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Synthesis and Pharmacological Characterization of Bicyclic Triple Reuptake Inhibitor 3-Aryl Octahydrocyclopenta[*c*]pyrrole Analogues

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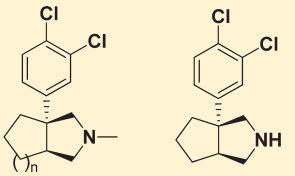
ABSTRACT: The present work expands the chemical space known to offer potent inhibition of the serotonin transporter (SERT), norepinephrine transporter (NET), and dopamine transporter (DAT) and discloses novel bicyclic octahydrocyclopenta[*c*]pyrrole and octahydro-1*H*-isoindole scaffolds as potent triple reuptake inhibitors (TRIs) for the potential treatment of depression. Optimized compounds **22a** (SERT, NET, DAT, IC_{50} = 20, 109, 430 nM), **23a** (SERT, NET, DAT, IC_{50} = 29, 85, 168 nM), and **26a** (SERT, NET, DAT, IC_{50} = 53, 150, 140 nM) were highly brain penetrant, active in vivo in the mouse tail suspension test at 10 and 30 mpk PO, and were not generally motor stimulants at doses ranging from 1 to 30 mpk PO. Moderate in vitro cytochrome P450 (CYP) and potassium ion channel Kv11.1 (hERG) inhibition were uncovered as potential liabilities for the chemical series.



Potent triple reuptake (SERT, NET, DAT) inhibitors *in vitro*
Active in tail suspension test at 10 and 30 mpk PO
Not motor stimulants at 1 to 30 mpk PO

INTRODUCTION

Antidepressants modulate levels of neurotransmitters (serotonin and norepinephrine) by either inhibiting reuptake (such as selective serotonin reuptake inhibitors, SSRIs; norepinephrine reuptake inhibitors, NRIs) or blocking metabolism pathways (monoamine oxidase inhibitors, MAOIs).[1] The major drawbacks to both reuptake inhibitors and MAOIs are slow onset[2] and low patient response rate.

Recent study results showed the linkage between the deficits in mesocorticolimbic dopaminergic function and anhedonia, which is a core symptom of depression.[3] Clinically, dopamine reuptake inhibitors (e.g., buproprion) and dopamine agonists (e.g., pramipexole) are already used to augment the effects of traditional antidepressants in treatment of refractory patients and also show improved side effect profiles.[4] As a result, there is considerable interest in designing and developing a triple reuptake inhibitor (TRI), i.e. a single compound that would simultaneously modulate levels of serotonin, norepinephrine, and dopamine levels for the treatment of major depression disorder (MDD).[5]

DOV Pharmaceuticals previously reported[6] on a novel aryl azabicyclo[3.1.0]hexane scaffold that potently inhibited the three monoamine transporters and showed antidepressant-like activity in the rat forced swim and mouse tail suspension tests. In addition, Breuer et al. (2008) later showed that DOV-216,303 lacked sexual side effects in rat.[7]

We previously reported on our work developing TRIs in the tetralone[8] and amino tetralone[9] families; our present work expands the chemical space known to offer potent triple reuptake inhibition. Starting from the DOV family of compounds

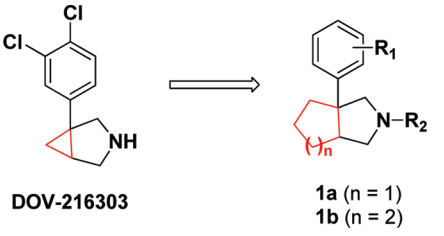


Figure 1. Novel DOV-like triple reuptake inhibitors: octahydrocyclopenta[*c*]pyrroles (**1a**) and octahydro-1*H*-isoindoles (**1b**).

(vida supra), we expanded the cyclopropyl ring of DOV-216303 to create two novel fused octahydrocyclopenta[*c*]pyrrole and octahydro-1*H*-isoindole scaffolds (Figure 1). Our goal was to probe the SAR of these novel chiral amines as potential triple reuptake inhibitors and create novel architectures that possessed varying degrees of reuptake inhibition at SERT, NET, and DAT. The synthesis, detailed SAR of the scaffolds, and their inhibition against SERT, NET, and DAT are detailed below.

RESULTS AND DISCUSSION

Chemistry. Synthetic access to the novel bicyclic aryl amines **1a** and **1b** was achieved by four different synthetic routes, which allowed for access to an diverse array of compounds of type **1a** and **1b** with a variety of amine substituents and aryl modifications

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on the bridging carbon of the fused ring systems. The developed synthetic pathways further allowed access to different chiral analogues, which were separated by chiral chromatography. The first route to our novel bicyclic aryl amines utilized Diels–Alder chemistry to construct the six-membered ring (Scheme 1). Condensation of 3-arylfuran-2,5-diones **2** with 1,3-butadiene gave the desired *cis* 6–5 carbon skeleton. Formation of the imide, hydrogenation, and reduction provided the *cis*-octahydro-1*H*-isoindoles **4**.

We also developed an alternate route to the bicyclic amines that enabled us to synthesize both octahydro-1*H*-isoindoles and octahydrocyclopenta[*c*]pyrroles, using lactone arylation chemistry we developed specifically for the scaffold (Scheme 2).[10] Palladium catalyzed arylation of lactone **5**/**6** with commercially available aryl bromides gave excellent yields of the desired bicylic aryl lactones, which could be opened by amine nucleophiles resulting in two alternative pathways: lithium anions of alkyl amines opened the lactone at the carbonyl to give amido-alcohols **9**/**10**, while potassium phthalimide opened the lactone at carbon to give phthalimido-acids **11**/**12**. Either precursor could be elaborated to the desired racemic *cis* bicyclic aryl amines **13**/**4** in two steps.

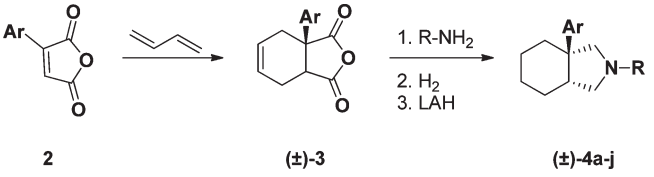
Synthesis of the corresponding *trans*-octahydro-1*H*-isoindoles required a Diels–Alder route that is shown in Scheme 3. Horner–Wadsworth–Emmons reaction of ester **14** with diethyl cyanomethylphosphonate in toluene provided a 3:1 *cis*:*trans* mixture of alkene **15**, which could be equilibrated to 100% *trans* after hydrolysis of the ester with aqueous base. Acid chloride **16** was the preferred dienophile for the Diels–Alder reaction with 1,3-butadiene. The product acid chloride nitrile was quenched with methanol to provide an intermediate methyl ester. Both the methyl ester and nitrile were reduced with LAH to provide amino alcohol **17**. Protection of the primary amine, hydrogenation, and mesylation provided penultimate compound **18**, which could be cyclized with NaOH after deprotection of the BOC group to provide racemic *trans* compound **19**.

Preparation of chirally pure *cis* and *trans* bicyclic aryl amines could be accomplished via chiral HPLC of the parent amines, derivatization (i.e., Boc_2O), and chiral separation, or separation of the amido-alcohol precursors **9**/**10** (Scheme 4). The route used depended on the bicyclic scaffold and substitution of the aryl portion and was determined experimentally. The absolute configuration was not determined for the single enantiomers but was assigned arbitrarily, as shown in the schemes.

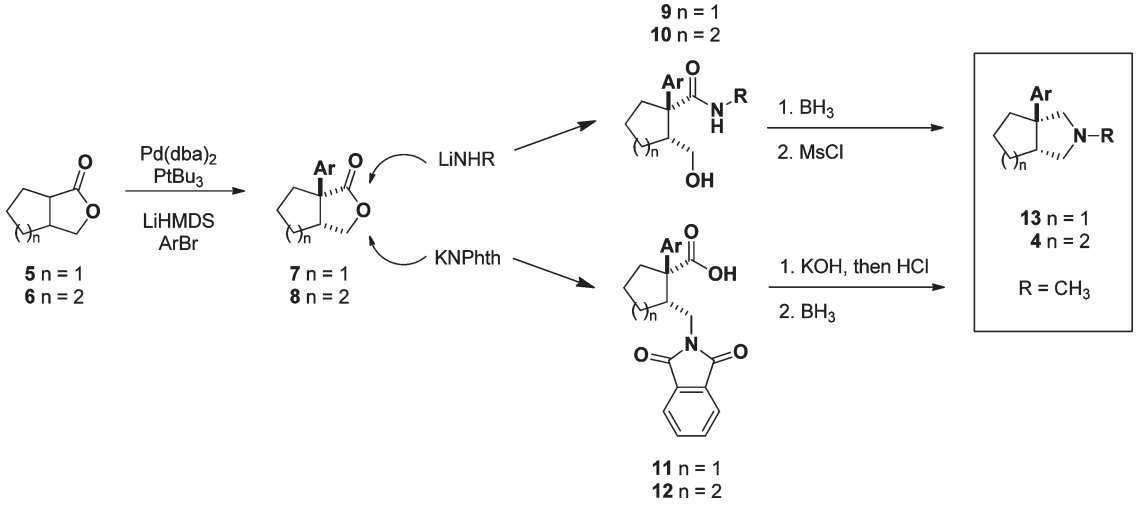
The last group of analogues we synthesized was the benzyl substituted amines shown in Scheme 5. Alkylation of imide **28** with 3,4-dichlorobenzyl bromide and reduction gave the desired racemic *N*-methyl amines, which could be resolved into constitutive isomers **29** and **30** via chiral HPLC. Demethylation (chloroethylchloroformate) converted the tertiary amines into secondary amines **31** and **32**.

Inhibition of Serotonin, Norepinephrine, and Dopamine Reuptake, SAR. The novel compounds described above were tested for their inhibition of functional uptake of serotonin, norepinephrine, or dopamine, using recombinant transporters expressed in HEK-293, MDCK, or CHO-K1 cells (Table 1, see Experimental Section for details). The first parameter we systematically varied was the aromatic substituent in the *N*-methyl octahydro-1*H*-isoindoles (Table 1). We used the racemic compounds to get an initial sense of the potential for triple reuptake inhibition before investing additional resources in chiral separation of the individual enantiomers. As Table 1 shows, the serotonin transporter showed a distinct preference for phenyl substituents at the 3 and 4-position, with 3,4-di-OCH_3 **4f**, 3-OCH_3 **4d**, and 4-OCH_3 phenyl **4e**, in addition to 2-naphthyl **4h**, all showing reasonably potent inhibition of serotonin reuptake. Potency against the NE and DA transporter was more difficult to achieve: the 2-naphthyl compound **4h** showed potent inhibition of both NE and DA, and the 1-naphthyl (**4g**) and 2,4-di-Cl (**4b**) phenyl compounds showed potency at NET and some potency at DAT. Given the literature data for the DOV compounds, we knew that a 3,4-dichlorophenyl aromatic would be a preferred substituent, so we carried out chiral separations of 3,4-dichlorophenyl (**4i**), 1-naphthyl (**4g**), and 2-napthyl (**4h**)

Scheme 1. Diels–Alder Route to Racemic *cis*-Octahydro-1*H*-isoindoles with Diverse Substitution Patterns ((±)-4a–j)



Scheme 2. Lactone Arylation Pathway to *cis*-Octahydro-1*H*-isoindole 4 or Octahydrocyclopenta[*c*]-pyrrole 13 Scaffolds



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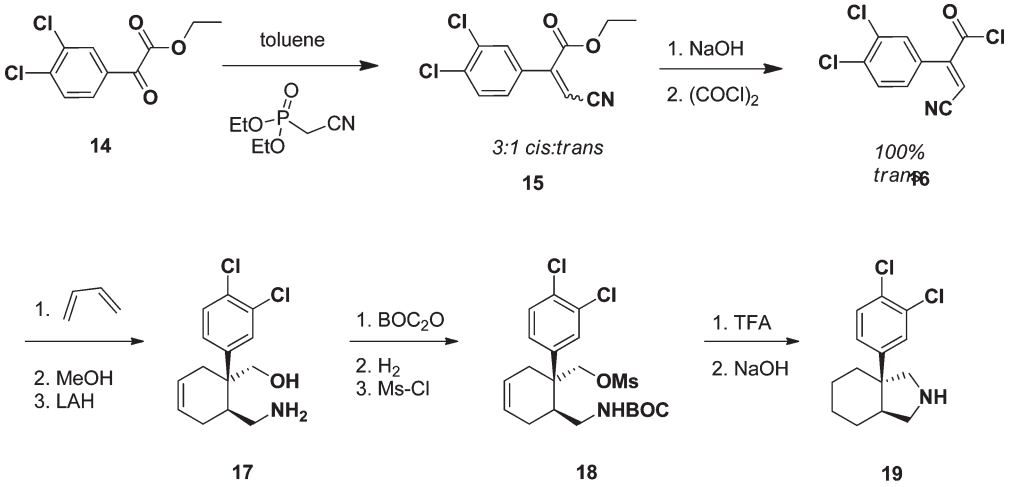
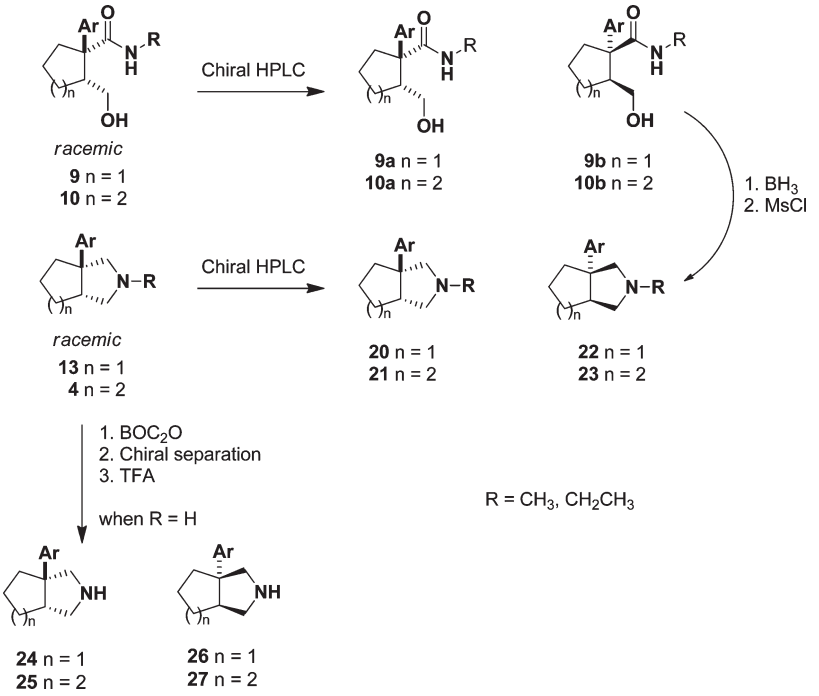
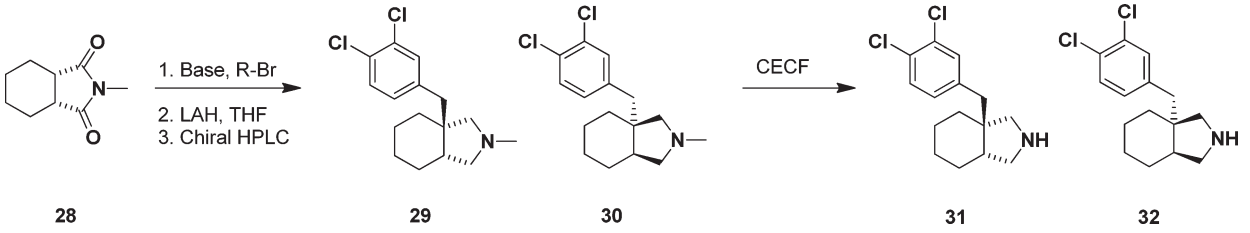
Scheme 3. Synthesis of Racemic *trans*-Octahydro-1*H*-isoindoles 19



Scheme 4. Chiral Separation Schematic to Prepare Single Enantiomers 20−27



Scheme 5. Synthesis of Benzyl Compounds 29−32



compounds and tested their constitutive isomers against SERT, NET, and DAT. 3,4-Dichlorophenyl enantiomer **23a** showed excellent inhibition against all three transporters and prompted us to make and separate the *N*-ethyl analogues (**21b**, **23b**), which

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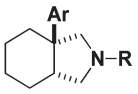
Table 1. *N*-Alkyl Octahydro-1*H*-isoindole Aryl Variation[a]

			AlogP	$pIC_{50} \pm SD$ (LLE) [b]		
Compound	**R**	**Ar**		**SERT**	**NET**	**DAT**
4a	CH_3	Ph	3.27	6.14 (2.87)	6.25 (2.98)	5.80 (2.53)
4b	CH_3	2,4-di-Cl Ph	4.59	6.46±0.01 (1.87)	6.04±0.15 (1.45)	5.89±0.043 (1.3)
4c	CH_3	3,4-di-F Ph	3.68	5.91±0.01 (2.23)	5.63±0.26 (1.95)	6.38±0.01 (2.7)
4d	CH_3	3-OCH_3 Ph	3.25	6.83±0.07 (3.58)	5.61±0.18 (2.36)	5.47±0.01 (2.22)
4e	CH_3	4-OCH_3 Ph	3.25	6.89±0.15 (3.64)	4.85±0.15 (1.6)	6±0.06 (2.75)
4f	CH_3	3,4-di-OCH_3 Ph	3.23	7.46±0.04 (4.23)	5.23±0.03 (2)	5.01±0.001 (1.78)
4g	CH_3	1-Naphthyl	4.17	6.79±0.56 (2.62)	6.38±0.26 (2.21)	5.88±0.11 (1.71)
4h	CH_3	2-Naphthyl	4.17	8.32±0.16 (4.15)	6.27±0.14 (2.1)	7.69±0.046 (3.52)
4i	CH_3	3,4-di-Cl Ph	4.59	n.t.	n.t.	n.t.
23a	CH_3	3,4-di-Cl Ph	4.59	8.01±0.67 (3.42)	7.25±0.42 (2.66)	7.26±0.63 (2.67)
21a	CH_3	3,4-di-Cl Ph	4.59	6.97±0.72 (2.38)	6.85±0.33 (2.26)	7.85±0.19 (3.26)
23b	CH_2CH_3	3,4-di-Cl Ph	4.94	7.34±0.24 (2.4)	7.57±0.43 (2.63)	7.60±0.11 (2.66)
21b	CH_2CH_3	3,4-di-Cl Ph	4.94	7.16±0.36 (2.22)	7.42±0.62 (2.48)	8.00±0.19 (3.06)
21c	CH_3	1-Naphthyl	4.17	6.01±0.36 (1.84)	<5	5.48±0.10 (1.31)
23c	CH_3	1-Naphthyl	4.17	7.48±0.20 (3.310	6.69±0.17 (2.52)	5.61±0.14 (1.44)
21d	CH_3	2-Naphthyl	4.17	8.16±0.20 (3.99)	6.14±0.02 (1.97)	6.51±0.13 (2.34)
23d	CH_3	2-Naphthyl	4.17	7.90±0.36 (3.73)	6.27±0.28 (2.1)	7.21±0.083 (3.04)

[a] SERT, NET, and DAT reuptake inhibition assays were done with at least $n = 2$ and five concentrations to generate inhibition curves, from which pIC_{50} values were determined. The indicated standard deviation (SD) values were determined from replicate independent concentration-dependence studies. Assay performance was monitored by the use of SERT reference compound fluoxetine (pIC_{50} = 8.3 (±0.1)), NET reference compound nisoxetine (pIC_{50} = 8.1 (±0.1)), and DAT reference compound nomifensine (pIC_{50} = 7.5 (±0.1)). An IC_{50} value can be calculated from pIC_{50} using the following formula: IC_{50} (nM) = $10^{(9-pIC50)}$. [b] LLE (lipophilic ligand efficiency) = $pIC_{50} - \log P$.

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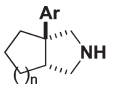
Table 2. Octahydrocyclopenta[*c*]pyrroles and Octahydro-1*H*-isoindoles[a]

			AlogP	$pIC_{50} \pm SD$ (LLE)[b]		
Compound	**n**	**Ar**		**SERT**	**NET**	**DAT**
4j	2	3,4-di-Cl Ph	4.06	6.94 (2.88)	6.95 (2.89)	6.69 (2.63)
27a	2	3,4-di-Cl Ph	4.06	7.04±0.23 (2.98)	6.69±0.01 (2.63)	7.08±0.13 (3.02)
25a	2	3,4-di-Cl Ph	4.06	7.01±0.22 (2.95)	6.75±0.01 (2.69)	6.34±0.37 (2.28)
19a	2	3,4-di-Cl Ph	4.06	5.74±0.26 (1.68)	5.22±0.09 (1.16)	5.99±0.03 (1.93)
19b	2	3,4-di-Cl Ph	4.06	5.72±0.34 (1.66)	5.67±0.37 (1.61)	6.34±0.11 (2.28)
24a	1	3,4-di-Cl Ph	3.6	6.30±0.04 (2.7)	6.68±0.38 (3.08)	6.85±0.16 (3.45)
26a	1	3,4-di-Cl Ph	3.6	7.31±0.2 (3.71)	6.97±0.43 (3.37)	6.91±0.22 (3.31)
24b	1	2-Naphthyl	4.05	7.87±0.2 (3.82)	7.85±0.04 (3.8)	7.47±0.07 (3.42)
26b	1	2-Naphthyl	4.06	6.92±0.14 (2.86)	7.22±0.09 (3.16)	7.52±0.04 (3.46)

[a] SERT, NET, and DAT reuptake inhibition assays were done with at least $n = 2$ and five concentrations to generate inhibition curves, from which pIC_{50} values were determined. The indicated standard deviation (SD) values were determined from replicate independent concentration-dependence studies. Assay performance was monitored by the use of SERT reference compound fluoxetine ($pIC_{50} = 8.3$ (± 0.1)), NET reference compound nisoxetine ($pIC_{50} = 8.1$ (± 0.1)), and DAT reference compound nomifensine ($pIC_{50} = 7.5$ (± 0.1)). An IC_{50} value can be calculated from pIC_{50} using the following formula: IC_{50} (nM) = $10^{(9-pIC50))}$. [b] LLE (lipophilic ligand efficiency = $pIC_{50} - \log P$.

showed potent inhibition against all three transporters as well. The 2-naphthyl enantiomers **21d** and **23d** showed nice profiles, although the potency at NET was a bit weaker than in the 3,4-dichlorophenyl series, and the 1-naphthyl enantiomers **21c** and **23c** showed a preference for the serotonin transporter over the dopamine and norepinephrine transporters. For the first series of compounds, **23a** showed the most potential for triple reuptake inhibition and was also close to the SERT/NET/DAT potency ratio we were seeking.

Table 2 shows our survey of octahydrocyclopenta[*c*]pyrroles and octahydro-1*H*-isoindoles with aromatic 3,4-dichlorophenyl and 2-naphthyl substitution, which showed excellent reuptake inhibition at all three transporters. Racemic 3,4-dichlorophenyl octahydro-1*H*-isoindole **4j** showed potent inhibition at all three transporters; resolution into constitutive isomers **27a** and **25a** showed that enantiomer **27a** was the standout, with potency against SERT and DAT below 100 nM and NET inhibition around 200 nM. We also examined if the *trans*-octahydro-1*H*-isoindole compounds showed any potential: neither **19a** nor enantiomer **19b** showed potent inhibition and clearly indicated that a *cis* ring fusion was necessary to potently inhibit the three monoamine transporters. In the octahydrocyclopenta[*c*]pyrrole series, we did not profile the racemic 3,4-dichlorophenyl and 2-naphthyl compounds but went straight to resolution into constitutive isomers: the excellent profiles we saw for all four compounds validated our reasoning. In particular, 3,4-dichlorophenyl amine **26a** and 2-naphthyl enantiomer **24b** showed potent reuptake inhibition at all three transporters and became candidates for additional in vitro ADMET characterization.

In the *N*-methyl octahydrocyclopenta[*c*]pyrrole aryl variation, the 3,4-dichlorophenyl **13a** and 2-naphthyl **13f** racemic compounds were standouts from a TRI perspective and led us to separate the racemic compounds into constitutive isomers. All four enantiomers (**20a**, **22a**, **20b**, and **22b**) showed potent inhibition at the three transporters, with a nice variety of ratios among the four compounds. 2-Naphthyl **20b** was among the most potent SERT inhibitors we profiled ($pIC_{50} = 8.637$, $IC_{50} =$ 2 nM) and maintained the general trends of superior potency for tertiary amines over second amines at the serotonin transporter and also of 2-naphthyl > 3,4-dichlorophenyl in absolute transporter potency: the most potent compounds we made were invariably 2-naphthyl substituted.

One other group of compounds we chose to profile were the enantiopure 3,4-dichlorobenzyl octahydro-1*H*-isoindoles (Table 4), which showed potent inhibition against SERT and DAT and dramatically reduced potency against NET compared with the 3,4-dichlorophenyl counterparts. The tertiary amines showed increased potency against SERT compared with the

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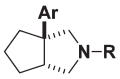
Table 3. *N*-Methyl Octahydrocyclopenta[*c*]pyrroles SAR[a]

			AlogP	$pIC_{50} \pm SD$ (LLE)[#]		
Compound	R	Ar		SERT	NET	DAT
13a	CH_3	3,4-di-Cl Ph	4.14	8.05 (3.91)	7.62 (3.48)	7.17 (3.03)
13b	CH_3	3,4-di-F Ph	3.22	5.96±0.03 (2.74)	5.19±0.10 (1.97)	5.55±0.23 (2.33)
13c	CH_3	4-OCH_3 Ph	4.93	8.35±0.1 (3.42)	<5	<5
13d	CH_3	3-OCH_3 Ph	4.93	6.16±0.16 (1.23)	<5	<5
13e	CH_3	3,5-di-Cl Ph	4.14	6.48±0.13 (2.34)	5.37 (1.23)	5.01±0.01 (0.87)
13f	CH_3	2-Naphthyl	3.72	8.45±0.31 (4.73)	6.33±0.2 (2.61)	7.0±0.02 (3.34)
20a	CH_3	3,4-di-Cl Ph	4.14	7.08±0.32 (2.94)	7.02±0.31 (2.88)	7.32±0.26 (3.18)
22a	CH_3	3,4-di-Cl Ph	4.14	8.44±0.50 (4.3)	7.65±0.28 (3.51)	6.83±0.25 (2.69)
20b	CH_3	2-Naphthyl	3.72	8.64±0.06 (4.92)	6.68±0.15 (2.96)	6.74±0.52 (3.02)
22b	CH_3	2-Naphthyl	3.72	7.84±0.34 (4.12)	6.72±0.09 (3)	7.54±0.05 (3.82)

[a] SERT, NET, and DAT reuptake inhibition assays were done with at least $n = 2$ and five concentrations to generate inhibition curves, from which pIC_{50} values were determined. The indicated standard deviation (SD) values were determined from replicate independent concentration-dependence studies. Assay performance was monitored by the use of SERT reference compound fluoxetine ($pIC_{50} = 8.3$ (± 0.1)), NET reference compound nisoxetine ($pIC_{50} = 8.1$ (± 0.1)), and DAT reference compound nomifensine ($pIC_{50} = 7.5$ (± 0.1)). An IC_{50} value can be calculated from pIC_{50} using the following formula: IC_{50} (nM) = $10^{(9-pIC50)}$. [b] LLE (lipophilic ligand efficiency = pIC_{50} − log *P*.

secondary amines in the benzyl series, keeping with the trends we observed with the phenyl analogues shown above.

Ligand Efficiency. Ligand efficiency[11] (LE) considerations during the lead optimization process are a current topic of great interest to medicinal chemists and were also factored into our thinking in the evaluation of our novel TRIs. LE refers to the potency of a compound normalized for its molecular weight (MW) and is used to try to avoid unnecessary increases in MW as a lever to increase potency. LE can be defined a number of ways, but in general is pK_i, pK_d, or pIC_{50}/no. of heavy atoms. Another useful metric that can be easily calculated is the lipophilic ligand efficiency[12], which is defined as LLE = pK_i or pIC_{50} − ClogP or ($ClogP_{7.4}$). LLE normalizes potency for lipophicity and attempts to tease our potency that is due to specific interactions between a ligand and a protein and potency that is simply due to entropy driven hydrophobic effects (burying lipophicity in a cavity of the protein to avoid water). Increases in MW and lipophilicity will increase unwanted off-target interactions and lead to, among other things, poor solubility, permeability, and oral bioavailability.[13] A review from 2010 also showed a nice correlation between LE for leads and approved drugs and showed that most approved drugs were not that different from their respective lead when the lead had a LE in the "ideal" range (i.e., 0.3).[14] For all the reasons stated above, we chose to also consider the LLE of our novel compounds when evaluating their potency (Table 1−4) and comment on it briefly below. Our LLE considerations were complicated slightly by the fact that we were trying to optimize potency at three different transporters simultaneously; in general, we saw the best LLE at SERT (range 3−4), while LLE at NET and DAT were closer to 2−3 for the majority of our compounds. In the octahydro-1*H*-isoindole series, dichlorophenyl *N*-methyl analogue **23a** showed a nice LLE of 3.42 at SERT, while 2-naphthyl *N*-CH_3 analogues **21d** and **23d** were at SERT LLEs of 3.99 and 3.73, respectively. Racemic octahydro-1*H*-isoindole methoxy phenyl substituted compounds **4e**, **4d**, and **4f** were also standouts in SERT LLE, with values of 3.58, 3.64, and 4.23 but no appreciable potency/LLE at NET and DAT. In the octahydrocyclopenta[*c*]pyrrole series, the LLE values were generally higher due to the lower log *P*s of the compounds compared to their octahydro-1*H*-isoindole counterparts and also maintained the general trend of better LLEs at SERT vs NET and DAT. Some standouts in the dichlorophenyl octahydro-1*H*-isoindole series were **22a**, with LLE at SERT of 4.3 and 3.51 at NET and **26a**, with LLE at SERT of 3.71, 3.37 at

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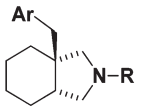
Table 4. Benzyl Substituted Bicyclic Amines[a]

			AlogP	$pIC_{50} \pm SD$ (LLE)[#]		
Compound	R	Ar		SERT	NET	DAT
29	CH_3	3,4-di-Cl Ph	5.05	6.47±0.26 (1.42)	5.35±0.22 (0.3)	6.31±0.1 (1.26)
30	CH_3	3,4-di-Cl Ph	5.05	8.26±0.45 (3.21)	5.42±0.27 (0.37)	6.32±0.14 (1.27)
31	H	3,4-di-Cl Ph	4.51	6.18±0.16 (1.67)	5.27±0.14 (0.76)	6.3±0.17 (1.79)
32	H	3,4-di-Cl Ph	4.51	7.31±0.51 (2.8)	5.53±0.17 (1.02)	6.80±0.29 (2.29)

[a] SERT, NET, and DAT reuptake inhibition assays were done with at least $n = 2$ and five concentrations to generate inhibition curves, from which pIC_{50} values were determined. The indicated standard deviation (SD) values were determined from replicate independent concentration-dependence studies. Assay performance was monitored by the use of SERT reference compound fluoxetine ($pIC_{50} = 8.3$ (±0.1)), NET reference compound nisoxetine ($pIC_{50} = 8.1$ (±0.1)), and DAT reference compound nomifensine ($pIC_{50} = 7.5$ (±0.1)). An IC_{50} value can be calculated from pIC_{50} using the following formula: IC_{50} (nM) = $10^{(9-pIC50)}$. [b] LLE (lipophilic ligand efficiency = $pIC_{50} - \log P$.

NET, and 3.31 at DAT. One surprise in the SAR was the excellent LLE for 2-naphthyl octahydrocyclopenta[*c*]pyrrole **20b** (*N*-CH3), with SERT LLE of 4.92 and for 2-naphthyl **24b** (*N*-H), with SERT LLE of 3.82, NET LLE of 3.8, and DAT LLE of 3.42.

In Vitro ADME: Microsomal Stability, CYP, and hERG Inhibition. Table 5 details the in vitro microsomal stability, CYP, and hERG inhibition data for a selection of our potent TRIs. In the 3,4-dichlorophenyl series, microsomal stability was best for the secondary amines and worse, albeit respectable, for both **23a** (*N*-CH_3) and **23b** (*N*-Et) compounds. Although *N*-ethyl compound **23b** showed potent CYP2D6 inhibition and **24a** showed potent CYP1A1 inhibition, the 3,4-dichlorophenyl compounds in general in both octahydrocyclopenta[*c*]pyrrole and octahydro-1*H*-isoindole scaffolds showed minimal CYP inhibition profiles. hERG inhibition was present at low potency for several of the dichlorophenyl compounds, but none showed inhibition below 1 μM. There was no clear distinction in stability, CYP inhibition, or hERG inhibition for either the octahydrocyclopenta[*c*]pyrrole and octahydro-1*H*-isoindoles dichlorophenyl compounds secondary amines (compare **26a** to **27a**): both compounds showed excellent stability and moderate CYP and hERG inhibition profiles. The standout in the dichlorophenyl series from an ADME perspective was secondary amine **26a**; for tertiary amines both **23a** and **22a** showed good profiles, albeit with decreased stability compared to secondary amine **26a**. The 2-naphthyl compounds **21d** and **23d** showed significantly decreased microsomal stability compared to the 3,4-dichlorophenyl counterparts for both *N*-H and *N*-Me and were not profiled extensively for that reason. Finally, the 3,4-dichlorobenzyl compounds **30** (*N*-CH_3) and **32** (*N*-H) showed excellent stability in both human and mouse liver microsomes but also significant (IC_{50} < 50 nM) inhibition of CYP2D6.

In Vivo Pharmacology. On the basis of ADMET, efficiency, and potency considerations, dichlorophenyl compounds **23a** (octahydro-1*H*-isoindoles, *N*-Me), **22a** (octahydrocyclopenta[*c*]pyrrole, *N*-Me), and **26a** (octahydrocyclopenta[*c*]pyrrole, *N*-H) were selected for profiling in the mouse tail suspension test (TST).[15] The assay is not a model of depression but is sensitive to the effects of several classes of antidepressants, including tricyclics, SSRIs, and SNRIs. The HCl salt of **26a** was prepared and dosed 3, 10, and 30 mg/kg PO in male mice in the TST. A 60 min pretreatment time was used based on exposure work prior to the assay, which showed maximal brain levels 60 min after PO dosing. **26a** dose dependently reduced in immobility, which was statistically significant compared to vehicle at 10 and 30 mg/kg doses (Figure 2a). The positive control, desipramine, also showed a statistically significant reduction in immobility at 100 mg/kg PO dosing. Terminal brain and plasma samples taken from the test animals showed that the brain to plasma (B/P) ratio was close to 30 at the 30 mg/kg dose, and approximately 50 μM of **26a** was present in the brain (Table 6). Given the high degree of protein binding measured for compound **26a** (96% protein binding using mouse plasma protein), there was an estimated 2 μM of free compound **26a** in brain, which exceeded the IC_{50}s at all three transporters. The effects of compound **26a** (Figure 2b) in the TST was not due to a general locomotor activation effect; the compounds did not significantly increase spontaneous locomotor activity in vivo in the first 5 min at the 10 or 30 mg/kg dose, as the 5 min time point was significant because that is the amount of time the compounds were evaluated in the TST.

3,4-Dichlorophenyl *N*-methyl amines **23a** (Figure 3a) and **22a** (Figure 4a) also showed dose-dependent reductions in immobility, which were statistically significant compared to vehicle at 10 and 30 mg/kg doses. In both experiments, the positive control, desipramine, also showed a statistically significant reduction in immobility at 100 mg/kg PO. The effects of **23a** and **22a** (Figure 3b and 4b) in the TST were also not due to a general locomotor activation effect; the compounds did not

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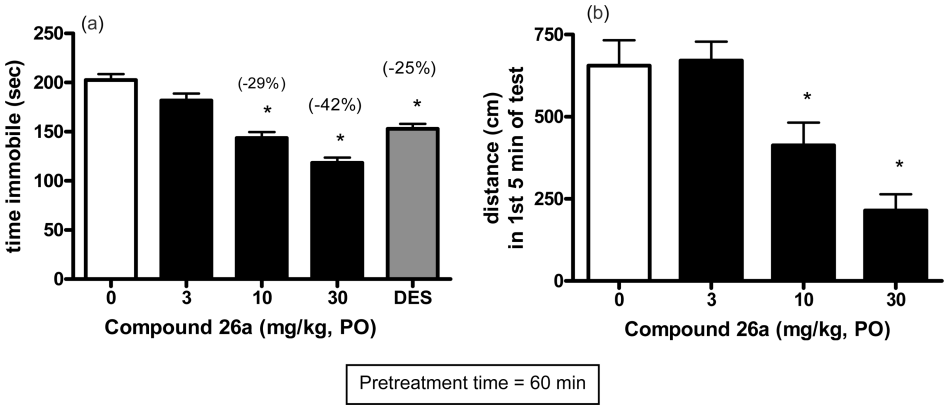


Figure 2. (a) Compound **26a** in the mouse TST at 3, 10, and 30 mg/kg PO. (b) Compound **26a** in mouse locomotor activity assay at 5 min time point, at 3, 10, and 30 mg/kg PO. * $p < 0.05$, one-way ANOVA, Dunnett's post test.

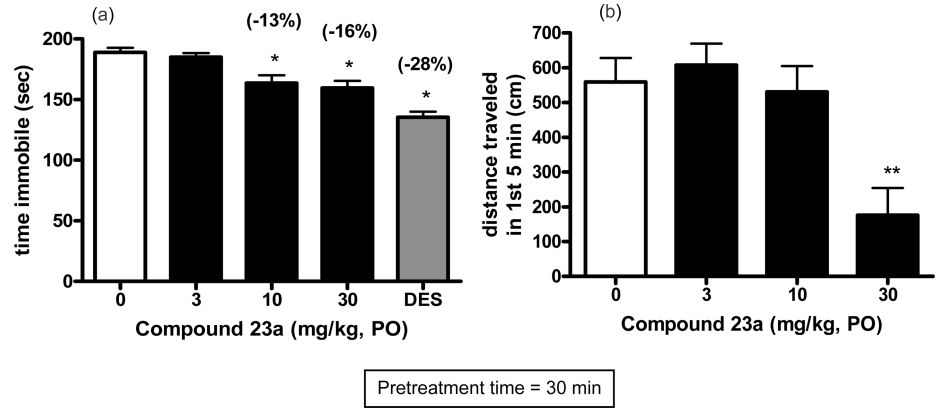


Figure 3. (a) Compound **23a** in the mouse TST at 3, 10, and 30 mg/kg PO. (b) Compound **23a** in mouse locomotor activity assay at 5 min time point, at 3, 10, and 30 mg/kg PO. * $p < 0.01$, one-way ANOVA, Dunnett's post test.

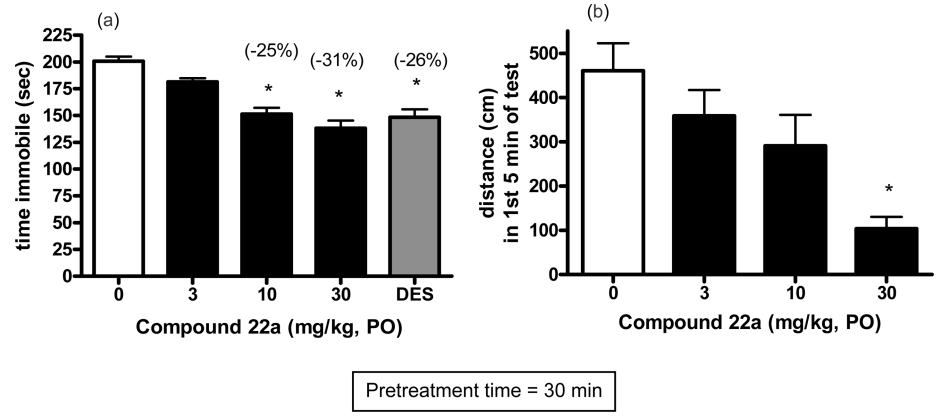


Figure 4. (a) Compound **22a** in the mouse TST at 3, 10, and 30 mg/kg PO. (b) Compound **22a** in mouse locomotor activity assay at 5 min time point, at 3, 10, and 30 mg/kg PO. * $p < 0.05$, one-way ANOVA, Dunnett's post test.

significantly increase spontaneous locomotor activity in vivo in the first 5 min at the 30 mg/kg dose. Terminal whole brain levels from test animals (Table 6) for **23a** showed significant amounts of both **23a** and its metabolite **27a** and a B/P ratio close to 5. Terminal brain levels from test animals for **22a** showed a significant amount of metabolite **26a** and a B/P ratio close to 20.

CONCLUSIONS

The present work expanded the chemical space known to offer potent inhibition of the serotonin, norepinephrine, and dopamine transporters and disclosed novel octahydrocyclopenta-[*c*]pyrrole and octahydro-1*H*-isoindole scaffolds as TRIs for the treatment of depression. We showed the synthesis and in vitro characterization of a wide range of aromatic amines in this

Table 5. In Vitro Microsomal Stability, CYP, and hERG Inhibition of Selected Bicyclic Amines[a]

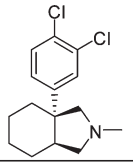
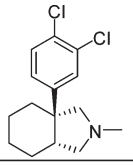
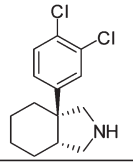
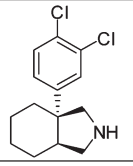
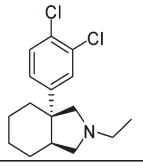
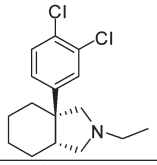
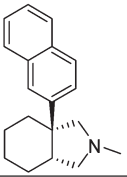
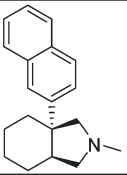
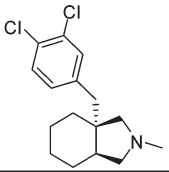
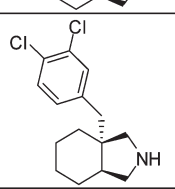
Structure	Compound	Microsomal Stability $t_{1/2}$ (min)		CYP450 Inhibition IC_{50} (μM)					hERG IC_{50} (μM)
		Human	Mouse	1A	2C9	2C19	2D6	3A4	
	**23a**	125	45	>25	>25	11.9	1.1	6.5	4.14
	**21a**	62	35	3	>25	>25	8	5.28	2.32
	**25a**	>300	>300	7.96	>25	>25	2.65	8.39	6.48
	**27a**	>300	>300	6.14	>25	>25	7.93	12.8	3.64
	**23b**	68	52	>25	>5	17.9	0.25	>5	4.73
	**21b**	55	33	4.23	>5	>25	2.92	>5	4.33
	**21d**	46	18	NT	NT	NT	0.56	NT	NT
	**23d**	17	7	NT	NT	NT	13.6	NT	NT
	**30**	121	25	NT	NT	NT	<0.05	NT	NT
	*32*	*>300*	*>300*	*NT*	*NT*	*NT*	*<0.05*	*NT*	NT

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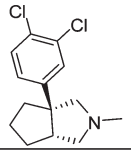
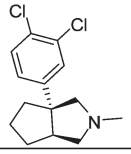
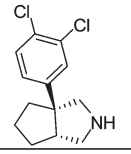
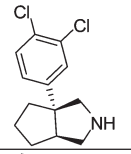
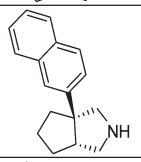
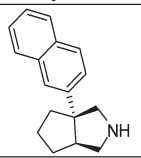
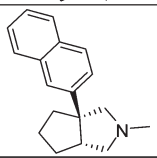
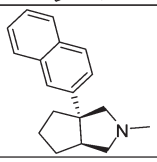
Table 5. Continued

Structure	Compound	Microsomal Stability $t_{1/2}$ (min)		CYP450 Inhibition IC_{50} (μM)					hERG IC_{50} (μM)
		Human	Mouse	1A	2C9	2C19	2D6	3A4	
	**20a**	28	27	1.49	9.83	2.22	4.35	12.7	5.64
	**22a**	37	27	>25	>25	7.22	5.21	>25	5.43
	**24a**	22	56	0.53	4.01	1.9	2.47	9.17	6.4
	**26a**	262	91	18.7	>25	11.9	6.96	>5	5.28
	**24b**	25	26	4.13	>25	>25	9.42	2.65	NT
	**26b**	28	56	0.71	14.4	7.19	10.8	10.7	NT
	**20b**	36	18	NT	NT	NT	2.61	NT	NT
	**22b**	19	18	NT	NT	NT	12.8	NT	NT

[a] NT = not tested.

new scaffold and probed the SAR around the serotonin, norepinephrine, and dopamine transporters. 3,4-Dichlorophenyl and 2-naphthyl emerged as preferred aromatic substituents for developing potency at the three transporters, and 3,4-dichlorophenyl showed greater potential from an ADME perspective. In vivo profiling of three 3,4-dichlorophenyl amines in the TST confirmed our hypothesis and showed that this scaffold holds potential for the development of novel antidepressants. Future publications will disclose our efforts to expand upon the favorable profile of 3a-(3,4-dichlorophenyl)octahydrocyclopenta[*c*]pyrrole, a new TRI for the potential treatment of major depressive disorder.

EXPERIMENTAL SECTION

Chemistry. *General Statement.* Reagents were commercial grade and were used as received unless otherwise noted. Reactions were run with anhydrous solvents under an atmosphere of nitrogen unless otherwise noted. Flash column chromatography was done using an

Table 6. Whole Brain and Plasma Levels of Compounds 23a, 26a, and 22a from TST Animals

compd	dose (mg/kg), PO	plasma levels (ng/mL)	brain levels (ng/g)
23a	3	14	294
(**27a** metabolite)		(114)	(440)
	10	50	1054
		(417)	(1878)
	30	181	4203
		(1228)	(6193)
26a	3	69	1747
	10	214	5950
	30	569	14050
22a	3	2	34
(**26a** metabolite)		(69)	(1531)
	10	7	113
		(211)	(4762)
	30	30	507
		(537)	(11433)

ISCO purification system on silica gel columns. The structures of all new compounds were consistent with their ^{1}H, ^{13}C NMR, and mass spectra and were judged to be >95% pure by LCMS. Chiral chromatography was done using the designated Chiral Technologies Chiracel 20 μm analytical (0.46 cm ID × 25 cm L) column, a flow rate of 1 mL/min with the designated solvent, with detection by UV at 220 and 254 nM. LCMS was performed on an Agilent 1100 series system connected to a Micromass Platform LC. GC-MS was performed on a Hewlett-Packard 6890 series GC system with an HP1 column (30 m, 0.15 μ film thickness) coupled to a Hewlett-Packard 5973 series mass selective detector. Accurate mass measurements were carried out on a Waters Q-TOF micro system.

General procedures are described below for synthesis of the novel bicyclic compounds with Ar = 3,4-dichlorophenyl. For Ar = Ph (**4a**, Table 1), 2,4-di-Cl-Ph (**4b**, Table 1), 3,4-di-F-Ph (**4c**, Table 1), 3-OCH_3 (**4d**, Table 1), 4-OCH_3 (**4e**, Table 1), 3,4-di-OCH_3 (**4f**, Table 1), 1-naphthyl (**4g**, Table 1), and 2-naphthyl (**4h**, Table 1), the arylation procedure described below for Ar = 3,4-di-Cl Ph was used, substituting in the appropriate aryl bromide.

*cis-3a-(3,4-Dichlorophenyl)octahydro-1H-isoindole (**4j**).* The lactam *cis*-7a-(3,4-dichlorophenyl)octahydro-1*H*-isoindol-1-one (65 mg, 0.2287 mmol) was diluted in THF (2 mL) and borane (0.7 mL, 1 M in THF, 3 equiv) and heated in the microwave for 15 min (max temp = 100 °C). After cooling, the mixture was stirred with 6 N HCl for 30 min and washed with MTBE. The aqueous layer was basified with KOH and extracted with MTBE. The organic phase was evaporated and filtered (aminopropyl cartridge) to give **4j** (15.5 mg, 24%) as a colorless oil. HRMS $[M + H]^+$: calculated for $C_{14}H_{18}Cl_2N$ 270.0811, found 270.0795. LCMS R_t = 7.60 min, m/z = 270 (M + 1). The purity was 98% by LCMS analysis. ^{1}H NMR ($CDCl_3$, δ): 7.43 (d, J = 2.3 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.19 (dd, J = 2.3, 8.5 Hz, 1H), 3.1 (m, 2H), 2.9 (m, 1H), 2.6 (m, 2H), 2.0−1.2 (m, 8H). ^{13}C NMR ($CDCl_3$, δ): 146.7, 132.3, 130.1, 129.7, 128.9, 126.2, 59.9, 49.6, 47.9, 41.0, 32.8, 24.2, 22.0, 21.4.

*cis-6a-(3,4-Dichlorophenyl)hexahydrocyclopenta[c]furan-1-one (**7**).* To a solution of lactone **5** (630 mg, 5 mmol), palladium dba (145 mg, 5 mol %), and toluene (6 mL), which was stirring under nitrogen in a sealed vial, was added tri-*t*-butylphosphine (250 uL, 5 mol %), lithium HMDS (6 mL, 1.2 equiv), and dichlorophenylbromide (1.69 g, 1.5 equiv). The solution was heated in the microwave for 15 min (max temp = 140 °C). After cooling, the mixture was diluted with hexane, washed with 3 N HCl, and evaporated. The crude brown oil was separated on silica gel to give the title compound (578 mg, 44%) as pale-brown oil. GC-MS R_t = 12.48 min, m/z = 270 (M+). The purity was 98% by LCMS analysis. ^{1}H NMR ($CDCl_3$, δ): 7.49 (d, J = 2.3 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.24 (dd, J = 2.3, 8.4 Hz, 1H), 4.50 (dd, J = 7.3, 9.6 Hz, 1H), 4.14 (dd, J = 2.2, 9.6 Hz, 1H), 3.1 (m, 1H), 2.60 (ddd, J = 3.0, 6.4, 12.5 Hz, 1H), 2.2−1.6 (m, 5H). ^{13}C NMR ($CDCl_3$): 179.7, 140.6, 132.8, 131.5, 130.6, 128.3, 125.8, 72.7, 59.4, 46.2, 40.3, 34.4, 25.8.

*cis-7a-(3,4-Dichlorophenyl)octahydroisoindol-1-one (**8**).* A mixture of the dichlorophenyl lactone **6** (580 mg, 2.049 mmol), potassium phthalimide (758 mg, 2 equiv), and DMF (4 mL) was stirred in a 150 °C bath for 24 h. Evaporation gave the crude acid. The crude material from above was diluted with 5 M KOH (10 mL) and heated in a 110 °C bath for 24 h. Extraction with ethyl acetate followed by evaporation gave the crude lactam. Separation on silica gel gave the pure lactam (67.5 mg, 12%) as colorless oil. GC-MS R_t = 13.77 min, m/z = 283 (M − 1). The purity was 98% by LCMS analysis. ^{1}H NMR ($CDCl_3$/DMSO-d_6, δ): 7.37 (d, J = 2.3 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 7.14 (dd, J = 2.3, 8.5 Hz, 1H), 4.02 (s, 1H), 3.07 (dd, J = 5.8, 9.9 Hz, 1H), 2.83 (dd, J = 3.6, 9.9 Hz, 1H), 2.6 (m, 1H), 1.9 (m, 1H), 1.7 (m, 1H), 1.6−1.2 (m, 6H). ^{13}C NMR ($CDCl_3$/DMSO-d_6, δ): 179.3, 142.5, 132.2, 130.6, 130.1, 128.8, 126.2, 51.6, 44.2, 40.3, 32.6, 26.9, 22.7, 22.6.

For Aryl = 2-naphthyl (**24b**/**26b**, Table 2; **20b**/**22b**, Table 3), 2-bromonaphthalene was used instead of dichlorophenylbromide. For Ar = 3,4-di-F Ph (**13b**, Table 3), 4-OCH_3 Ph (**13c**, Table 3), 3-OCH_3 Ph (**13d**, Table 3), ans 3,5-di-Cl Ph (**13e**, Table 3), the appropriate aryl bromide was employed instead of dichlorophenylbromide.

*cis-1-(3,4-Dichlorophenyl)-2-(hydroxymethyl)-N-methylcyclopentanecarboxamide (**9**).* To a solution of methylamine (1.5 mL, 2 M in THF, 2 equiv) at −78 °C was added *n*-BuLi (1.2 mL, 2.5 M in hexanes, 2 equiv) dropwise. After 5 min, a solution of the lactone **7** (413 mg, 1.529 mmol) in THF (3 mL) was added in one portion. The mixture was stirred at low temperature for 5 min and at ambient temperature for 2 h. The solution was quenched with NH_4Cl, extracted with MTBE, and evaporated. The residue was purified on silica to give the title compound as pale-yellow oil (351.0 mg, 76%). GC-MS R_t = 12.4 min, m/z = 283 (M − H_2O). The purity was 98% by LCMS analysis. ^{1}H NMR ($CDCl_3$, δ): 7.51 (d, J = 2.3 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.25 (dd, J = 2.3, 8.5 Hz, 1H), 3.8 (bs, 1H), 3.7 (m, 2H), 3.5 (s, 1H), 2.73 (d, J = 4.8 Hz, 3H), 2.7−2.4 (m, 2H), 2.1−1.5 (m, 4H). ^{13}C NMR ($CDCl_3$, δ): 175.7, 144.4, 132.5, 131.0, 130.4, 129.1, 126.8, 63.9, 61.3, 50.0, 37.9, 27.8, 26.7, 22.1.

cis-2-(3,4-Dichlorophenyl)-2-((methylamino)methyl)cyclopentyl)-methanol. To a solution of **9** (110 mg, 0.442 mmol) in THF (1.3 mL) was added borane−THF (1.3 mL). After 2 min, the solution was heated in the microwave for 30 min (max temp = 100 °C). After cooling, the reaction was quenched cautiously with a few drops of methanol followed by 3 N HCl (4 mL) and stirred for 30 min. The solution was washed with 50%MTBE/hexanes, chilled, basified with KOH, and extracted with MTBE. After evaporation, the compound was filtered through an aminopropyl cartridge to give the amine (315.9 mg, 95% yield) as a pale-yellow oil. LCMS R_t = 5.98 min, m/z = 288 (M + 1). ^{1}H NMR ($CDCl_3$, δ): 7.6 (m, 1H), 7.4 (m, 2H), 6.8 (bs, 1H), 3.7 (m, 2H), 2.8 (m, 3H), 2.32 (s, 3H), 2.1−1.2 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 147.3, 132.5, 130.2, 130.1, 129.3, 126.9, 63.7, 58.3, 52.9, 47.1, 41.6, 36.0, 28.6, 22.1.

The amino alcohol was separated on a ChiralTech AD column using 2:3:95:0.1 MeOH/EtOH/Hex/DEA. The faster moving enantiomer eluted at 6.5 min and the slower moving enantiomer eluted at 8.5 min. The purity was 98% and 96%, respectively, by HPLC analysis.

*cis-3a-(3,4-Dichlorophenyl)-2-methyloctahydrocyclopenta[c]pyrrole (**20a**).* The aminol *cis*-2-(3,4-dichlorophenyl)-2-((methylamino)methyl)-cyclopentyl)methanol (47.3 mg, 0.164 mmol) was dissolved in 1 mL of DCM and allowed to react with mesyl chloride (19 μL, 1.5 equiv) in the presence of diisopropylethylamine (86 μL, 3 equiv) for 2 h. The mixture

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was quenched with aqueous potassium carbonate and extracted with MTBE. The crude residue after evaporation was passed through an aminopropyl column to afford **20a** (43.5 mg, 99%) as a clear oil. HRMS $[M + H]^+$: calculated for $C_{14}H_{18}Cl_2N$ 270.0811, found 270.0809. LCMS R_t = 8.56 min m/z = 270 (M + 1). The purity was >99% by LCMS analysis. ^{1}H NMR ($CDCl_3$, δ): 7.41 (d, J = 2.3 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.17 (dd, J = 2.3, 8.4 Hz, 1H), 2.87 (t, J = 8.4 Hz, 1H), 2.7 (m, 1H), 2.6 (m, 2H), 2.3 (m, 1H), 2.32 (s, 3H), 2.0−1.5 (m, 6H). ^{13}C NMR ($CDCl_3$, δ): 150.4, 131.9, 129.9, 128.2, 125.7, 70.4, 64.6, 58.3, 50.2, 42.0, 41.0, 33.6, 25.8.

Enantiomer **22a** was prepared as above in 100% yield. HRMS $[M + H]^+$: calculated for $C_{14}H_{18}Cl_2N$ 270.0811, found 270.0820. The purity was 97% by LCMS analysis.

cis-3a-(3,4-Dichlorophenyl)-2-ethyloctahydro-1H-isoindole (***21b***). Compound **21b** was prepared as above with starting material *cis*-2-(3,4-dichlorophenyl)-2-((ethylamino)methyl)cyclohexyl)methanol in 53% yield. LCMS R_t = 7.87 min, m/z = 298 (M + 1). The purity was >99% by LCMS analysis. ^{1}H NMR ($CDCl_3$, δ): 7.47 (d, J = 2.2 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.18 (dd, J = 2.4, 8.5 Hz, 1H), 3.0 (m, 2H), 2.85 (t, J = 9.0 Hz, 1H), 2.7−2.5 (m, 4H), 2.0 (m, 2H), 1.8−1.4 (m, 6H), 1.11 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, δ): 147.7, 132.1, 129.9, 129.5, 128.9, 126.2, 68.1, 56.7, 51.0, 46.9, 39.9, 34.6, 24.6, 21.9, 21.1, 14.0.

cis-3a-(3,4-Dichlorophenyl)-2-methyloctahydro-1H-isoindole (***23a***). Compound **23a** was prepared as above with starting material *cis*-2-(3,4-dichlorophenyl)-2-((methylamino)methyl)cyclohexyl)methanol in 75% yield. HRMS $[M + H]^+$: calculated for $C_{15}H_{20}Cl_2N$ 284.0967, found 284.0973. LCMS R_t = 8.20 min, m/z = 284 (M + 1). The purity was 99% by LCMS analysis. ^{1}H NMR ($CDCl_3$, δ): 7.43 (d, J = 2.3 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.19 (dd, J = 2.3, 8.5 Hz, 1H), 2.9 (m, 2H), 2.78 (t, J = 9.2 Hz, 1H), 2.7−2.6 (m, 2H), 2.42 (s, 3H), 2.0−1.8 (m, 2H), 1.8−1.6 (m, 2H), 1.6−1.4 (m, 3H), 1.2−1.0 (m, 1H). ^{13}C NMR ($CDCl_3$, δ): 146.9, 132.2, 130.1, 129.8, 128.8, 126.1, 69.8, 58.8, 47.7, 43.3, 40.5, 34.3, 24.6, 21.7, 20.9.

cis-3a-(3,4-Dichlorophenyl)octahydrocyclopenta[c]pyrrole (***24a***). Methyl amine **20a** (20 mg) was dissolved in 1-chloroethyl chloroformate (250 μL) and heated to 80 °C for 15 h. The reaction was cooled and evaporated. The residue was dissolved in methanol and heated at 80 °C for an additional 3 h. After evaporation, the residue was diluted in DCM, washed with K_2CO_3, and filtered (aminopropyl cartridge). The product (50% conversion) was then separated from unreacted starting material by chromatography (Chiracel AD; 95:5:0.1 IPA/Hex/DEA). HRMS $[M + H]^+$: calculated for $C_{13}H_{16}Cl_2N$ 256.0654, found 256.0666. LCMS R_t = 7.14 min m/z = 256 (M + 1). The purity was 97% by LCMS analysis. ^{1}H NMR ($CDCl_3$, δ): 7.3 (m, 2H), 7.12 (dd, J = 2.3, 8.4 Hz, 1H), 3.3 (m, 1H), 3.00 (s, 2H), 2.7 (m, 2H), 2.0−1.9 (m, 3H), 1.8−1.5 (m, 2H), 1.5 (m, 1H). ^{13}C NMR ($CDCl_3$, δ): 150.2, 132.1, 130.0, 129.4, 128.1, 125.7, 62.2, 60.0, 56.1, 50.7, 40.5, 33.7, 25.8.

In Vitro ADMET. In vitro microsomal stability, hERG inhibition, and CYP inhibition assays were performed at Cyprotex, Macclesfield, UK, using their standard assay protocols, as described below.

CYP-450 Inhibition. The five cytochrome P450 isoforms of interest (CYP1A, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were investigated. Isoform-specific substrates were incubated individually with human liver microsomes and a range of test compound concentrations (typically 0.1−25 μM). At the end of the incubation, the formation of metabolite was monitored by LC-MS/MS (or fluorescence in the case of CYP1A using ethoxyresorufin as substrate) at each of the test compound concentrations. A decrease in the formation of the metabolites compared to vehicle control was used to calculate an IC_{50} value (test compound concentration which produced 50% inhibition).

Human and Mouse Liver Microsomes. The microsomes (0.5 mg/mL) were incubated with 3 μM test compound at 37 °C in the presence of the cofactor, NADPH, which initiated the reaction. The reaction was terminated by the addition of methanol containing internal standard. Following centrifugation, the supernatant was analyzed by LC-MS/MS. The disappearance of test compound was monitored over a 45 min time period. The in peak area ratio (compound peak area/internal standard peak area) was plotted against time and the gradient of the line determined. Using this information and the formula below, half-life in minutes could be calculated.

$$\text{elimination rate constant } (\mathbf{k}) = (-\text{gradient})$$

$$\text{half-life } (t_{1/2})\ (\text{min}) = 0.693/k$$

hERG Inhibition. The hERG inhibition assay used a high throughput single cell planar patch clamp approach (Ionworks HT System from Molecular Devices). Chinese hamster ovary cells transfected with the hERG gene (CHO-*h*ERG) were dispensed into the PatchPlate. Amphotericin was used as a perforating agent to gain electrical access to the cells. The hERG tail current was measured prior to the addition of the test compound by perforated patch clamping. Following addition of the test compound (typically 0.008, 0.04, 0.2, 1, 5, and 25 μM, n = 4 cells per concentration, final DMSO concentration = 0.25%), a second recording of the hERG current was performed. Postcompound hERG currents were expressed as a percentage of precompound hERG currents (% control current) and plotted against concentration for each compound. Where concentration dependent inhibition was observed, the Hill equation was used to fit a sigmoidal line to the data and an IC_{50} (concentration at which 50% inhibition was observed) was determined.

In Vitro Pharmacology. The novel compounds described above were tested for their inhibition of functional uptake of 5-HT,[16] NE,[17] or DA,[18] using recombinant transporters expressed in HEK-293, MDCK, or CHO-K1 cells as described in the literature. Compounds were tested initially at 10 μM in duplicate, and if ≥50% inhibition of uptake was observed, they were tested further at 10 different concentrations in duplicate in order to obtain full inhibition curves. IC_{50} values (concentration inhibiting control activity by 50%) were then determined by nonlinear regression analysis of the inhibition curves. Replicate studies done independently on a set of compounds allowed the determination of values for minimum significant ratios (MSR). MSR is a statistical measure for how many-fold different IC_{50} values for two compounds (determined only once) must be for those two IC_{50} values to be considered significantly different with 95% confidence limits.[19] From replicate studies of the effects of 13 reuptake inhibitors assayed with the human transporters at MDS Pharma on two separate occasions, MSR values were 2, 2, and 3, respectively, for SERT, NET, and DAT. Similar analysis of the assays performed internally at Sunovion provides MSR values of 4, 3, and 2, respectively, for SERT, NET, and DAT. These correspond to average SD values (on pIC_{50}s) of 0.2−0.3.

In Vivo Pharmacology. *Mouse Tail Suspension Test.* The method, which detects antidepressant activity, follows that described by Stéru et al.[15] Rodents, suspended by the tail, rapidly become immobile. Antidepressants decrease the duration of immobility. The behavior of the animal was recorded automatically for 5 min using a computerized device (Med-Associates Inc.) similar to that developed by Stéru et al.[20] Ten−twelve mice were tested in each group. The test was performed blind. The dosing solution was test compound dissolved in 5% transcutol (2-(2-ethoxyethoxy)ethanol) in saline. Compounds were typically evaluated at 3 doses (1−30 mg/kg), administered orally one time: 30−120 min before the test, and compared with a vehicle control group. Desipramine (100 mg/kg), administered under the same experimental conditions, was used as the positive reference substance.

Data were analyzed by one way analysis of variance (ANOVA) followed by posthoc comparisons where appropriate. An effect was considered significant if $p < 0.05$.

Locomotor Activity. To ensure effects of the compounds on immobility time were not related to a general stimulant effect on baseline motor activity, locomotor activity was assessed using photocell

monitored cages (Med-Associates Inc.). Each test chamber was equipped with infrared photocell beams to measure movement of the animals. Horizontal and vertical activities were measured.

Rats or mice were pretreated with vehicle or test compounds and placed back in home cage, following which they were individually placed in locomotor cages and activity was monitored in 5 min intervals for up to 60 min.

Data were analyzed by one way analysis of variance (ANOVA) followed by posthoc comparisons where appropriate. An effect was considered significant if $p < 0.05$.

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ABBREVIATIONS USED

SERT, serotonin transporter; NET, norepinephrine transporter; DAT, dopamine transporter; TRI, triple reuptake inhibitor; hERG, potassium ion channel Kv11.1; CYP, cytochrome P450; HLM, human liver microsomes; RLM, rat liver microsomes; TST, tail suspension tests; MAOIs, monoamine oxidase inhibitors; SSRIs, selective serotonin reuptake inhibitors; SNRIs, dual serotonin and norepinephrine reuptake inhibitors; 5-HT, serotonin; NE, norepinephrine; DA, dopamine; B/P ratio, brain/plasma ratio; LE, ligand efficiency; LLE, lipophilic ligand efficiency; MW, molecular weight

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