI) *m/z* 393 (M^+). The oxalate salt precipitated from EtOAc; mp 139–141 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61-7.48 (m, 8H), 7.16 (m, 3H), 5.20 (dd, *J* = 12.8, 12.8 Hz, 1H), 5.17 (dd, *J* = 12.8, 12.8 Hz, 1H), 2.96 (m, 2H), 2.62 (s, 6H), 2.20 (m, 2H), 1.47 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 143.7, 141.9, 140.2, 133.6, 127.6, 127.5, 123.6, 123.0, 120.6, 115.9, 115.6, 90.6, 72.2, 57.5, 43.0, 38.0, 20.2; ^{19}F NMR (376 MHz, DMSO-d_6) δ −113.5, 117.0; IR (powder) 1159, 1199, 1222 cm^{-1}; Anal. ($C_{25}H_{25}F_2NO\cdot C_2H_2O_4\cdot 1/4\ H_2O$) C, H, N.

3-(5-(3-Chlorophenyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (16)

Compound **16** was prepared by following the general procedure B using **5** (0.39, 1 mmol) and 3-chlorophenylboronic acid (0.19 g, 1.1 mmol), eluting with chloroform/MeOH (30:1) to give product (0.39 g) in 95% yield. GC-MS (EI) *m/z* 409 (M^+). The oxalate salt was precipitated from acetone; mp 110–111 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (s, 1H), 7.58 (m, 6H), 7.47 (t, *J* = 6.8 Hz, 1 H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.16 (dd, *J* = 7.2 Hz, 2H), 5.20 (dd, *J* = 12.4, 12.8Hz, 1H), 5.17 (dd, *J* = 11.6, 12.0 Hz, 1H), 2.96 (t, *J* = 7.2 Hz, 2H), 2.62 (s, 6H), 2.20 (m, 2H), 1.50 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 144.4, 142.8, 141.7, 140.3, 139.2, 134.6, 131.6, 128.2, 127.9, 127.7, 127.3, 126.2, 126.3, 123.2, 120.4, 115.8, 115.5, 90.9, 72.2, 57.3, 43.0, 20.3; ^{19}F NMR (376 MHz, DMSO-d_6) δ −116.7; IR (powder) 1170, 1222 cm^{-1}; Anal. ($C_{25}H_{25}ClFNO\cdot C_2H_2O_4\cdot 1/4\ H_2O$) C, H, N.

(*E*)-3-(5-(4-Chlorostyryl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (17)

Compound **17** was prepared by following the general procedure B using **5** (0.38, 1 mmol) and trans-4-chlorophenylvinylboronic acid (0.18 g, 1 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give product (0.4 g) in 90% yield; GC-MS (EI) *m/z* 435 (M^+). The oxalate salt was precipitated from acetone; mp 113–115 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42-7.57 (m, 7H), 7.40 (d, *J* = 7.6 Hz, 2 H), 7.24 (m, 2H), 7.13 (dd, *J* = 8.4, 8.4 Hz, 2H), 5.13 (dd, *J* = 12.8, 13.6Hz, 1H), 5.12 (dd, *J* =12.4, 13.6 Hz, 1H), 2.96 (t, *J* = 7.2 Hz, 2H), 2.61 (s, 6H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.49 (m, 1H), 1.45 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2, 163.1, 160.6, 144.1, 141.8, 140.0, 137.4, 136.6, 132.7, 129.5, 129.4, 128.8, 128.0, 127.6, 127.5, 127.3, 122.8, 120.0, 115.8, 115.6, 90.6, 72.0, 57.3, 42.8, 38.0, 31.4, 20.0; ^{19}F NMR (376 MHz, DMSO-d_6) δ −117.5; IR (powder) 1159, 1222, 1718 cm^{-1}; Anal. ($C_{27}H_{27}ClFNO\cdot C_2H_2O_4\cdot 1/3\ H_2O$) C, H, N.

3-(1-(4-Fluorophenyl)-4-phenyl-1,3-dihydroisobenzofuran-1-yl)-*N,N*-dimethylpropan-1-amine (18)

Compound **18** was prepared by following the general procedure B using **6** (0.2 g, 0.53 mmol) and phenyl boronic acid (0.065 g, 0.53 mmol), eluting with chloroform/MeOH (10:1)

to give product (0.2 g) in 95% yield. GC-MS (EI) *m/z* 375 (M^+). The oxalate salt was precipitated from 2-propanol; mp 152–154 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (m, 2H), 7.50-7.35 (m, 8H), 7.14 (dd, *J* = 8.8, 9.2 Hz, 2H), 5.32 (d, *J* = 12 Hz, 1H), 5.16 (d, *J* = 13.6 Hz, 1H), 2.89 (m, 2H), 2.57 (s, 6H), 2.29 (m, 2H), 1.41 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.0, 139.9, 128.7, 128.6, 127.9, 127.7, 127.6, 127.1, 127.0, 120.8, 115.0, 114.8, 90.3, 71.5, 57.9, 42.2, 37.6, 19.9; ^{19}F NMR (376 MHz, CD_3OD) δ −118.4; IR (powder) 1170, 1234 cm^{-1}; GC-MS (EI) *m/z* 375 (M^+). Anal. ($C_{25}H_{26}FNO\cdot C_2H_2O_4\cdot 1/4\ H_2O$) C, H, N.

(E)-3-(1-(4-Fluorophenyl)-4-styryl-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (19)

Compound **19** was prepared by following the general procedure B using **6** (0.28 g, 0.74 mmol) and 2-phenylvinyl boronic acid (0.12 g, 0.75 mmol), eluting with chloroform/ MeOH (30:1, 10:1) to give the product (0.27 g) in 91% yield. GC-MS (EI) *m/z* 401(M^+). The oxalate salt was made from Acetone. Recrystallization from methanol gave pure **19**; mp 175–176 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59-7.16 (m, 14H), 5.38 (dd, *J* = 13.6, 11.6 Hz, 1H), 5.36 (dd, *J* =11.2, 12.0 Hz, 1H), 2.97 (t, *J* = 8.0 Hz, 2H), 2.62 (s, 6H), 2.19 (t, *J* = 7.6 Hz, 2H), 1.51 (m, 1H), 1.45 (m, 1H);^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 145.0, 137.5, 136.9, 131.9, 129.4, 129.0, 127.6, 127.5, 127.4, 126.3, 126.0, 115.8, 115.6, 90.8, 72.1, 57.5, 46.8, 43.1, 20.2; ^{19}F NMR (376 MHz, DMSO-d_6) δ −116.7; IR (powder) 1158, 1228, 1718 cm^{-1}; GC-MS (EI) *m/z* 401 (M^+). Anal. ($C_{27}H_{28}FNO\cdot C_2H_2O_4\cdot 1/4\ H_2O$) C, H, N.

3-(5-(3-Fluorophenyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (20)

Compound **20** was prepared by following the general procedure B using **6** (0.2, 0.53 mmol) and 3-fluorophenylvinylboronic acid (0.09 g, 0.64 mmol), eluting with chloroform/MeOH (30:1, 10:1) to give the product (0.2 g) in 95% yield. GC-MS (EI) *m/z* 393 (M^+). The oxalate salt was precipitated from acetone; mp 1441-146 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (dd, *J* = 4.8, 5.2 Hz, 2H), 7.53-7.38 (m, 4H), 7.30 (dd, *J* = 10.4 Hz, 7.6 Hz, 2H), 7.17 (m, 3H), 5.34 (d, *J* = 12.8 Hz, 1H), 5.18 (d, *J* = 13.2 Hz, 1H), 2.93 (t, *J* = 8 Hz, 2H), 2.60 (s, 6H), 2.18 (t, *J* = 7.6 Hz, 2H), 1.45 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 145.5, 141.7, 136.9, 134.7, 131.5, 131.4, 129.4, 128.4, 127.7, 127.6, 124.7, 122.2, 115.8, 115.6, 115.4, 115.1, 90.8, 71.9, 57.5, 43.0, 38.1, 20.1; ^{19}F NMR (376 MHz, DMSO-d_6) δ −113.6, −117.2; IR (powder) 1169, 1220 cm^{-1}; Anal. ($C_{25}H_{25}F_2NO\cdot C_2H_2O_4$) C, H, N.

3-(1-(4-Fluorophenyl)-5-iodo-1,3-dihydroisobenzofuran-1-yl)-N,N-dimethylpropan-1-amine (21)

A suspension of **5** (0.38, 1 mmol), KI (2.46 g, 15 mmol) and CuI (0.95 g, 5 mmol) in HMPA (3 mL) was heated at 150 °C for 3 h. The reaction mixture was purified by flash column chromatography eluting with chloroform/MeOH (30:1, 5:1) to give the product (0.2 g) in 47% yield; GC-MS (EI) *m/z* 425 (M^+). The oxalate salt was precipitated from EtOAc; mp 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.42 (dd, *J* = 5.6 Hz, 7.6 Hz, 2H), 7.21 (d, *J* = 8 Hz, 1H), 7.02 (dd, *J* = 8.8 Hz, 8.8 Hz, 2H), 5.00 (dd, *J* = 13.2 Hz, 13.6 Hz, 1H), 4.96 (dd, *J* = 13.2 Hz, 13.6 Hz, 1H), 2.82 (t, *J* = 7.2 Hz, 2H), 2.37 (s, 6H), 2.03 (t, *J* = 7.6 Hz, 2H), 1.35 (m, 1H), 1.29 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 143.9, 141.8, 136.6, 130.8, 127.2, 124.5, 123.4, 115.5, 115.3, 90.4, 71.2, 57.1, 42.7, 37.5, 19.7; ^{19}F NMR (376 MHz, DMSO-d_6) δ −112.8; IR (powder) 1152, 1222 cm^{-1}; GC-MS (EI) *m/z* 425 (M^+). Anal. ($C_{19}H_{21}FINO\cdot C_2H_2O_4$) C, H, N.

4-(4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl)benzonitrile (22)

Compound **22** was prepared by following the general procedure A using 5-cyanophthalidite (6.36 g, 40 mmol) to give the crude diol, which was purified by chromatography eluting with chloroform /MeOH (10:1) to give pure product (11 g) in 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61-7.54 (m, 3H), 7.31-7.26 (m, 2H), 6.97 (dd, *J* = 8.4, 8.8 Hz, 2H), 4.42 (d, *J* = 12 Hz, 1H), 4.16 (d, *J* = 12 Hz, 1H), 2.37 (m, 4H), 2.22 (s, 6H), 1.66 (m, 1H), 1.57 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4, 149.6, 142.9, 130.7, 130.3, 128.4, 127.6, 119.8, 115.0, 110.3, 77.4, 60.5, 59.8, 45.6, 41.1, 22.1; GC-MS (EI) *m/z* 342 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) with the chemical shift reagent (*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol (TFAE, 1/1 weight ratio), area ratio of the peaks with chemical shift 2.17 : 2.15 = 48 : 52 (*ee* = 4%).

(*S*)-(−)-4-(4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl)benzonitrile (S-22)

Compound **S-22** was prepared by following the general procedure C using **22** (3.5 g, 10 mmol) and (+)-di-*p*-toluoyl-*D*-tartaric acid monohydrate (1 g, 2.5 mmol) to give the enantiomerically pure free base (0.5 g) in 14 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) with the chemical shift reagent (*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol (TFAE, 1/2 weight ratio), area ratio of the peaks with chemical shift 2.19 : 2.17 = 99/1 (*ee* = 98%). $[\alpha]_D26$ = −61.09 ± 0.075 (c = 2.01, MeOH).

(*R*)-(+)-4-(4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl)benzonitrile (R-22)

Compound **R-22** was prepared by following the general procedure C using **22** (7.8 g, 23 mmol) and (−)-di-*p*-toluoyl-*L*-tartaric acid monohydrate (2.3 g, 5.75 mmol) to give the enantiomerically pure free base (1.27 g) in 15% yield. ^{1}H NMR (400 MHz, $CDCl_3$) with the chemical shift reagent (*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol (TFAE, 1/2 weight ratio), area ratio of the peaks with chemical shift 2.19 : 2.17 = 1/99 (*ee* = 98%). $[\alpha]_D24$ = +59.02 ± 0.074 (c =3.98, MeOH).

4-(4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl)benzobromide (23)

Compound **23** was prepared by following the general procedure A using 5-bromophthalide (8.5 g, 40 mmol). The reaction mixture became a green cloudy suspension after the addition of two Grignard reagents. The pure di-magnesium salt was filtered and treated with aq NH_4Cl (sat.) to give the pure diol (2.38 g) in 15% yield. More diol was obtained from the filtrate left by following the same work-up procedure in general procedure A. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41, 7.32 (2 m, 5H), 6.94 (dd, *J* = 8.8, 8.8 Hz, 2H), 4.33 (d, *J* = 12 Hz, 1H), 4.07 (d, *J* = 12 Hz, 1H), 2.45 (m, 2H), 2.32 (m, 2H), 2.22 (s, 6H), 1.68 (m, 1H), 1.54 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9, 160.5, 145.2, 143.9, 143.8, 143.3, 135.7, 130.1, 128.4, 127.9, 127.8, 121.5, 114.9, 114.7, 64.6, 60.2, 45.0, 44.3, 22.5; ^{19}F NMR (376 MHz, DMSO-d_6) δ −115.7; GC-MS (EI) *m/z* 394 (M^+); ^{1}H NMR (400 MHz, $CDCl_3$) with the chemical shift reagent (*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol (TFAE, 1/2 weight ratio), area ratio of the peaks with chemical shift 2.22 : 2.20 = 49 : 51(*ee* = 2%).

(*S*)-(−)-4-(4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl)benzobromide (S-23)

Compound **S-23** was prepared by following the general procedure C using **23** (9.6 g, 24 mmol) and (+)-di-*p*-toluoyl-*D*-tartaric acid monohydrate (2.44 g, 6 mmol) to give the pure salt (2.65 g) in 28% yield. $[\alpha]_D25$ = 11.0 ± 0.74 (c = 1.00, MeOH); Free base was checked

by ^{1}H NMR (400 MHz, $CDCl_3$) with the chemical shift reagent (*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol (TFAE, 1/1 weight ratio), only one peak with chemical shift 2.22 (*ee* > 99%). GC-MS (EI) *m/z* 394 (M^+).

(*R*)-(+)-4-(4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl)benzobromide (R-23)

Compound **R-23** was prepared by following the general procedure C using **23** (9 g, 23 mmol) and (−)-di-*p*-toluoyl-*L*-tartaric acid monohydrate (2.18 g, 5.8 mmol) to give the pure salt (2.4 g) in 27% yield. $[\alpha]_D25 = -11.2 \pm 0.62$ (c = 0.65, MeOH); Free base was checked by ^{1}H NMR (400 MHz, $CDCl_3$) with the chemical shift reagent (*R*)-(−)-1-(9-Anthryl)-2,2,2-trifluoroethanol (TFAE, 1/1 weight ratio), only one peak with chemical shift 2.21 (*ee* > 99%).

Biology

Scintillation Proximity-Based Binding Studies

Binding of ^{3}H-leucine (140 Ci/mmol; Moravek) to purified LeuT was performed by means of the scintillation proximity assay (SPA) as described6, 11 with 4 nmol of purified protein and 20 nM ^{3}H-leucine per assay in buffer composed of 50 mM Tris, Mes (pH 8.0), 100 mM NaCl, 1 mM TCEP, 20% glycerol, 1 mM DDM and concentration (10^{-7} - 10^{-2} M) of the indicated compounds. Note that the 3H–Leu and test compounds were added simultaneously.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

SERT	serotonin transporter
NET	norepinephrine transporter
DAT	dopamine transporter
LeuT	leucine transporter
NSS	neurotransmitter:sodium (Na^+/Cl^-) symporter
SSRI	selective serotonin reuptake inhibitor

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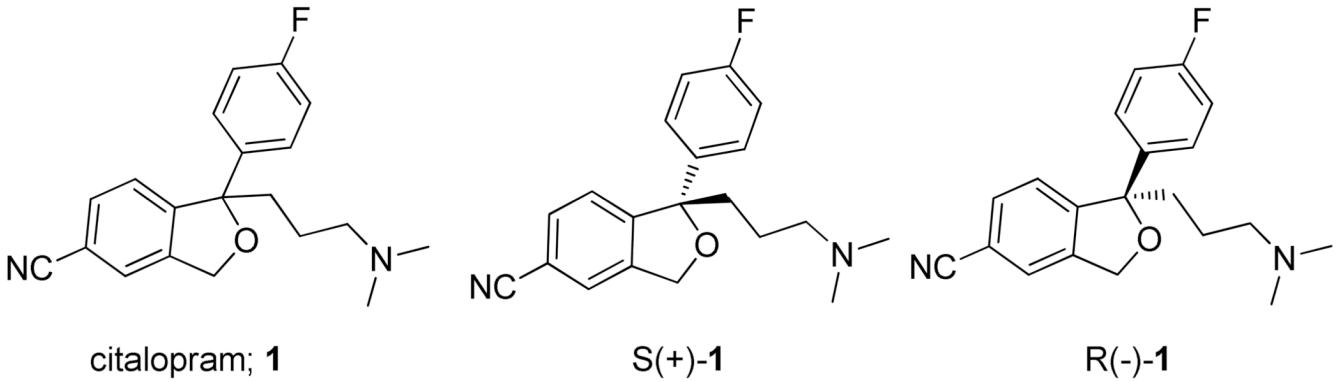


Figure 1.
Citalopram and its enantiomers S-1 and R-1.

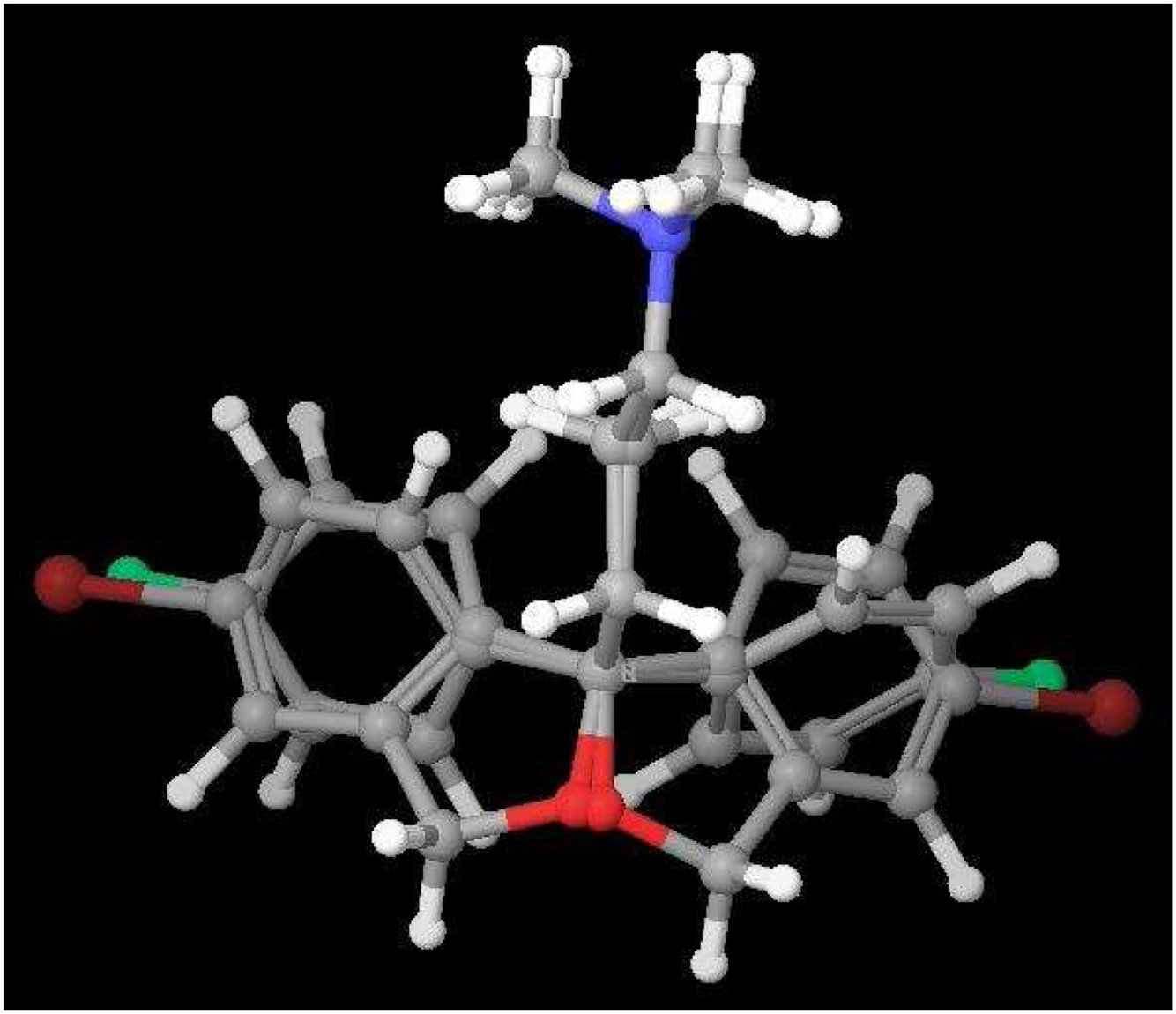


Figure 2.
3D-superimposition of S- and R-538
3D–superimposition of S- and R-**5** suggests the interchangeability of the halogen groups in compound **5** (Br *v*. F).

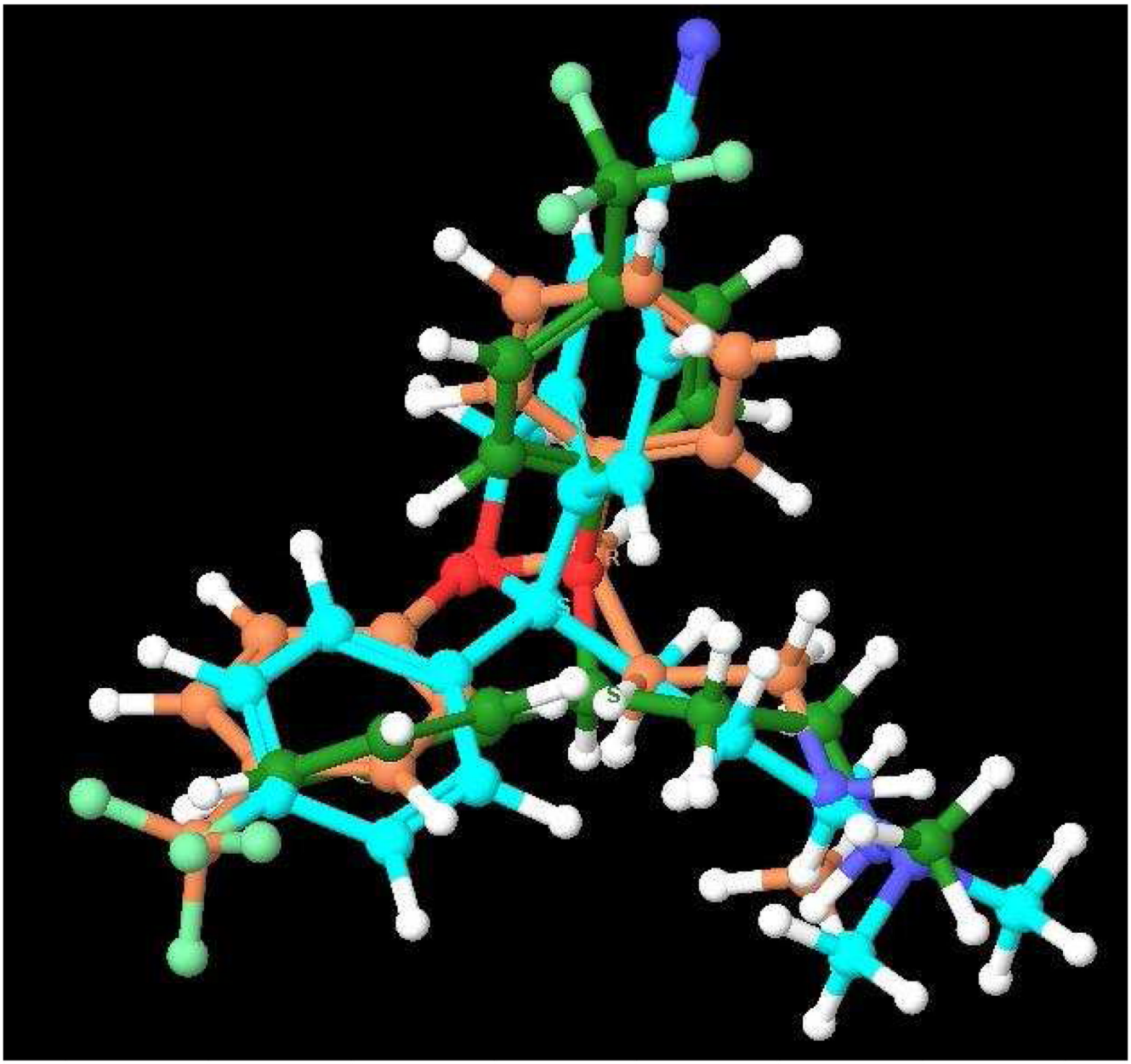
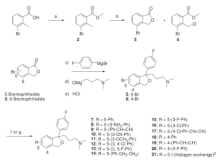


Figure 3.

3D-superimposition of S-1 and R- and S-fluoxetine38

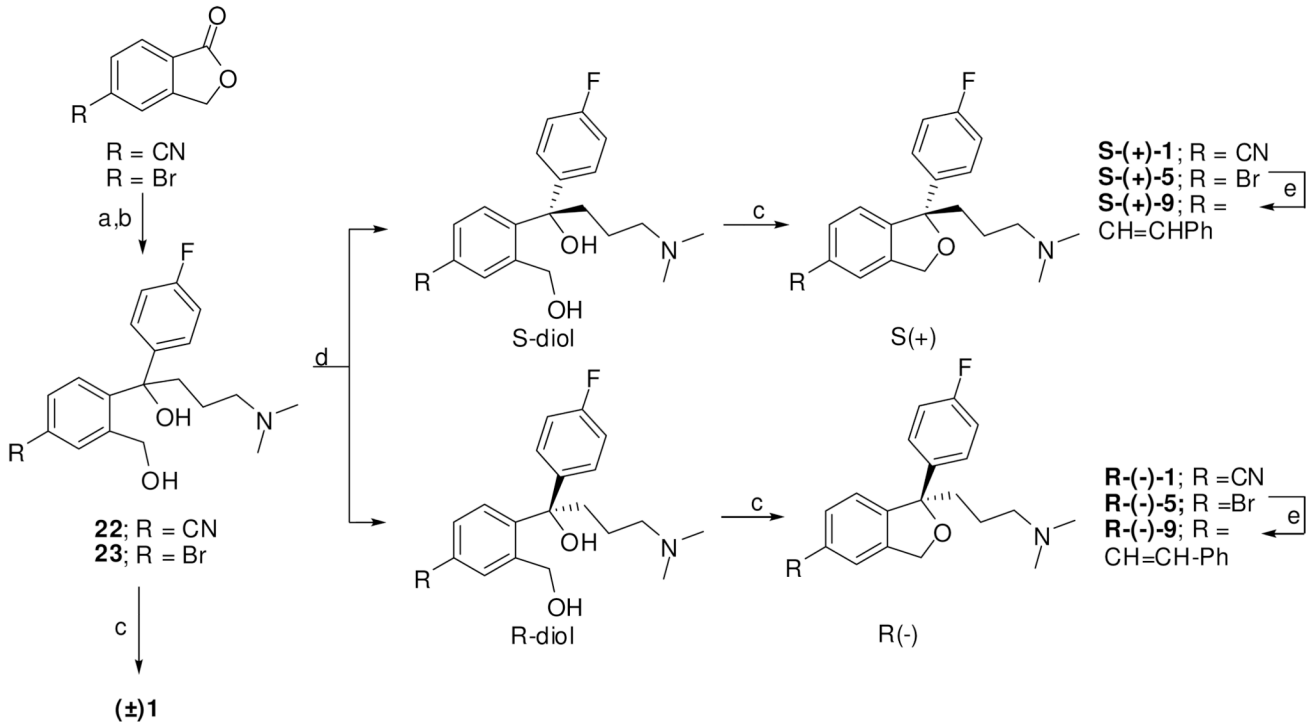
3D-superimposition of R-, S-**24** and S-**1** suggests the interchangeability of the F with the CN of **1** could be an explanation for the canceling effect on enantioselectivity. Note: The carbon chain of S-**1** is shown in turquoise blue, S-**24** in green and R-**24** in orange for clarity.



Scheme 1.

Synthesis of (±)4- and 5-substituted citalopram analogues[a]

[a] Reagents and conditions: (a) MeOH, conc. H_2SO_4, reflux, 12 h; (b) chromyl chloride, CCl_4, 0°C ~ reflux, 20 h; (c) THF, 0°C ~ rt, 3 h; (d) THF, 0°C ~ rt, overnight; (e) HCl/EtOH (1:1), 10 min; (f) Boronic acids, Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, 70–80°C, overnight; (g) CuI, KI, HMPA, 150°C, 3h.



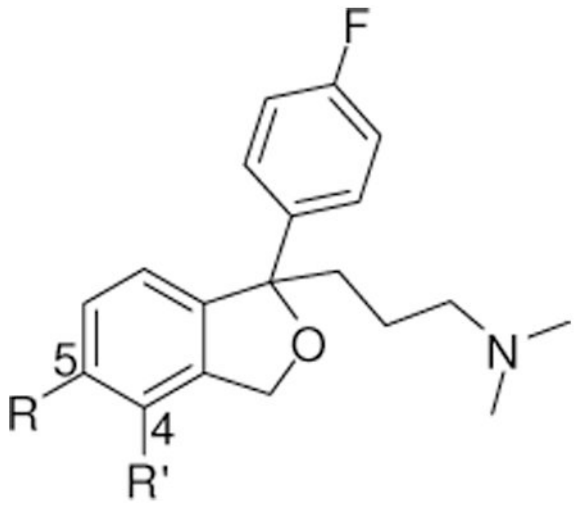
Scheme 2.

Synthesis of Chiral Analoguesa

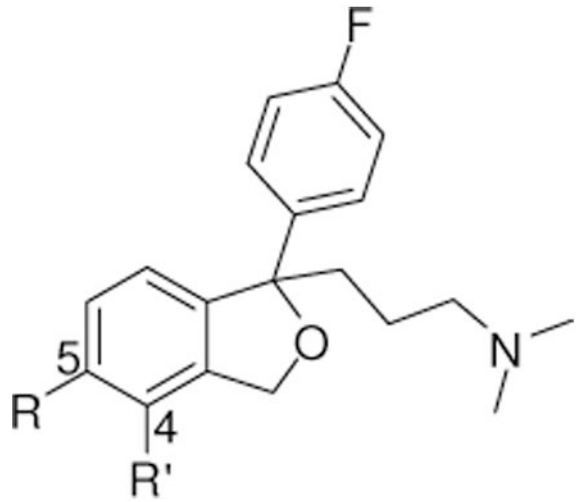
[a] Reagents and conditions: (a) (4-Fluorophenyl)magnesium bromide, THF, 0°C ~ rt, 3h; (b) (3-(Dimethylamino)propyl)magnesium chloride, THF, 0 °C ~ rt, overnight; (c) Triethylamine, MsCl, 0 °C, 3h; (d) Resolution with (+)-di-p-toluoyl-D-tartaric acid or (−)-di-p-toluoyl-L-tartaric acid monohydrate; (e) trans-phenylvinylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, 70–80 °C, overnight.

Table 1

In Vitro Data for (±)1 and its (±)analogues[a]



compound	R	R'	SERT Ki ± SEM (nM)	NET Ki ± SEM (nM)	DAT Ki ± SEM (nM)	Ki(NET)/ Ki (SERT)	Ki(DAT)/ Ki (SERT)
1	CN	H	1.94 ± 0.198	5950 ± 77.4	9270 ± 872	3070	4780
5[b]	Br	H	1.04 ± 0.126	28400 (global fit no SEM)	1650 ± 112	>10,000	1590
6	H	Br	3.87 ± 0.575	6170 ± 521	216 ± 18.1	1590	56
7	Ph	H	40.0 ± 4.01	16900 ± 1380	1800 ± 167	423	45
8	3-NH_2-Ph	H	10.4 ± 1.34	3820 ± 318	1690 ± 231	367	163
9	Ph-CH=CH	H	9.32 ± 1.36	11400 ± 1090	836 ± 14.9	1220	90
10	3-CN-Ph	H	6.70 ± 0.983	4980 ± 115	737 ± 68.5	743	110
11	3-OCH_3-Ph	H	9.20 ± 1.32	2610 ± 301	NT	284	
12	3,4-diCl-Ph	H	143 ± 21.2	12700 ± 1290	2510 ± 71.3	89	18
13	3,5-diF-Ph	H	108 ± 16.2	2000 ± 260	NT	19	
14	Ph-CH_2CH_2	H	38.1 ± 1.58	4630 ± 255	NT	122	
15	3-F-Ph	H	21.8 ± 1.09	4200 ± 489	NT	193	



compound	R	R’	SERT Ki ± SEM (nM)	NET Ki ± SEM (nM)	DAT Ki ± SEM (nM)	Ki(NET)/ Ki (SERT)	Ki(DAT)/ Ki (SERT)
16	3-Cl-Ph	H	61.2 ± 6.11	5710 ± 640	1390 ± 172	93	23
17	4-Cl-Ph-CH=CH	H	33.6 ± 4.48	25100 ± 3220	2000 ± 132	747	60
18	H	Ph	5.07 ± 0.350	7160 ± 791	542 ± 39.1	1410	107
19	H	Ph-CH=CH	46.4 ± 4.91	11900 ± 534	1140 ± 52.5	256	25
20	H	3-F-Ph	8.22 ± 1.12	7670 ± 1100	490 ± 26.2	933	60
21[c]	I	H	1.42 ± 0.155	32500 ± 4630	1350 ± 72.9	>10,000	950

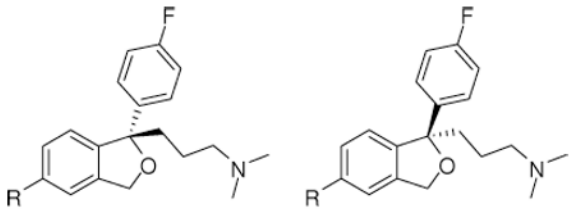
[a] These novel analogues were assessed by radioligand binding displacement of [^{3}H]citalopram (for SERT), [^{3}H]nisoxetine (for NET) and [^{3}H]WIN 35, 428 in rat brain stem, frontal cortex and caudate-putamen, respectively.36

[b] Published compound.32

[c] Published compound.37

Table 2

In Vitro Data for (±)1 and its chiral analogues[a]



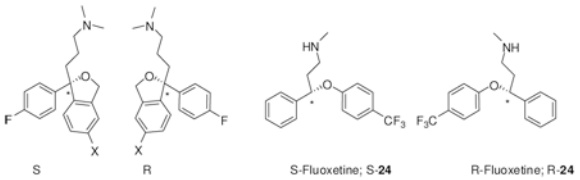
compound	R'	SERT Ki ± SEM (nM)	NET Ki ± SEM (nM)	DAT Ki ± SEM (nM)	Ki(NET)/Ki (SERT)	Ki(DAT)/Ki (SERT)
(±)-**1**	CN	1.94 ± 0.198	5950 ± 77.4	9270 ± 872	3070	4780
S(+)-**1**	CN	0.89 ± 0.132	10500 ± 893	8150 ± 314	11800	9160
R(−)-**1**	CN	28.3 ± 3.62	4980 ± 515	7980±646	178	282
(±)-**5**[b]	Br	1.04 ± 0.126	28400 (global fit no SEM)	1650 ± 112	>10,000	1590
S(+)-**5**	Br	0.92 ± 0.056	8410 ± 202	2250 ± 115	9140	2450
R(−)-**5**	Br	23.6 ± 1.76	21600 ± 3210	1350 ± 44	915	57
(±)-**9**	Ph-CH=CH	9.32 ± 1.36	11400 ± 1090	836 ± 14.9	1220	90
S(+)-**9**	Ph-CH=CH	10.6 ± 1.09	16800 ± 1360	1810 ± 46.8	1590	171
R(−)-**9**	Ph-CH=CH	113 ± 14.7	5190 ± 427	541 ± 40.7	46	4.79

[a] These novel analogues were assessed by radioligand binding displacement of [^{3}H]citalopram (for SERT), [^{3}H]nisoxetine (for NET) and [^{3}H]WIN 35, 428 in rat brain stem, frontal cortex and caudate-putamen, respectively.36

[b] Published compound.32

Table 3

LeuT Binding data



Compound	X	IC_{50} (mM)[a]
S-**1**	CN	1.56
R-**1**	CN	1.70
S-**5**	Br	0.40
R-**5**	Br	0.42
S-**24**	--	355[b]
R-**24**	--	2.54[b]

[a] This competition was done at 4 nM Protein, 20 nM ^{3}H-Leucine.

[b] Published data.8