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Development of potent dopamine-norepinephrine uptake inhibitors (DNRI) based on a (2*S*,4*R*,5*R*)-2-benzhydryl-5-((4-methoxybenzyl)amino)tetrahydro-2*H*-pyran-4-ol molecular template

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Abstract

Current therapy of depression is less than ideal with remission rates of only 25–35% and response rates of 45–60%. It has been hypothesized that a dysfunctional dopaminergic system in the mesocorticolimbic pathway in depressive disorder may lead to development of anhedonia associated with loss of pleasure and interest along with loss of motivation. The current antidepressants do not address dopamine dysfunction which might explain their low efficacy. In this report, we have described an SAR study on our pyran-based triple reuptake inhibitors (TRIs) which are being investigated as the next-generation antidepressants. In the present work we demonstrate that our lead TRIs can be modified with appropriate aromatic substitutions to display a highly potent SSRI profile for compounds **2a** and **4a** (K_i (SERT); 0.71 and 2.68 nM, respectively) or a potent DNRI profile for compounds **6b** and **6h** (K_i (DAT/NET); 8.94/ 4.76 and 13/ 7.37 nM, respectively). Compounds **4g–4i** exhibited potencies at all three monoamine transporters. The results provide insights into the structural requirements for developing selective dual- and triple-uptake inhibitors from a unique pyran molecular template for an effective management of depression and related disorders.

Introduction

Major depressive disorder (MDD) is a debilitating illness affecting 15–20% of the population in the United States.^[1] Depression is characterized by symptoms like insomnia,

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Supporting Information Available: Elemental analysis data for all final targets is available. This material is available free of charge via the internet.

loss of appetite, and psychomotor agitation. MDD is a severe form of depression defined by multiple episodes of depressed mood that persisting for at least 2 weeks accompanied by at least four other symptoms.^[2, 3] According to the WHO by 2020 MDD would be the second-most leading cause of disability worldwide, affecting 121 million people, thus making it a global health problem.^[4]

Modulation of serotonin (5-HT) and noradrenergic (NE) systems is at the core of the “monoamine deficiency” hypothesis of depression that postulates impaired monoaminergic transmission, either due to a deficit of monoamine neurotransmitters in synapses and surrounding extracellular space, or disturbed monoamine receptor signaling.^[5, 6] Therefore, most current antidepressants block serotonin and norepinephrine transporters (SERTs and NETs), either as serotonin-norepinephrine reuptake inhibitors (SNRIs) or as selective serotonin reuptake inhibitors (SSRIs).^[7–10] Converging evidences suggest that inhibitors targeting additionally NE neurotransmission may be more efficacious than those acting selectively on 5-HT systems in MDD.^[11–15] In a *meta-analysis*, venlafaxine (Figure 1), an SNRI, exhibited greater response and remission rates than an SSRI.^[11] Although a plethora of antidepressants are on the market, there still remains a significant unmet need for improved therapy, as large numbers of depressed people are still refractory to the current existing drugs. Thus, current therapy is less than ideal with remission rates of only 25–35% and response rates of 45–60%.^[16] Furthermore, slow onset of action of the current therapies along with other associated side effects call for improvements in MDD therapy.

Dopamine has been linked to depression for quite some time.^[17–20] The medial prefrontal cortex has been shown to be associated with depressed mood and sadness and neuroimaging studies indicate deficiencies of neuronal activity in this brain area in depressed subjects.^[21] This region receives innervations from all three monoamines; thus, restoration in the imbalanced level of monoamines by antidepressants has been shown to improve symptoms of depression.^[22] Since dopamine controls mood and emotion, a dysfunctional dopaminergic system in the mesocorticolimbic pathway may lead to development of anhedonia associated with loss of pleasure and interest along with loss of motivation.^[19] The persistence of anhedonia makes it one of the most treatment-resistant symptoms of MDD with a tendency for anhedonia to increase over the course of the disorder.^{23–25} Addition of dopaminergic activity to an antidepressant with serotonergic and norepinephrine activity should alleviate these symptoms provided the additional dopamine component does not introduce abuse liability.¹⁹ Interestingly, in a recent study DOV 21,947 (amitifadine), a TRI, has been shown to produce little to no abuse liability.²⁶ Be that as it may, any novel monoamine transporters blocker which includes dopaminergic activity such as a TRI, should undergo extensive abuse liability testing.

It has been hypothesized that inhibitors with ability to block dopamine reuptake in addition to NE reuptake (DNRI) should also have robust therapeutic effects by addressing dopamine deficit-related anhedonia in depression.^{27, 28} Given the side effects associated with clinically used SSRIs, exploration of dual dopamine-norepinephrine reuptake inhibitors (DNRI) is important, and understanding the relationship between dopaminergic and noradrenergic systems is of significant interest in addressing the pathophysiology of MDD.²⁹ In this regard, it is important to mention that there are only few compounds which are known to

exhibit a DNRI-type profile. One well known example of this is bupropion, used as an antidepressant agent in the clinic.^[17, 30, 31] It should be noted that the mechanism of antidepressant action of bupropion is quite complex and involves other targets in addition to dopaminergic and noradrenergic systems.³⁰ It should be noted that a successful adjunct therapeutic approach involving use of bupropion and an SSRI was found to be more efficacious in patients refractory to SSRI.^[17, 20] Nomifensine is another DNRI that has antidepressant activity and is as effective as imipramine.^[32, 33] It should be noted that DNRI, including nomifensine, have also been reported to exert a therapeutic effect on attention-deficit hyperactivity disorder (ADHD), a related mental disorder.^[34] In this regard, atomoxetine, a NET inhibitor, is the only non-stimulant drug approved for ADHD.^[35] It is perceived that a dual DNRI with a potency order of NET>DAT might offer better alternatives to current ADHD therapies.^[36]

In recent years triple reuptake inhibitors (TRIs) inhibiting all three monoamine transporters have been hypothesized to produce greater efficacy than SSRIs or dual uptake inhibitors. A number of TRIs, e.g. DOV 21,947 (amitifadine), PRC200-SS, JNJ-7925476 and GSK-372,475 have been developed (Figure 1) and have been characterized in animal models for depression.^[37, 38] Recently, DOV 21,947, now known as amitifadine, has undergone Phase IIb clinical trial which demonstrated promising efficacy in a treatment-resistant MDD patient group.

In our effort to address the unmet need in antidepressant therapy, we have been working to develop unique asymmetric pyran based inhibitors of monoamine uptake systems.^{39–43} Thus, our drug development work led to discovery of novel TRIs which interacted with all three transporters. In this manuscript, we report a structure-activity relationship (SAR) study describing modifications of the TRI profile of our compounds resulting in potent SSRI- and DNRI-type transporter inhibitors.

Chemistry

Amines **1** (disubstituted pyran), **3**, and **5** (trisubstituted pyran) were synthesized according to procedures we have published in our earlier reports.^[39, 40] Reductive amination of amines **1**, **3**, and **5** (Schemes 1, 2, and 3, respectively) with appropriate aldehydes in presence of sodium triacetoxyborohydride or sodiumcyanoborohydride and catalytic amount of acetic acid in 1,2-dichloroethane afforded final target compounds **2a**, **4a–4i**, **6a–6h** in appreciable yields.

Results and Discussion

Our recent endeavors have resulted in the discovery of several TRIs that exhibited potencies at all three monoamine transporters. In this regard, our lead TRIs, **D-142** and **D-161** (Figure 1) were shown to be efficacious, as evidenced by significant reduction of immobility, in both rat forced swim tests (FSTs) and mouse tail suspension tests (TSTs) that are established models for preclinical testing of potential antidepressants.^[44, 45] Recently, we have reported development of an orally active TRI, D-473 (Figure 1), which exhibited efficacious activity in FST and elevated level of all three monoamines in a microdialysis study.⁴⁶

In our quest to develop effective therapy for the treatment of MDD, we herein report an extensive SAR study on our trisubstituted pyran derivatives leading to the discovery of novel potent DNRI. Their functional effect at monoamine transporters was assessed by monitoring inhibition of substrate uptake in synaptosome-enriched fractions from rat brain (striatum for DAT assays and cerebral cortex for SERT and NET assays). Subtle structural modifications of our pyran-based inhibitors, interestingly, led to significantly different uptake profiles. Compound **2a** exhibited highly potent and selective serotonin reuptake inhibition (Table 1) with K_i values of 82 nM, 0.71 nM, and 25 nM, at DAT, SERT, and NET, respectively. It should be noted that compound **2a** is one of the most potent SERT inhibitors known to date. Compound **2a** was 20 times more active at SERT than fluoxetine^[31] with DAT, SERT, and NET inhibitory ratios of 115:1:35. Its trisubstituted counterpart, compound **4a**, was also selective for SERT and much weaker at DAT (K_i : 234, 2.7, and 34 nM for DAT, SERT, and NET, respectively). The exceptional potency at SERT might be due to aromatic π -stacking interactions of benzofuran moiety in **4a**. This notion was supported by the observation of a similar π - π stacking interaction between fluoxetine and Tyr176, in the SERT binding site.^[47] Moreover, mutation of Tyr176 also has been shown to significantly affect the binding of fluoxetine indicating the importance of this interaction. Since furan oxygen can also participate as H-bond acceptor, the role of Hbonding interactions at this position was further explored. In this regard compounds **4b–4d** were synthesized and biologically evaluated. Compound **4b** showed moderate affinity at DAT and SERT but was potent at NET (K_i : 167, 223, and 34 nM for DAT, SERT, and NET, respectively). Compound **4c** exhibited appreciable potencies at DAT (K_i : 58 nM) and NET (K_i : 30 nM) and was weakly active at SERT (K_i : 281 nM). Inhibition of SERT uptake was further weakened in compound **4d** (K_i : 209, 385, and 44 nM for DAT, SERT, and NET, respectively). Thus, the binding data of compounds **4b–4d** suggest that for DAT inhibition methoxy was most favorable at the *para*-position of the *N*-benzyl group. For inhibition of norepinephrine uptake hydroxyl and methoxy moieties could be substituted at the *meta*- and *para*-positions with retention of activities; However, hydroxyl, methoxy disubstitution led to significant reduction in SERT inhibition. It can be inferred that an H-bond donor particularly in the presence of another potential H-bonding group, resulted in weaker potencies at SERT. In accordance, the dihydroxy substituted compound **4e** was inactive at SERT (K_i : 3.0 μ M) and only moderately active at DAT (K_i : 259 nM) and NET (K_i : 152 nM). In contrast, a monosubstituted compound **4f**, which has a methoxy group at the *meta*-position, is favored at SERT (K_i : 27 nM) and NET (K_i : 5.8 nM) but detrimental at DAT (K_i : 376 nM) inhibition. Thus, **4f** displayed an SNRI profile. Interestingly, compound **4g** exhibited a balanced TRI profile (K_i : 44, 42, and 38 nM for DAT, SERT, and NET, respectively) suggesting that phenyl in the *N*-benzyl moiety could be replaced by a heterocyclic pyridine ring. The methoxy group in compounds **4f** and **4g** can participate in electronic interactions and can also act as a weak H-bond acceptor. The pyridine ring in compound **4g** imparts a polar character which is tolerated well at DAT, SERT and NET. Our next exploration was aimed at understanding the effect of fluorine substitution on the *N*-benzyl group with respect to inhibition of neurotransmitter uptake by the three monoamine transporters. Compound **4h** also exhibited TRI activity (K_i : 48, 9.0, and 42 nM for DAT, SERT, and NET, respectively). The improved activity of **4h** at SERT may be a result of polar interactions of electronegative fluorine at the *meta*-position. Next, the 2-fluoro substituted compound **4i** also retained TRI

activity (K_i : 52, 64, and 9.3 nM for DAT, SERT, and NET, respectively). It should also be noted that enhancement of SERT activity resulting in production of the TRI profile of compounds **4g–4i** may be due to the presence of methoxy substituent at the *para*-position. Taken together with our earlier SAR studies, trisubstituted unsubstituted benzhydryl pyran-based TRI could be designed by appropriate *N*-benzyl aromatic substitutions, at least with: (1) substitution of a *p*-methoxy or hydroxyl group, which suggest a H-bonding interaction, at the *para*-position; (2) presence of polar or H-bond acceptor groups at the *ortho*- and *meta*-positions; (3) accordingly, absence of hydrophobic or H-bond donor moieties at the *meta*-position and; (4) not more than one Hbond donor or two hydrogen bonding groups on the *N*-benzyl phenyl ring.

Difluoro benzhydryl pyran derivatives were recently developed as novel TRIs by our laboratory.^[40] In general, we found from our earlier SAR studies that difluoro substituted compounds favorably interact with DAT and tend to show somewhat decreased activity at SERT. In this SAR study we have further explored the effect of introduction of the difluoro substituent on the benzhydryl moiety. Compounds **6e** (K_i : 37, 400, and 10 nM for DAT, SERT, and NET, respectively) and **6c** (K_i : 17, 54, and 26 nM for DAT, SERT, and NET, respectively) conferred (i) an improved inhibitory potency at DAT and NET, and (ii) a weaker SERT inhibitory activity than their non-fluoro counterparts as we have reported in our earlier study.⁴⁰ This effect is further exemplified by comparing the inhibitory activities of compounds **6a** and **4a**. Thus, as predicted compound **6a** produced much greater potency at DAT compared to **4a** with reduction of potency at SERT (K_i values of 29 nM, 68 nM, and 26 nM for DAT, SERT, and NET, respectively). Compound **6f**, as predicted, was weaker at SERT (K_i of 129 nM) compared to non-fluorinated analogue **4f** but showed improved DAT potency (K_i : 56 nM). It should be noted that NET activity is generally well tolerated in both non-fluoro and disubstituted fluorinated benzhydryl pyran analogs. Based on such extensive SAR data, as well as results from our previous studies, the next series of compounds was designed aiming for DAT and NET, i.e. as novel DNRI, a relatively unexplored class of antidepressants. As mentioned before, a DNRI can also be potentially applied in the treatment of ADHD, without the liability of acting as a stimulant if its potency rankorder is NET>DAT. Thus, compounds **6d** and **6b** were designed as DNRI by incorporating: (a) difluoro substitution on the benzhydryl group; and (b) two hydrogen bonding substituents, at specific positions, on the *N*-benzyl moiety. Based on the SAR studies, it was expected that a hydroxyl, methoxy disubstitution on the *N*-benzyl group coupled with difluoro benzhydryl moiety was highly detrimental for SERT inhibition. In concordance to our design, **6d** (K_i : 26, 577, and 4.9 nM for DAT, SERT, and NET, respectively) and **6b** (K_i : 8.9, 107, and 4.8 nM for DAT, SERT, and NET, respectively) turned out to be potent DNRI. From compounds **6b** and **4f** it appears that the unfavorable effect of a *meta*-methoxy substituent at DAT in **4f** is compensated by two favorable structural features, namely hydroxyl at *para*-position on the *N*-benzyl group and the difluoro substituent on the benzhydryl moiety. These structural features on the other hand impart reduction in SERT activity with retention of activity at NET and gain in potency for DAT, thus, producing a DNRI type-effect. Finally, compounds **6h** (K_i : 13, 334, and 7.3 nM for DAT, SERT, and NET, respectively) and **6g** (K_i : 20, 258 and 28 nM for DAT, SERT, and NET, respectively) also displayed DNRI properties. Further comparison of the binding profiles of compounds **6g** and **4h** validate our design of

modifying a TRI to produce a DNRI. Figure 2 represents an updated model of pyran derivatives developed for TRI, SSRI, and DNRI properties. Thus, in the present series, compounds **6b** and **6h** were the most potent and selective DNRI discovered; both compounds exhibited favorable physicochemical properties for brain penetration as shown in Table 2.

Conclusions

This report describes an SAR study on our pyran-based TRIs, investigated as the next-generation antidepressants. In the present work we demonstrate that our lead TRIs could be transformed into compounds with SSRI and DNRI profiles by proper aromatic substitutions. Compounds **2a** and **4a** were discovered as some of the most potent SSRIs developed to date. Compounds **4g–4i** exhibited balanced potencies at all three monoamine transporters, whereas compounds **6d–6g** were developed as novel DNRI. Given the absence of 3D-structures for the three monoamine transporters, and the homology existing between them, it is challenging to exploit subtle differences and similarities in their drug binding sites. The SAR approach taken in the present study provides insights into the structural requirements for developing selective, dual, and triple-uptake inhibitors from a unique pyran molecular template which with optimal pharmacokinetic properties should provide a potential for a more effective management of depression and related disorders than afforded by current drug therapies.

Experimental Section

Chemistry

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Dry solvents were obtained according to the standard procedures. All reactions were performed under inert atmosphere (N_2) unless otherwise noted. Analytical silica gel-coated TLC plates (silica gel 60 F254) were purchased from EM Science and were visualized with UV light or by treatment with either phosphomolybdic acid (PMA) or ninhydrin. Flash chromatography was carried out on Baker Silica Gel 40 μ m. 1H NMR and ^{13}C spectra were routinely recorded with a Varian 400 spectrometer operating at 400 and 100 MHz, respectively. The NMR solvent used was either $CDCl_3$ or CD_3OD as indicated. TMS was used as an internal standard. NMR and rotation of free bases were recorded. Salts of free bases were used for biological characterization. Elemental analyses were performed by Atlantic Microlab Inc. and were within $\pm 0.4\%$ of the theoretical value. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter.

Procedure A

(3S,6S)-6-benzhydryl-N-(benzofuran-5-ylmethyl)tetrahydro-2H-pyran-3-amine (2a)—To a stirred solution of amine **1** (60 mg, 0.22 mmol) and 1-benzofuran-5-carbaldehyde (35 mg, 0.24 mmol) in 1,2-dichloroethane (6 mL) was added glacial acetic acid (13 μ L, 0.22 mmol). After being stirred for 30 minutes, $NaCNBH_3$ (28 mg, 0.44 mmol) was added portion wise followed by methanol (1 mL). The reaction mixture was stirred for overnight. The reaction mixture was quenched with saturated $NaHCO_3$ solution at 0 $^\circ$ C and

extracted with dichloromethane (3 X 75 mL). The combined organic layer was washed with water, brine, dried over Na₂SO₄, and the solvent was removed under reduced pressure. Crude product was purified by column chromatography using 70% ethyl acetate in hexanes to give compound **2a (D-484)** (60 mg, 67%) as thick syrup. $[\alpha]_D^{25} = (-)78.2^\circ$ (c 0.5, MeOH). ¹H NMR (400 MHz, CDCl₃): δ 1.25–1.38 (m, 1H), 1.50–1.70 (m, 2H), 1.82–2.02 (m, 1H), 2.68 (br s, 1H), 3.56 (dd, *J* = 1.6, 12.0 Hz, 1H), 3.80–4.12 (m, 6H), 6.73 (d, *J* = 1.2 Hz, 1H), 7.14–7.46 (m, 12H), 7.56 (s, 1H), 7.61 (d, *J* = 2.0 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 25.43, 27.76, 50.46, 50.98, 57.42, 70.40, 79.50, 106.75, 111.37, 120.78, 124.93, 126.47, 126.66, 127.70, 128.54, 128.74, 128.80, 135.11, 142.44, 142.70, 145.44, 154.42. The product was converted into the corresponding hydrochloride salt; mp: 140–142 °C. Anal. (C₂₇H₂₇NO₂·HCl·0.7H₂O) C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((benzofuran-5-ylmethyl)amino)tetrahydro-2H-pyran-4-ol (4a)—Amine **3** (60 mg, 0.21 mmol) was reacted with 1-benzofuran-5-carbaldehyde (34 mg, 0.23 mmol), glacial acetic acid (12 μL, 0.21 mmol), and NaCNBH₃ (26 mg, 0.42 mmol) in 1,2-dichloroethane (6 mL) using procedure A. The residue was purified by column chromatography using ethyl acetate to afford compound **4a (D-485)** (60 mg, 69%) as a white solid. $[\alpha]_D^{25} = (-)64.8^\circ$ (c 0.5, MeOH). ¹H NMR (400 MHz, CDCl₃): δ 1.43 (dt, *J* = 3.2, 14.0 Hz, 1H), 1.70–1.80 (m, 1H), 2.48 (d, *J* = 2.4 Hz, 1H), 3.76–3.84 (m, 2H), 3.88–4.16 (m, 4H), 4.51 (dt, *J* = 2.4, 10.4 Hz, 1H), 6.73 (d, *J* = 1.6 Hz, 1H), 7.12–7.38 (m, 11H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.52 (s, 1H), 7.61 (d, *J* = 2.4 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 33.68, 51.65, 56.74, 56.78, 65.05, 67.72, 73.85, 106.73, 111.45, 120.82, 124.90, 126.56, 126.76, 127.74, 128.62 (3C), 128.67 (3C), 128.87 (3C), 134.86, 142.27, 142.36, 145.53, 154.48. The product was converted into the corresponding hydrochloride salt; mp: 204–206 °C. Anal. (C₂₇H₂₇NO₃·HCl·0.5H₂O) C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((4-hydroxy-3-methoxybenzyl)amino)tetrahydro-2H-pyran-4-ol (4b)—Amine **3** (60 mg, 0.21 mmol) was reacted with vanillin (39 mg, 0.25 mmol), glacial acetic acid (12 μL, 0.21 mmol), and NaCNBH₃ (26 mg, 0.42 mmol) in 1,2-dichloroethane (6 mL) using procedure A. The residue was purified by column chromatography using 5% methanol in ethyl acetate to afford compound **4b (D-501)** (65 mg, 73%) as a thick syrup. $[\alpha]_D^{25} = (-)80.4^\circ$ (c 0.5, MeOH). ¹H NMR (400 MHz, CDCl₃): δ 1.38–1.48 (m, 1H), 1.68–1.78 (m, 1H), 2.52 (br s, 1H), 3.66 (d, *J* = 12.8 Hz, 1H), 3.78 (s, 3H), 3.76–3.86 (m, 2H), 3.88–4.04 (m, 3H), 4.52 (dt, *J* = 2.4, 8.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.86 (s, 1H), 7.12–7.37 (m, 10H). ¹³C NMR (100 MHz, CDCl₃): δ 33.53, 51.18, 56.05, 56.63, 56.68, 64.30, 66.82, 74.02, 111.42, 114.78, 119.20, 121.56, 126.61, 126.82, 128.62 (3C), 128.66 (3C), 128.91 (3C), 142.23, 145.29, 147.09. The product was converted into the corresponding hydrochloride salt; mp: 203–205 °C. Anal. (C₂₆H₂₉NO₄·HCl·0.5H₂O) C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((3-hydroxy-4-methoxybenzyl)amino)tetrahydro-2H-pyran-4-ol (4c)—Amine **3** (60 mg, 0.21 mmol) was reacted with 3-hydroxy-4-methoxybenzaldehyde (39 mg, 0.25 mmol), glacial acetic acid (12 μL, 0.21 mmol), and NaCNBH₃ (26 mg, 0.42 mmol) in 1,2-dichloroethane (6 mL) using procedure A. The

residue was purified by column chromatography using 5% methanol in ethyl acetate to afford compound **4c (D-502)** (65 mg, 73%) as a thick syrup. $[\alpha]_D^{25} = (-)78.4^\circ$ (c 0.5, MeOH). ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{MeOH}-d_4$): δ 1.36–1.44 (m, 1H), 1.54–1.64 (m, 1H), 2.45 (d, $J = 2.8$ Hz, 1H), 2.85 (br s, 1H), 3.58 (d, $J = 12.8$ Hz, 1H), 3.72–3.78 (m, 2H), 3.81 (s, 3H), 3.84–3.94 (m, 3H), 4.47 (dt, $J = 2.4, 10.8$ Hz, 1H), 6.69 (dd, $J = 1.6, 8.4$ Hz, 1H), 6.74 (d, $J = 8.0$ Hz, 1H), 6.83 (d, $J = 1.6$ Hz, 1H), 7.08–7.32 (m, 10H). The product was converted into the corresponding hydrochloride salt; mp: 168–170 °C. Anal. ($\text{C}_{26}\text{H}_{29}\text{NO}_4 \cdot \text{HCl} \cdot \text{H}_2\text{O}$) C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((3-hydroxy-5-methoxybenzyl)amino)tetrahydro-2H-pyran-4-ol (4d)—Amine **3** (60 mg, 0.21 mmol) was reacted with 3-hydroxy-5-methoxybenzaldehyde (38 mg, 0.25 mmol), glacial acetic acid (12 μL , 0.21 mmol), and NaCNBH_3 (26 mg, 0.42 mmol) in 1,2-dichloroethane (6 mL) using procedure A. The residue was purified by column chromatography using 5% methanol in ethyl acetate to afford compound **4d (D-503)** (65 mg, 73%) as a thick syrup. $[\alpha]_D^{25} = (-)75.6^\circ$ (c 0.5, MeOH). ^1H NMR (400 MHz, CDCl_3): δ 1.34–1.42 (m, 1H), 1.52–1.64 (m, 1H), 2.41 (br s, 1H), 3.52 (d, $J = 13.2$ Hz, 1H), 3.64 (s, 3H), 3.60–3.75 (m, 2H), 3.78–3.93 (m, 2H), 4.45 (dt, $J = 1.6, 10.0$ Hz, 1H), 4.56 (br s, 2H), 6.26 (s, 1H), 6.32 (s, 1H), 6.33 (s, 1H), 7.10–7.32 (m, 10H). ^{13}C NMR (100 MHz, CDCl_3): δ 33.35, 50.85, 55.47, 56.34, 56.51, 63.94, 66.58, 74.14, 101.09, 106.51, 108.32, 126.67, 126.86, 128.54 (3C), 128.71 (3C), 128.94 (3C), 140.79, 142.04, 142.18, 158.00, 161.25. The product was converted into the corresponding hydrochloride salt; mp: 160–162 °C. Anal. ($\text{C}_{26}\text{H}_{29}\text{NO}_4 \cdot \text{HCl} \cdot 0.8\text{H}_2\text{O}$) C, H, N.

5-(((3R,4R,6S)-6-benzhydryl-4-hydroxytetrahydro-2H-pyran-3-yl)amino)methyl)benzene-1,3-diol (4e)—Amine **3** (60 mg, 0.21 mmol) was reacted with 3,5-dihydroxybenzaldehyde (29 mg, 0.21 mmol), glacial acetic acid (12 μL , 0.21 mmol), and NaCNBH_3 (26 mg, 0.42 mmol) in 1,2-dichloroethane (6 mL) using procedure A. The residue was purified by column chromatography using 7% methanol in dichloromethane to afford compound **4e (D-542)** (65 mg, 76%) as a thick syrup. $[\alpha]_D^{25} = (-)74.2^\circ$ (c 0.5, MeOH). ^1H NMR (400 MHz, CDCl_3): δ 1.34–1.42 (m, 1H), 1.55 (dt, $J = 2.4, 10.8$ Hz, 1H), 2.43 (br s, 1H), 3.30 (s, 1H), 3.51 (d, $J = 12.8$ Hz, 1H), 3.60–3.70 (m, 2H), 3.78–3.90 (m, 3H), 4.31 (br s, 3H), 4.45 (t, $J = 8.4$ Hz, 1H), 6.16 (s, 1H), 6.21 (s, 1H), 6.22 (s, 1H), 7.02–7.28 (m, 10H). ^{13}C NMR (100 MHz, CDCl_3): δ 33.06, 50.68, 55.99, 56.73, 63.94, 65.73, 74.08, 102.18, 107.35, 126.51, 126.72, 128.46 (3C), 128.48 (2C), 128.55, 128.77(3C), 139.97, 141.97, 142.18, 158.26. The product was converted into the corresponding hydrochloride salt; mp: 168–170 °C. Anal. ($\text{C}_{25}\text{H}_{27}\text{NO}_4 \cdot \text{HCl} \cdot \text{H}_2\text{O}$) C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((3-methoxybenzyl)amino)tetrahydro-2H-pyran-4-ol (4f)—Amine **3** (60 mg, 0.21 mmol) was reacted with 3-methoxybenzaldehyde (35 mg, 0.25 mmol), glacial acetic acid (12 μL , 0.21 mmol), and NaCNBH_3 (27 mg, 0.42 mmol) in 1,2-dichloroethane (6 mL) using procedure A. The residue was purified by column chromatography using 3% methanol in ethyl acetate to afford compound **4f (D-523)** (65 mg, 76%) as a thick syrup. $[\alpha]_D^{25} = (-)84.2^\circ$ (c 0.5, MeOH). ^1H NMR (400 MHz, CDCl_3): δ 1.38–1.46 (m, 1H), 1.66–1.78 (m, 1H), 2.16 (br s, 2H), 2.44 (d, $J = 2.4$ Hz, 1H), 3.70 (d, $J =$

13.6 Hz, 1H), 3.76–3.82 (m, 1H), 3.79 (s, 3H), 3.84–3.98 (m, 4H), 4.50 (dt, $J = 2.4, 10.4$ Hz, 1H), 6.80 (dd, $J = 1.6, 8.0$ Hz, 1H), 6.86–6.92 (m, 2H), 7.14–7.38 (m, 11H), ^{13}C NMR (100 MHz, CDCl_3): δ 33.40, 51.26, 55.22, 56.50, 56.64, 64.76, 67.30, 73.61, 112.53, 113.64, 120.43, 126.35, 126.55, 128.40 (3C), 128.66 (3C), 129.45 (3C), 141.64, 142.04, 142.10, 159.75. The product was converted into the corresponding hydrochloride salt; mp: 197–199 °C. Anal. ($\text{C}_{26}\text{H}_{29}\text{NO}_3 \cdot \text{HCl}$) C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((6-methoxypyridin-3-

yl)methyl)amino)tetrahydro-2H-pyran-4-ol (4g)—Amine **3** (68 mg, 0.24 mmol) was reacted with 6-methoxynicotinaldehyde (40 mg, 0.29 mmol), glacial acetic acid (12 μL , 0.21 mmol), and NaCNBH_3 (92 mg, 0.43 mmol) in 1,2-dichloroethane (6 mL) using procedure A. The residue was purified by column chromatography using 3% methanol in ethyl acetate to afford compound **4g** (**D-576**) (51 mg, 51%) as a thick syrup, $[\alpha]_{\text{D}}^{25} = (-)44.7^\circ$, $c = 1$ in MeOH. ^1H NMR (500 MHz, CDCl_3): δ 8.04 (s, 1 H), 7.55 (dd, $J = 2.1, 8.6$ Hz, 1 H), 7.06–7.40 (m, 10 H), 6.69 (d, $J = 8.6$ Hz, 1 H), 4.42–4.55 (m, 1 H), 3.85–4.00 (m, 4 H), 3.74–3.84 (m, 2 H), 3.66 (d, $J = 13.1$ Hz, 1 H), 2.42 (br s, 1 H), 1.65–1.76 (m, 1 H), 1.37–1.47 (m, 1 H). ^{13}C NMR (125 MHz, CDCl_3): δ 163.5, 146.1, 142.0, 141.9, 139.0, 128.6 (3C), 128.4 (3C), 128.3 (3C), 126.5, 126.3, 110.7, 73.5, 67.4, 64.7, 56.7, 56.2, 53.4, 48.1, 33.4. The product was converted into the corresponding hydrochloride salt; mp: 190–195 °C. Anal. Calcd for $[\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_3 \cdot 2\text{HCl} \cdot 0.2\text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((3-fluoro-4-methoxybenzyl)amino)tetrahydro-2H-pyran-4-ol (4h)—Amine **3** (60 mg, 0.21 mmol) was reacted with 3-fluoro-4-

methoxybenzaldehyde (36 mg, 0.23 mmol), glacial acetic acid (16 μL , 0.27 mmol), and NaCNBH_3 (80 mg, 0.36 mmol) in 1,2-dichloroethane (3 mL) using procedure A. The residue was purified by column chromatography by using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **4h** (**D-580**) as colorless syrup (60 mg, 68%). ^1H NMR (500 MHz, CDCl_3): δ 7.12–7.39 (m, 10 H), 7.07 (dd, $J = 12.2, 1.8$ Hz, 1 H), 6.97 (d, $J = 8.2$ Hz, 1 H), 6.87 (t, $J = 8.6$ Hz, 1 H), 4.49 (dt, $J = 10.4, 2.1$ Hz, 1 H), 3.87–3.98 (m, 3 H), 3.86 (s, 3 H), 3.80 (d, $J = 13.4$ Hz, 1 H), 3.75 (d, $J = 11.9$ Hz, 1 H), 3.63 (d, $J = 13.1$ Hz, 1 H), 2.41 (s, 1 H), 1.85 (br s, 1 H), 1.66–1.76 (m, 1 H), 1.37–1.47 (m, 1 H). ^{13}C NMR (125 MHz, CDCl_3): δ 153.4, 151.4, 146.6, 146.5, 142.1, 142.0, 133.5, 133.4, 128.6, 128.4, 128.3, 126.5, 126.3, 123.6, 123.5, 115.8, 115.7, 113.3, 73.6, 67.5, 64.8, 56.6, 56.4, 56.3, 50.4, 33.4. $[\alpha]_{\text{D}}^{25} = (-)53.9^\circ$, $c = 1$ in CH_2Cl_2 . The product was converted into the corresponding hydrochloride salt; mp: 190–195 °C. Anal. Calcd for $[\text{C}_{26}\text{H}_{28}\text{FNO}_3 \cdot \text{HCl} \cdot \text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-2-benzhydryl-5-((2-fluoro-4-methoxybenzyl)amino)tetrahydro-2H-pyran-4-ol (4i)—Amine **3** (60 mg, 0.21 mmol) was reacted with 2-fluoro-4-

methoxybenzaldehyde (36 mg, 0.23 mmol), glacial acetic acid (16 μL , 0.27 mmol), and $\text{Na(OAc)}_3\text{BH}$ (80 mg, 0.36 mmol) in a mixture of 1,2-dichloroethane (3 mL) and methanol (1 mL) by following Procedure A. The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **4i** (**D-581**) as a colorless syrup (60 mg, 68%). ^1H NMR (500 MHz, CDCl_3): δ 7.11–7.38 (m, 11 H), 6.64 (dd, $J = 8.6, 2.1$ Hz, 1 H), 6.58 (dd, $J = 11.9, 2.4$

Hz, 1 H), 4.48 (dt, $J = 10.1, 2.4$ Hz, 1 H), 3.91–3.98 (m, 2 H), 3.88 (dd, $J = 11.9, 2.1$ Hz, 1 H), 3.80 (d, $J = 13.4$ Hz, 1 H), 3.76 (s, 3 H), 3.68–3.75 (m, 2 H), 2.42 (m, 1 H), 1.86 (s, 1 H), 1.65–1.78 (m, 1 H), 1.35–1.46 (m, 1 H). ^{13}C NMR (125 MHz, CDCl_3): δ 162.5, 160.6, 160.0, 159.9, 142.1, 142.0, 130.7, 130.6, 128.6, 128.4, 128.3, 128.3, 126.5, 126.3, 119.0, 118.9, 109.8, 109.7, 101.6, 101.4, 73.6, 67.3, 65.0, 56.7, 56.4, 55.5, 44.4, 33.4.

$[\alpha]_{\text{D}}^{25} = (-)54.1^\circ$, $c = 1$ in CH_2Cl_2 . The product was converted into the corresponding hydrochloride salt; mp: 170–175 °C. Anal. Calcd for $[\text{C}_{26}\text{H}_{28}\text{FNO}_3 \cdot \text{HCl} \cdot \text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-5-((benzofuran-5-ylmethyl)amino)-2-(bis(4-fluorophenyl)

methyl)tetrahydro-2H-pyran-4-ol (6a)—Amine **5** (50 mg, 0.16 mmol) was reacted with benzofuran-5-carbaldehyde (27 mg, 0.19 mmol), glacial acetic acid (13 μL , 0.21 mmol), and NaCNBH_3 (17 mg, 0.27 mmol) in a mixture of 1,2-dichloroethane (4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6a (D-508)** (55 mg, 81%) as a light yellow syrup. ^1H NMR (500 MHz, CDCl_3): δ 7.61 (d, $J = 2.1$ Hz, 1 H), 7.51 (s, 1 H), 7.43 (d, $J = 8.5$ Hz, 1 H), 7.25–7.30 (m, 2 H), 7.23 (d, $J = 8.5$ Hz, 1 H), 7.11–7.18 (m, 2 H), 6.91–7.03 (m, 4 H), 6.69–6.75 (m, 1 H), 4.34–4.44 (m, 1 H), 3.97–4.03 (m, 4 H), 3.81 (d, $J = 12.8$ Hz, 2 H), 2.48 (s, 1 H), 1.65–1.77 (m, 1 H), 1.40 (d, $J = 14.4$ Hz, 1 H). ^{13}C NMR (125 MHz, CDCl_3): δ 162.4, 160.5, 154.2, 145.3, 137.7, 137.4, 134.5, 129.9, 129.8, 129.7, 129.6, 127.5, 124.6, 120.5, 115.5, 115.3, 115.2, 115.1, 111.2, 106.4, 73.5, 67.4, 64.9, 56.3, 54.9, 51.4, 33.2. $[\alpha]_{\text{D}}^{25} = (-)39.3^\circ$, $c = 1$ in CH_2Cl_2 . The product was converted into the corresponding hydrochloride salt; mp: 160–165 °C. Anal. Calcd for $[\text{C}_{27}\text{H}_{25}\text{F}_2\text{NO}_3 \cdot \text{HCl} \cdot \text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-2-(bis(4-fluorophenyl)methyl)-5-((4-hydroxy-3-methoxybenzyl)

amino)tetrahydro-2H-pyran-4-ol (6b)—Amine **5** (60 mg, 0.19 mmol) was reacted with 3-hydroxy-4-methoxybenzaldehyde (34 mg, 0.23 mmol), glacial acetic acid (17 μL , 0.28 mmol), and NaCNBH_3 (20 mg, 0.32 mmol) in a mixture of 1,2-dichloroethane (4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6b (D-527)** (65 mg, 76%) as a colorless syrup. ^1H NMR (400 MHz, CDCl_3): δ 7.26 (dd, $J = 8.2, 5.6$ Hz, 2 H), 7.14 (dd, $J = 8.5, 5.6$ Hz, 2 H), 6.89–7.20 (m, 4 H), 6.78–6.88 (m, 2 H), 6.71–6.77 (m, 1 H), 4.34–4.46 (m, 1 H), 4.0–4.08 (m, 1 H), 3.76–3.97 (m, 7 H), 3.67 (d, $J = 12.9$ Hz, 1 H), 2.51 (s, 1 H), 1.64–1.77 (m, 1 H), 1.37–1.48 (m, 1 H). ^{13}C NMR (100 MHz, CDCl_3): δ 162.7, 160.3, 146.6, 144.9, 137.6, 137.4, 130.6, 129.9, 129.8, 129.7, 129.6, 121.1, 115.6, 115.4, 115.3, 115.1, 114.3, 110.9, 73.6, 66.8, 64.3, 56.3, 55.8, 54.8, 50.9, 33.1. $[\alpha]_{\text{D}}^{25} = (-)28.7^\circ$, $c = 1$ in MeOH. The product was converted into the corresponding hydrochloride salt; mp: 190–195 °C. Anal. Calcd for $[\text{C}_{26}\text{H}_{27}\text{F}_2\text{NO}_4 \cdot \text{HCl} \cdot 0.8 \text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-2-(bis(4-fluorophenyl)methyl)-5-((2,3-dihydrobenzofuran-5-

yl)methyl)amino)tetrahydro-2H-pyran-4-ol (6c)—Amine **5** (50 mg, 0.16 mmol) was reacted with 2,3-dihydrobenzofuran-5-carbaldehyde (28 mg, 0.19 mmol), glacial acetic acid (13 μL , 0.21 mmol), and NaCNBH_3 (17 mg, 0.27 mmol) in a mixture of 1,2-dichloroethane

(4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6c (D-507)** (56 mg, 79%) as a light yellow syrup. ^1H NMR (400 MHz, CDCl_3): δ 7.23–7.31 (m, 2 H), 7.10–7.19 (m, 3 H), 6.89–7.03 (m, 5 H), 6.71 (d, J = 8.1 Hz, 1 H), 4.55 (t, J = 8.8 Hz, 2 H), 4.33–4.47 (m, 1 H), 3.95–4.03 (m, 1 H), 3.85–3.94 (m, 2 H), 3.74–3.83 (m, 2 H), 3.63 (d, J = 12.7 Hz, 1 H), 3.17 (t, J = 8.8 Hz, 2 H), 2.46 (d, J = 2.2 Hz, 1 H), 1.63–1.74 (m, 1 H), 1.36–1.46 (m, 1 H). ^{13}C NMR (100 MHz, CDCl_3): δ 162.7, 160.2, 159.2, 137.7, 137.4, 131.8, 129.9, 129.8, 129.7, 129.6, 127.9, 127.2, 124.8, 115.5, 115.3, 115.3, 115.1, 108.9, 73.5, 71.2, 67.2, 64.7, 56.2, 54.8, 51.0, 33.2, 29.6.

$[\alpha]_{\text{D}}^{25} = (-)39.8^\circ$, c = 1 in CH_2Cl_2 . The product was converted into the corresponding hydrochloride salt; mp: 145–150 °C. Anal. Calcd for $[\text{C}_{27}\text{H}_{27}\text{F}_2\text{NO}_3 \cdot \text{HCl} \cdot 0.5\text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-2-(bis(4-fluorophenyl)methyl)-5-((3-hydroxy-5-methoxybenzyl)

amino)tetrahydro-2H-pyran-4-ol (6d)—Amine **5** (50 mg, 0.16 mmol) was reacted with 3-hydroxy-5-methoxybenzaldehyde (29 mg, 0.19 mmol), glacial acetic acid (13 μL , 0.21 mmol), and $\text{Na}(\text{OAc})_3\text{BH}$ (125 mg, 0.59 mmol) in a mixture of 1,2-dichloroethane (4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6d (D-526)** (65 mg, 76%) as a colorless syrup. ^1H NMR (400 MHz, CDCl_3): δ 7.17–7.24 (m, 2 H), 7.02–7.12 (m, 2 H), 6.83–6.98 (m, 4 H), 6.42 (s, 1 H), 6.34 (s, 1 H), 6.26 (s, 1 H), 4.60 (br s, 1 H), 4.37 (t, J = 9.1 Hz, 1 H), 4.07 (s, 1 H), 3.82–3.94 (m, 2 H), 3.71–3.81 (m, 2 H), 3.67 (s, 3 H), 3.63 (d, J = 13.2 Hz, 1 H), 2.56 (s, 1 H), 1.65 (t, J = 11.5 Hz, 1 H), 1.39 (d, J = 14.9 Hz, 1 H). ^{13}C NMR (100 MHz, CDCl_3): δ 162.7, 161.1, 160.2, 157.8, 139.1, 137.3 (2C), 129.7 (4C), 115.6, 115.4 (2C), 115.1, 108.1, 106.6, 101.1, 73.9, 65.7, 63.4, 56.2, 55.2, 54.5, 50.4, 32.9. $[\alpha]_{\text{D}}^{25} = (-)46.4^\circ$, c = 1 in CH_2Cl_2 . The product was converted into the corresponding hydrochloride salt; mp: 150–155 °C. Anal. Calcd for $[\text{C}_{26}\text{H}_{27}\text{F}_2\text{NO}_4 \cdot \text{HCl} \cdot 0.5\text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-5-((benzo[d][1,3]dioxol-5-ylmethyl)amino)-2-(bis(4-fluorophenyl)-

methyl)tetrahydro-2H-pyran-4-ol (6e)—Amine **5** (40 mg, 0.13 mmol) was reacted with benzo[d][1,3]dioxole-5-carbaldehyde (23 mg, 0.15 mmol), glacial acetic acid (13 μL , 0.21 mmol), and NaCNBH_3 (14 mg, 0.22 mmol) in a mixture of 1,2-dichloroethane (4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6e (D-506)** (47 mg, 84%) as a colorless syrup.

^1H NMR (400 MHz, CDCl_3): δ 7.23–7.32 (m 2 H), 7.11–7.19 (m, 2 H), 6.90–7.02 (m, 4 H), 6.82 (s, 1 H), 6.67–6.78 (m, 2 H), 5.92 (s, 2 H), 4.29–4.46 (m, 1 H), 3.84–4.0 (m, 3 H), 3.70–3.82 (m, 2 H), 3.61 (d, J = 12.9 Hz, 1 H), 2.42 (d, J = 2.4 Hz, 1 H), 1.99 (br s, 1 H), 1.61–1.73 (m, 1 H), 1.32–1.42 (m, 1 H). ^{13}C NMR (100 MHz, CDCl_3): δ 162.7, 160.2, 147.7, 146.5, 137.7, 137.4, 133.9, 129.9, 129.8, 129.7, 129.6, 121.0, 115.5, 115.3, 115.3, 115.0, 108.5, 108.0, 100.9, 73.4, 67.2, 64.8, 56.1, 54.9, 51.0, 33.2. $[\alpha]_{\text{D}}^{25} = (-)23.9^\circ$, c = 1 in MeOH. The product was converted into the corresponding hydrochloride salt; mp: 150–155 °C. Anal. Calcd for $[\text{C}_{26}\text{H}_{25}\text{F}_2\text{NO}_4 \cdot \text{HCl} \cdot 0.3\text{H}_2\text{O}]$ C, H, N.

(2S,4R,5R)-2-(bis(4-fluorophenyl)methyl)-5-((3-methoxybenzyl) amino)

tetrahydro-2H-pyran-4-ol (6f)—Amine **5** (126 mg, 0.40 mmol) was reacted with 3-methoxybenzaldehyde (31 mg, 0.23 mmol), glacial acetic acid (16 μ L, 0.26 mmol), and NaCNBH₃ (20 mg, 0.32 mmol) in a mixture of 1,2-dichloroethane (4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6f (D-524)** (55 mg, 66%) as a colorless syrup. ¹H NMR (400 MHz, CDCl₃): δ 7.10–7.36 (m, 5 H), 6.83–7.03 (m, 6 H), 6.75–6.83 (m, 1 H), 4.33–4.45 (m, 1 H), 3.65–4.01 (m, 9 H), 2.45 (s, 1 H), 1.98 (br s, 1 H), 1.64–1.77 (m, 1 H), 1.39–1.48 (m, 1 H). ¹³C NMR (100 MHz, CDCl₃): δ 162.7, 160.2, 159.7, 141.6, 137.7, 137.4, 129.8, 129.8, 129.7, 129.6, 129.4, 120.3, 115.5, 115.3, 115.3, 115.1, 113.6, 112.3, 73.5, 67.3, 64.9, 56.3, 55.2, 54.9, 51.2, 33.2.

$[\alpha]_D^{25} = (-)77.6^\circ$, $c = 1$ in CH₂Cl₂. The product was converted into the corresponding hydrochloride salt; mp: 190–195 °C. Anal. Calcd for [C₂₆H₂₇F₂NO₃·HCl] C, H, N.

(2S,4R,5R)-2-(bis(4-fluorophenyl)methyl)-5-(((6-methoxypyridin-3-yl)methyl)

amino)tetrahydro-2H-pyran-4-ol (6g)—Amine **5** (60 mg, 0.19 mmol) was reacted with 6-methoxynicotinaldehyde (31 mg, 0.23 mmol), glacial acetic acid (13 μ L, 0.22 mmol), and NaCNBH₃ (22 mg, 0.34 mmol) in a mixture of 1,2-dichloroethane (4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6g (D-537)** (60 mg, 72%) as colorless syrup. ¹H NMR (500 MHz, CDCl₃): δ 8.03 (d, $J = 2.1$ Hz, 1 H), 7.55 (dd, $J = 2.4, 8.6$ Hz, 1 H), 7.23–7.31 (m, 2 H), 7.09–7.18 (m, 2 H), 6.88–7.02 (m, 4 H), 6.70 (d, $J = 8.2$ Hz, 1 H), 4.34–4.43 (m, 1 H), 3.85–3.99 (m, 6 H), 3.75–3.83 (m, 2 H), 3.64 (d, $J = 13.1$ Hz, 1 H), 2.42 (s, 1 H), 2.10 (br s, 1 H), 1.62–1.73 (m, 1 H), 1.35–1.45 (m, 1 H). ¹³C NMR (125 MHz, CDCl₃): δ 163.54, 162.4, 160.5, 146.1, 139.0, 137.6, 137.4, 129.9, 129.8, 129.7, 129.6, 127.9, 115.6, 115.4, 115.3, 115.1, 110.7, 73.5, 67.1, 64.7, 56.1,

55.0, 53.4, 48.0, 33.2. $[\alpha]_D^{25} = (-)85.0^\circ$, $c = 1$ in CH₂Cl₂. The product was converted into the corresponding hydrochloride salt; mp: 180–185 °C. Anal. Calcd for [C₂₅H₂₆F₂N₂O₃·2HCl·0.1C₄H₁₀O] C, H, N.

(2S,4R,5R)-2-(bis(4-fluorophenyl)methyl)-5-((3-fluoro-4-hydroxybenzyl)amino)

tetrahydro-2H-pyran-4-ol (6h)—Amine **5** (50 mg, 0.16 mmol) was reacted with 3-fluoro-4-hydroxybenzaldehyde (29 mg, 0.20 mmol), glacial acetic acid (16 μ L, 0.27 mmol), and NaCNBH₃ (17 mg, 0.27 mmol) in a mixture of 1,2-dichloroethane (4.5 mL) and methanol (1.5 mL). The residue was purified by gradient silica gel column chromatography using a mixture of dichloromethane and methanol (100:1 to 6:1) to afford corresponding compound **6h (D-531)** (50 mg, 80%) as a colorless semi solid. ¹H NMR (500 MHz, CDCl₃): δ 7.22–7.32 (m, 2 H), 7.09–7.18 (m, 2 H), 6.84–7.02 (m, 5 H), 6.80 (d, $J = 7.9$ Hz, 1 H), 6.68 (t, $J = 8.5$ Hz, 1 H), 5.0 (br s, 1 H), 4.43 (t, $J = 9.6$ Hz, 1 H), 4.11 (s, 1 H), 3.98 (d, $J = 11.3$ Hz, 1 H), 3.93 (d, $J = 8.9$ Hz, 1 H), 3.87 (d, $J = 12.2$ Hz, 1 H), 3.81 (d, $J = 12.5$ Hz, 1 H), 3.63 (d, $J = 12.5$ Hz, 1 H), 2.62 (s, 1 H), 1.62–1.80 (m, 1 H), 1.44 (d, $J = 14.3$ Hz, 1 H). ¹³C NMR (125 MHz, CDCl₃): δ 162.6, 160.5, 152.3, 150.4, 143.8, 137.4, 137.3, 129.9, 129.8, 129.7, 129.6, 124.9, 118.2, 116.1, 115.6, 115.4, 115.3, 115.2, 73.8, 65.9, 63.3, 56.2, 54.8, 49.9, 32.9. $[\alpha]_D^{25} = (-)41.0^\circ$, $c = 1$ in MeOH. The product was converted into the

corresponding hydrochloride salt; mp: 150–155 °C. Anal. Calcd for $[C_{25}H_{24}F_3NO_3 \cdot HCl \cdot 0.2H_2O \cdot 0.6C_4H_{10}O]$ C, H, N.

Functional transporter assays

The ability of test compounds to inhibit substrate uptake by monoamine transporters in synaptosome-enriched fractions from rat brain was monitored exactly as described by us previously.^{40, 41} [3H]DA ([ring 2,5,6- 3H]dopamine (45.0 Ci/mmol, Perkin-Elmer, Boston, MA, U.S.A) was used for monitoring DAT (rat striatum) and NET (rat cerebral cortex). Regarding the latter, it is worth mentioning that DA is an excellent substrate for NET (for our previous discussion see Santra et al., 2012=ref 40); the use of [3H]DA instead of [3H]norepinephrine for rat NET greatly reduced nonspecific uptake, and control experiments with a number of test compounds did not detect significant differences between K_i values obtained with [3H]dopamine and [3H]norepinephrine. [3H]5-HT ([1,2- 3H]serotonin (27.9 Ci/mmol, Perkin-Elmer) was the radioligand for monitoring SERT (rat cerebral cortex).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MDD	Major depressive disorder
SSRI	Selective Serotonin reuptake inhibitors
SNRI	Serotonin/norepinephrine reuptake inhibitors
DAT	Dopamine transporter
SERT	Serotonin transporter
NET	Norepinephrine transporters
TRI	Triple reuptake inhibitor
SAR	Structure activity relationship

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Figure 1.
Molecular structures of known antidepressants and pyran-based TRIs.

Figure 2.

An updated model of pyran derivatives developed for TRI, SSRI, and DNRI properties.

Scheme 1.

Reagents and condition: (a) RCHO, NaCNBH₃ or Na(OAc)₃BH, AcOH, 1,2-dichloroethane, rt, overnight.

Scheme 2.

Reagents and condition: (a) RCHO, NaCNBH₃ or Na(OAc)₃BH, AcOH, 1,2-dichloroethane, rt, overnight.

Scheme 3.

Reagents and condition: (a) RCHO, NaCNBH₃ or Na(OAc)₃BH, AcOH, 1,2-dichloroethane, rt, overnight.

Table 1 K_i values for inhibiting neurotransmitter uptake in rat brain.^[a]

Compound	K_i [nM]		
	DAT ^[b]	SERT ^[c]	NET ^[b]
D-161 ^[d]	42.0 ± 3.3	29.1 ± 3.5	30.5 ± 7.8
D-142 ^[e]	32.8 ± 2.8	15.4 ± 3.4	14.6 ± 3.5
D-476 ^[f]	28.8 ± 2.7	334 ± 126	13.4 ± 5.6
2a (D-484)	81.9 ± 17.9	0.71 ± 0.085	25.2 ± 5.3
4a (D-485)	234 ± 11	2.68 ± 0.094	33.6 ± 18.7
4b (D-501)	167 ± 36	223 ± 34	33.7 ± 7.9
4c (D-502)	58.6 ± 11.8	281 ± 37	30.2 ± 7.6
4d (D-503)	209 ± 25	385 ± 42	44.2 ± 9.4
4e (D-542)	259 ± 44	3,017 ± 341	152 ± 29
4f (D-523)	376 ± 68	27.2 ± 3.0	5.85 ± 1.27
4g (D-576)	44.1 ± 2.9	42.2 ± 6.4	38.1 ± 3.6
4h (D-580)	48.2 ± 6.1	9.02 ± 2.26	41.6 ± 8.9
4i (D-581)	51.8 ± 9.9	64.0 ± 14	9.33 ± 1.7
6e (D-506)	37.0 ± 7.5	400 ± 80	10.2 ± 0.6
6c (D-507)	17 ± 1.7	54.0 ± 7.8	25.8 ± 5
6a (D-508)	29.3 ± 2.4	68.4 ± 11.4	26.5 ± 6.6
6f (D-524)	56.8 ± 7.6	129 ± 25	28.8 ± 4.3
6d (D-526)	25.6 ± 5.8	577 ± 94	4.91 ± 0.63
6b (D-527)	8.94 ± 2.20	107 ± 11	4.76 ± 1.72
6h (D-531)	13.1 ± 5.0	334 ± 54	7.37 ± 2.11
6g (D-537)	20.1 ± 5.3	258 ± 26	28.4 ± 4.0
Fluoxetine ^[e]	1092 ± 98	12.2 ± 2.4	158 ± 58
Reboxetine ^[e]	>10,000	503 ± 61	0.69 ± 0.21

^[a]Values are the mean ± SEM of n= 3–8 independent experiments performed in triplicate.^[b]Uptake measured by [³H]DA accumulation.^[c]Uptake measured by [³H]5-HT accumulation.^[d]Data taken from our previous work^[42].^[e]Data taken from our previous work^[41].^[f]Data taken from our previous work^[40].

Table 2Physicochemical parameters of lead DNRIIs.^[a]

Compd	Mr [Da]	HBA ^[b]	HBD ^[c]	RB ^[d]	TPSA[Å ²] ^[e]	log <i>P</i>	Log PB ^[f]
6b	455.49	5	3	7	70.95	3.69	0.24
6h	443.46	4	3	6	61.72	4.21	−0.32
6g	440.48	5	2	7	63.61	3.83	−0.15

^[a] Physico chemical parameters were calculated with ACD/ilabs software.^[b] Number of H-bond acceptors.^[c] Number of H-bond donors.^[d] Number of rotatable bonds.^[e] Topological polar surface area.^[f] extent of brain penetration.