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Discovery of *N*-methyl-1-(1-phenylcyclohexyl)ethanamine, a novel triple serotonin, norepinephrine and dopamine reuptake inhibitor

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This Letter dedicated to the memory of Professor William von E. Doering

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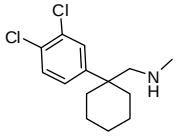
ABSTRACT

Novel chiral cyclohexylaryl amines were developed with potent reuptake inhibition against the serotonin, norepinephrine and dopamine transporters and activity at 10 and 30 mpk PO in the mouse tail suspension test. Prototype compound **31** (SERT, NET, DAT $IC_{50} \leqslant 1$, 21, 28 nM) was highly brain penetrant, had minimal CYP and hERG inhibition, and represents a previously undisclosed architecture with potential for treatment of major depressive disorder.

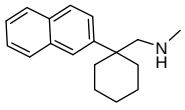
Commonly used antidepressants increase synaptic availability of biogenic amines by blocking their transport or reuptake into nerve terminals. Unfortunately, increasing monoamine levels at the synapse does not immediately result in alleviation of depressive symptoms in the majority of the patient population because depression may not be the result of a serotonin (5-HT) or norepinephrine (NE) deficiency. This therapeutic lag[1] is currently believed to be the result of slowly improving information processing in neural circuits via increased neurogenesis or increased plasticity of brain networks, stimulated over time by increased monoamine levels from current therapies.[2] One strategy to reduce the therapeutic lag and/or improve efficacy is the addition of a dopamine component to a dual reuptake inhibitor to create a 'triple' reuptake inhibitor.[3] Although addition of dopamine reuptake inhibitors to existing therapies is not a new concept,[4] there is mounting evidence that intervention resulting in decreasing catecholamine metabolites does produce a worsening of depressive symptoms in patients being treated with SSRIs and NRIs.[5] Whether the next generation of improved antidepressant comes in the form of reduction of therapeutic lag or increased compliance due to a reduction of anhedonia and sexual dysfunction associated with typical SSRI use, new monotherapies that exploit dopamine reuptake inhibition could be the next frontier in improving patient outcomes in this significant and persistent disease state.

Our initial lead optimization efforts directed toward a novel triple reuptake inhibitor for depression uncovered a novel cyclohexane alkyl amine chemotype. Exemplified by dichlorophenyl methyl amine **1** and 2-naphthyl methyl amine **2** (Fig. 1), we developed compounds with good potency for all three monoamine transporters, acceptable in vitro metabolic stability, brain penetration, and, most importantly, efficacy in an in vivo model predictive of antidepressant activity in humans. The most potent in vitro compounds in our initial set were *N*,*N*-dimethyl amines, which also suffered



1

169 85 21 (SERT, NET, DAT IC_{50} nM)
250, 32 (HLM, RLM $t_{1/2}$ *min*)



2

34 295 90 (SERT, NET, DAT IC_{50} nM)
136, 4 (HLM, RLM $t_{1/2}$ *min*)

Figure 1. Profile of leads **1** and **2**.

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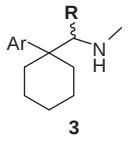


Figure 2. α-Alkyl amine analogs **3**.

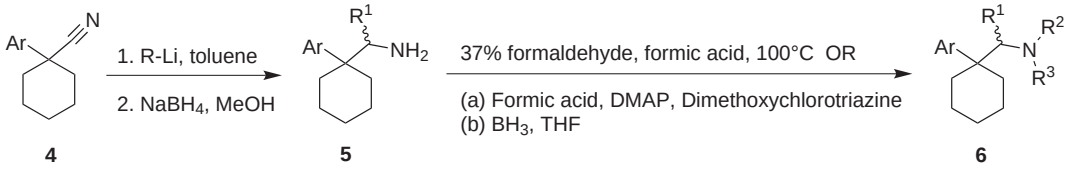
rapid metabolism to the *N*-mono methyl derivatives (in vitro and in vivo). To address this deficiency we synthesized a group of analogs with alkyl substitution proximal to the amine (compound **3**, Fig. 2)—our aim was to alter metabolism of the amine via steric shielding or through a subtle change in basicity.

The α-alkyl amines **3** were synthesized from the corresponding aryl cyclohexane acetonitriles (**4**) via addition of alkyl lithiums followed by $NaBH_4$ addition to the intermediate imines (Scheme 1). Functionalization of the primary amines **5** to mono or dimethyl amines could be accomplished by either Eschweiler–Clarke alkylation[6] with formaldehyde and formic acid or a literature method[7] that utilized an *N*-formyl intermediate.

The α-alkyl amine analogs were tested in vitro against the human recombinant serotonin,[8] norepinephrine[9] and dopamine[10] transporters and also for metabolic stability using human and mouse liver microsomes. Our goal was a compound that was superior to lead **1** or **2** in the in vitro metabolic stability assay in HLM and MLM and had transporter reuptake potency in the single digit nanomolar range for SERT and NET and in the range of 50 nM for DAT (Table 1).

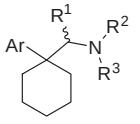
Our initial survey of analogs focused broadly on the composition of the α-alkyl group and also on the structure of the western aromatic group. In the 3,4-dichlorophenyl series, simple (racemic) α-methyl (**7**) and α-ethyl (**8**) substitution provided primary amines with reasonable potency at SERT, NET and DAT, while α-isobutyl substitution (**9**) gave an analog with single digit nanomolar potency at DAT. In the α-methyl 3,4-dichlorophenyl series, mono- (**10**) and dimethylation (**12**) provided analogs with excellent potency at all three transporters and good stability for **10** ($t_{1/2}$ = 281 min in HLM and 154 min in MLM). The racemic α-methyl *N,N*-dimethyl analog **12** did not show any stability enhancement compared to the non-alpha substituted compound (not shown). The α-isobutyl secondary (**11**) and tertiary amines (**13**) did not have profiles against the three transporters that warranted further study or separation of the racemic mixture to constituative isomers. Our scan of aryl groups showed that the 2-napthyl, α-methyl analog was comparable to 3,4-dichlorophenyl in terms of potency at the three transporters for both primary amine (**15**) and tertiary amine (**20**). Racemic 2-napthyl, α-CH_3, tertiary amine **20** showed subnanomolar potency at SERT and potency at NET and DAT below 100 nM. Metabolic stability in the 2-naphthyl series was comparable to 3,4-dichlorophenyl analogs in the α-methyl series; the excellent potency in both series led us to separate the racemic compounds into their respective constituative isomers (Table 2).

In the dichlorophenyl series, α-methyl *N*-mono methyl enantiomer **24** showed an excellent profile at the three transporters (IC_{50} = 81, 57, 30 nM at SERT, NET and DAT) and good stability in human and mouse liver microsomes ($t_{1/2} \sim 50$ min); potency for the α-methyl dimethyl derivative **25**, from the other enantiomeric



Scheme 1. Synthesis of α-alkyl amine derivatives.

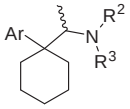
Table 1
In vitro results of α-alkyl amines



Compound	Ar	R^1	R^2	R^3	IC_{50} (nM)			In vitro microsomal stability ($t_{1/2}$, min)	
					5-HT	NE	DA	Human	Mouse
7	3,4-Cl_2Ph	CH_3	H	H	540	320	69	>300	163
8	3,4-Cl_2Ph	CH_2CH_3	H	H	660	120	29	96	56
9	3,4-Cl_2Ph	*iso*-Butyl	H	H	330	890	<1	83	59
10	3,4-Cl_2Ph	CH_3	CH_3	H	65	37	86	281	154
11	3,4-Cl_2Ph	*iso*-Butyl	CH_3	H	5900	960	9	23	18
12	3,4-Cl_2Ph	CH_3	CH_3	CH_3	15	7	64	23	22
13	3,4-Cl_2Ph	*iso*-Butyl	CH_3	CH_3	>10 K	>10 K	350	18	>300
14	1-Naphthyl	CH_3	H	H	1700	4000	4000	>300	80
15	2-Naphthyl	CH_3	H	H	440	330	120	140	16
16	4-OCF_3Ph	CH_3	H	H	110	3200	2300	>300	100
17	4-OCH_3Ph	CH_3	H	H	7100	5000	1520		
18	2-Thiophene	CH_3	H	H	>10 K	>10 K	1000	152	137
19	4-Biphenyl	CH_3	H	H	260	4000	530	248	40
20	2-Naphthyl	CH_3	CH_3	CH_3	<1	61	72	33	22

SERT, NET and DAT reuptake inhibition assays were done with at least n = 2 and five concentrations to generate inhibition curves, from which IC_{50} values were determined. 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)).

Table 2
In vitro potency for α-methyl amines in 3,4-dichlorophenyl and 2-naphthyl series



Compound	Enant	Ar	R^2	R^3	IC_{50} (nM)			In vitro microsomal stability ($t_{1/2}$, min)	
					5-HT	NE	DA	Human	Mouse
21	E1	3,4-Cl_2Ph	H	H	410	170	180	>300	84
22	E2	3,4-Cl_2Ph	H	H	820	1000	770	>300	66
23	E1	3,4-Cl_2Ph	CH_3	H	150	440	300	71	54
24	E2	3,4-Cl_2Ph	CH_3	H	81	57	30	53	52
25	E1	3,4-Cl_2Ph	CH_3	CH_3	12	10	36	28	25
26	E2	3,4-Cl_2Ph	CH_3	CH_3	240	570	220	28	28
27	E1	2-Naphthyl	H	H	190	530	66	150	11
28	E2	2-Naphthyl	H	H	60	780	110	164	14
29	E1	2-Naphthyl	CH_3	H	6	23	63	90	12
30	E2	2-Naphthyl	CH_3	H	340	180	100	101	11
31	E1	2-Naphthyl	CH_3	CH_3	<1	21	28	32	19
32	E2	2-Naphthyl	CH_3	CH_3	63	470	140	33	23

In all cases the compounds were single enantiomers of unknown absolute configuration. SERT, NET and DAT reuptake inhibition assays were done with at least $n = 2$ and five concentrations to generate inhibition curves, from which IC_{50} values were determined. 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)).

Table 3
In vitro CYP and hERG inhibition profile for lead compounds **24**, **25** and **31**

Compound	hERG inhibition (IC_{50}, μM)	CYP inhibition (isoform, IC_{50}, μM)
24	23	1A, 2C19, 2C9, 3A4 (mid), 3A4 (test) >25; 2D6 = 8.5
25	40	1A, 2C19, 2C9, 2D6 3A4 (mid) >25
31	4.3	1A, 3A4 (test) >25; 2C19, 2C9, 2D6, 3A4 (mid), >5

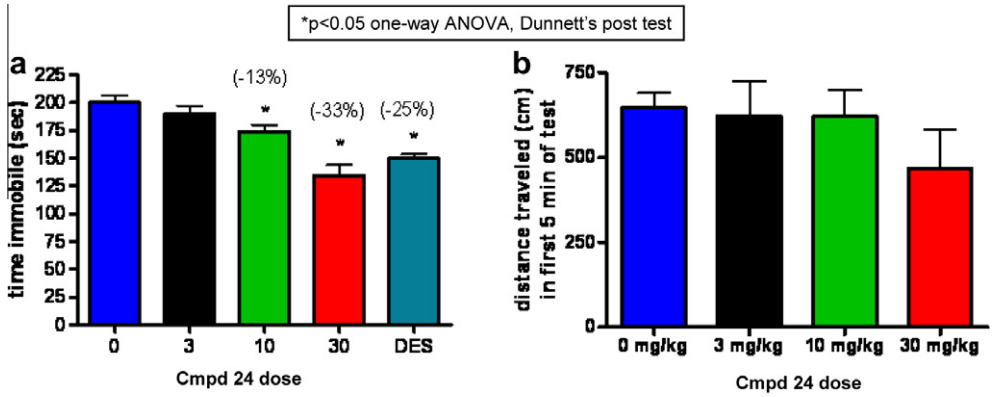


Figure 3. (a) Compound **24** in the mouse TST at 3, 10 and 30 mpk po. DES = desipramine 100 mpk po (b) Compound **24** in mouse locomotor activity assay at 5 min time point, at 3, 10 and 30 mpk po.

series, was better ($IC_{50} = 12$, 10, 36 nM at SERT, NET and DAT), albeit at the expense of stability ($t_{1/2} \sim 30$ min). In the 2-naphthyl series, one single enantiomeric series was the standout (E1), with α-CH_3 mono methyl **29** ($IC_{50} = 6$, 23, 63 nM at SERT, NET and DAT) and α-methyl dimethyl derivative **31** ($IC_{50} \leqslant 1$, 21, 28 nM at SERT, NET and DAT) showing profiles that warranted further examination. Compounds **24**, **25** and **31** were selected for evaluation in the mouse tail suspension test[11] based on their potent reuptake inhibition. In addition, we profiled the most promising compounds in the hERG and CYP inhibition in vitro assays to determine if any significant safety issues were present (Table 3).

HCl salts of compounds **24**, **25** and **31** were prepared and dosed 3, 10 and 30 mg/kg po in male mice. A 30 min pre-treatment time was used based on exposure work prior to the assay which showed maximal brain levels 30 min after po dosing. All three compounds showed a dose-dependent reduction in immobility at the 30 mpk dose, and both mono-methyl amine **24** and dimethylamine **31** showed dose-dependent reductions in immobility at the 10 mpk dose (Fig. 3a (**24**), Fig. 4a (**25**) and Fig. 5a (**31**)) All three compounds showed whole brain concentrations at the effective doses that were significantly higher than their IC_{50}'s at all three transporters (Table 3). The effects of compound **24** (Fig. 3b), **25** (Fig. 4b) and **31** (Fig. 5b) in the TST were also 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 mpk dose—the 5 min time point was significant because that is the amount of time the compounds were evaluated in the TST (Table 4).

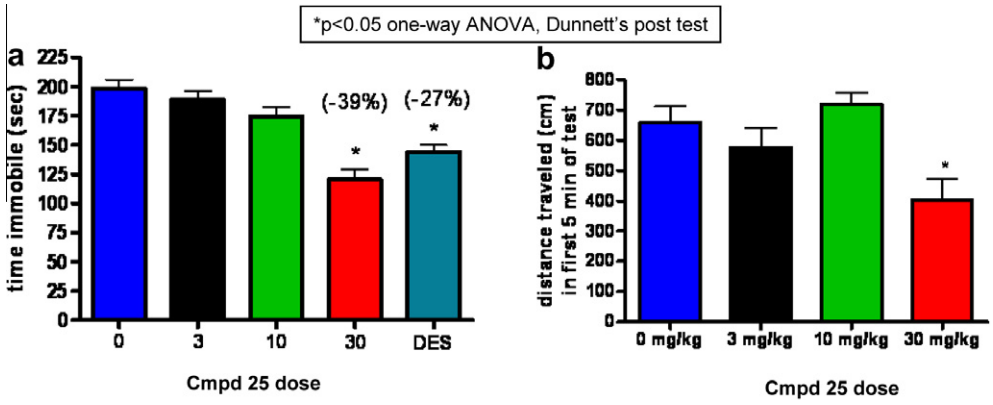


Figure 4. (a) Compound **25** in the mouse TST at 3, 10 and 30 mpk po. DES = desipramine 100 mpk po (b) Compound **25** in mouse locomotor activity assay at 5 min time point, at 3, 10 and 30 mpk po.

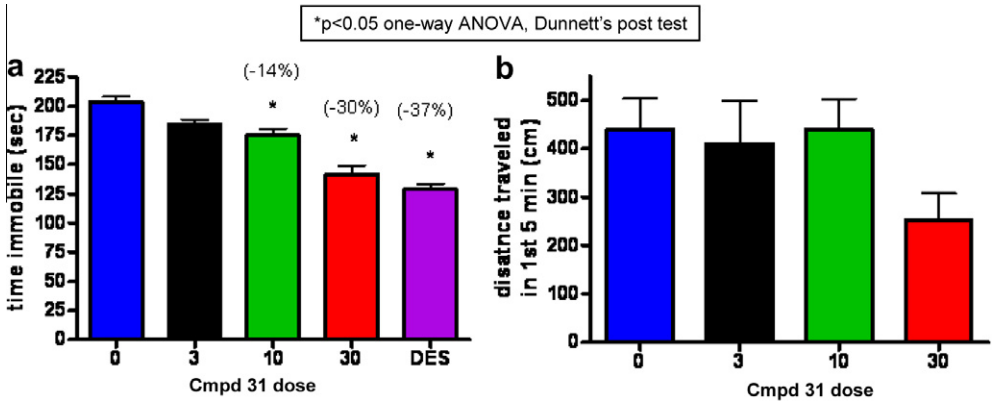


Figure 5. (a) Compound **31** in the mouse TST at 3, 10 and 30 mpk po. DES = desipramine 100 mpk po (b) Compound **31** in mouse locomotor activity assay at 5 min time point, at 3, 10 and 30 mpk po.

Table 4
Brain and plasma levels of compounds **24**, **25** and **31** from TST animals

Compound	Dose (mg/kg), po	Plasma levels (ng/ml)	Brain levels (ng/g)
24	3	9	145
	10	44	782
	30	544	7071
25	3	2	19
	10	12	114
	30	103	845
31	3	2	25
	10	13	130
	30	68	564

Our lead optimization campaign directed toward a novel triple reuptake inhibitor for depression moved into the chiral realm with the synthesis and profiling of novel α-alkyl cyclohexane aryl amines. We evaluated a variety of aryl and alkyl groups in our cycloalkane scaffold and determined that 3,4-dichlorophenyl and 2-naphthyl were the preferred substituents for the aryl component, α-methyl was the preferred group proximal to the amine, and both mono- and dimethyl amines provided compounds with potent triple reuptake inhibition in vitro and brain exposure in vivo. The effects we uncovered in vitro led us to single enantiomers **24**, **25** and **31**, which showed dose-dependent reductions in immobility in the tail suspension test in mice, an effect that is predictive of antidepressant activity in man. Additional vectors for optimizations of our desirable triple reuptake inhibitor scaffold include pyrrolidine amines and substituted cycloalkanes—results along those lines will be reported in due course.

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