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

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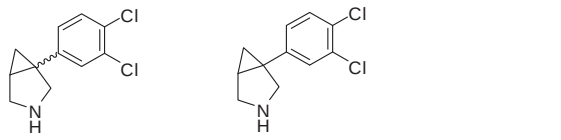
ABSTRACT

The current work discloses a novel cyclohexylarylamine chemotype with potent inhibition of the serotonin, norepinephrine, and dopamine transporters and potential for treatment of major depressive disorder. Optimized compounds **1** (SERT, NET, DAT, IC_{50} = 169, 85, 21 nM) and 42 (SERT, NET, DAT IC_{50} = 34, 295, 90 nM) were highly brain penetrant, active in vivo in the mouse tail suspension test at 30 mpk po and were not general motor stimulants.

Understanding the impact of norepinephrine (NE) and dopamine (DA) interactions on the treatment of major depressive disorder (MDD) is currently an area of active research.[1] Dual dopamine and norepinephrine reuptake inhibitors such as Bupropion increase the concentrations of NE and DA in (depression) relevant brain regions,[2] and have been found to be effective at treating MDD when used alone or in combination with SNRIs like mirtazepine (Remeron) or duloxetine (Cymbalta).[3] Dopaminergic agonists such as pramipexole have demonstrated efficacy as antidepressants.[4] Additionally, D_2 receptor partial agonists like aripiperazole, typically employed as antipsychotics, when given at sub-therapeutic doses for psychosis along with SNRIs like venlafaxine have demonstrated efficacy at increasing remission rates in resistant patient populations.[1]

As evidence continues to mount regarding the improved efficacy resulting from increasing DA levels, one strategy is the addition of a dopamine component to a dual reuptake inhibitor to create a 'triple' reuptake inhibitor.[5] The 'triple' reuptake inhibition theory was reduced to practice pre-clinically with DOV-21947[6] and DOV-216303,[7] (Fig. 1) which both potently inhibit the three monamine transporters (IC_{50} for hSERT, hNET, and hDAT for DOV-21947 = 12, 23, 96 nM and for DOV-216303 = 14, 20, 78, respectively). Both DOV compounds also dose-dependently reduce the duration of immobility in the forced swim and tail suspension tests in rats, two models that are highly predictive of antidepressant efficacy in humans. More importantly, the triple reuptake inhibition theory was validated in a Phase II efficacy trial in depressed patients. According to press releases from DOV Pharmaceuticals, DOV-216303 was shown in a multicenter Phase II study of patients with moderate to severe major depressive disorder (n = 67) to be safe and as effective as citalopram.[8] Taken together, the body of preclinical and clinical evidence for triple reuptake inhibitors such as the DOV compounds indicates that it is a validated and promising approach for the treatment of depression. Herein we wish to disclose our own efforts to develop triple reuptake inhibitors for MDD.

Screening of rationally designed compounds resulted in compound **1** (Fig. 2), which had a desirable profile at the three transporters and a long in vitro half life in human liver microsomes.



DOV-216,303

DOV-21,947
(+)-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]-hexane

Figure 1. Structures of DOV-216303 and 21947.

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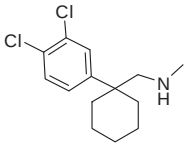


Profile of hit 1
SERT = 169 nM
NET= 85 nM
DAT = 21 nM
HLM $t_{1/2}$ = 250 min
CYP inhibition (μM): 1A, 2C19, 2C9, 3A4 > 25 μM; 2D6 3.76 μM
Brain exposure in mice after 10 mpk PO dosing

Figure 2. Profile of hit 1.

The compound was also brain penetrant in mice after oral dosing, had a relatively simple architecture, and made an excellent starting point for lead optimization efforts. Our goals for lead optimization were to maintain the brain penetration of the scaffold, maintain the low liability profile of the scaffold (CYP and hERG inhibition), increase the functional potency for SERT, NET and DAT, and show efficacy in multiple in vivo models.

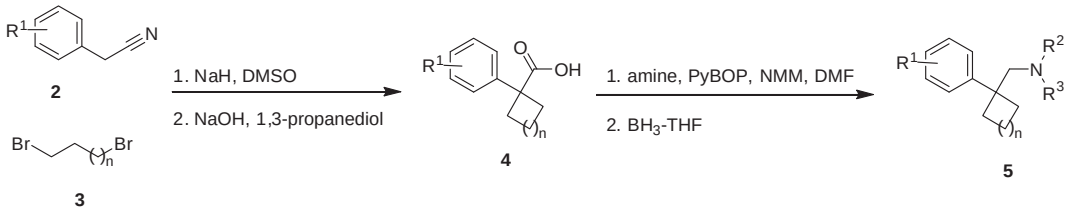
Synthesis of analogs of amine **1** followed the general synthetic route shown in Scheme 1, which was adapted from the literature.[9] Briefly, alkylation of commercially available aryl acetonitriles **2** with dibromoalkanes (**3**) in DMSO gave intermediate phenyl cycloalkanecarbonitriles (not shown), which were hydrolyzed with base to give acid **4**. Peptide coupling and subsequent reduction of the resulting amides with borane in THF gave the target amine **5**.

An alternative protocol was also used for the synthesis of *N*-methyl and *N,N*-dimethyl amines from the primary amine precursors **7** (Scheme 2). Eschweiler–Clarke alkylation[10] with formaldehyde and formic acid cleanly delivered the dimethyl amine derivative **8**, and a literature method[11] provided access to methyl amine derivatives via the *N*-formyl precursors.

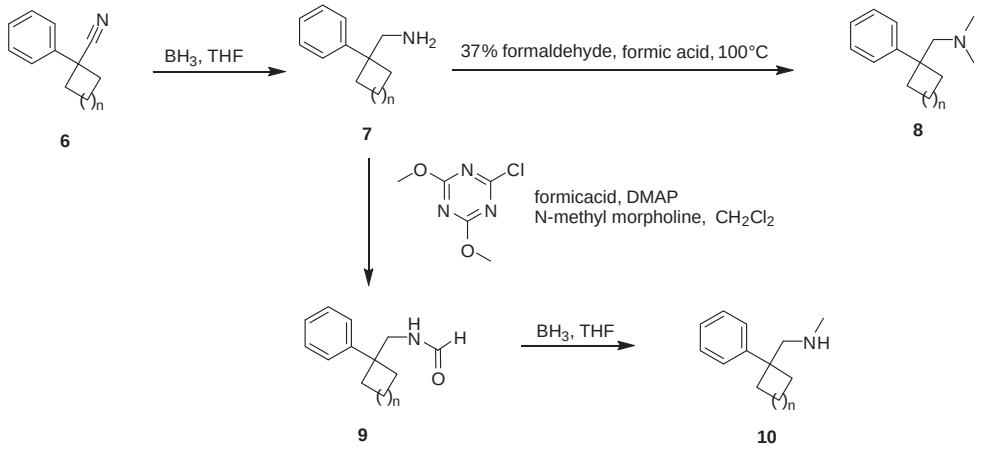
A variety of analogs were prepared to probe the role that the western aryl group, cycloalkane ring size and amine composition had on potency at the human recombinant serotonin,[12] norepinephrine,[13] and dopamine[14] transporters (Table 1). Inhibition of all three transporters was the assay used to develop SAR; in vitro metabolic stability, liability SAR and brain penetration were checked routinely on active analogs.

One of the first parameters that was optimized in the series was the ring size of the cycloalkane. The cyclopentane (**22**) and cyclohexane (**14**) dimethyl amine derivatives both inhibited all three monoamines at sub-micromolar concentrations, while the cycloheptane (**21**, primary amine, compare to **11**) and cyclobutane (**23**) derivatives showed diminished potency at the three transporters to varying degrees. In the cyclohexane dichlorophenyl series potency was best for the dimethyl derivative (**14**) compared to the monomethyl (**1**) and primary amine (**11**) derivatives, a trend which held up for other cyclohexane ring sizes and aryl substitutions (data not shown). For amine variety in the cyclohexane dichlorophenyl series, the NH-ethyl derivative **12** showed potent DAT inhibition and reasonable inhibition of SERT and NET. The cyclohexane cyclic amine derivatives were not potent SERT inhibitors, but pyrrolidine (**17**), piperidine (**18**), and *N*-methyl piperazino (**20**) analogs showed excellent potency against NET and DAT. Some other interesting cyclohexane analogs were 3-Cl-4-F-phenyl dimethylamine **29** and 3,4-difluorophenyl **31** which inhibited all three transporters with varying potency, but all less than 100 nM. In the data shown in Table 1, the most potent derivative at the three transporters was dimethylamine cyclohexane dichlorophenyl compound **14**, while compound **1** showed a favorable balance of reuptake potency across the three transporters and in vitro metabolic stability. In general, we found the primary (and some secondary) amine derivatives to be the most stable (longest microsomal $t_{1/2}$) and the dimethylamine derivatives to be least stable, presumably due to rapid N-demethylation.

In an effort to probe the size and electrostatic limitations of the aromatic region of our pharmacophore, several alternative aromatics were synthesized and tested for reuptake inhibition (Table 2),

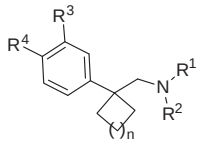


Scheme 1. General synthetic scheme for synthesis of aryl cycloalkyl amines **5**.



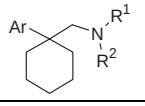
Scheme 2. Synthesis of monomethyl and dimethyl derivatives from primary amines.

Table 1
IC_{50} values for inhibition of human recombinant SERT, NET, and DAT transporters and human and rat liver microsome metabolic stability ($t_{1/2}$, min) for analogs of lead **1**



Compd	*N*	R^1	R^2	R^3	R^4	IC_{50} (nM)			In vitro microsomal stability ($t_{1/2}$, min)	
						5-HT	NE	DA	Human	Rat
1	3	H	CH_3	Cl	Cl	169	85	21	250	32
11	3	H	H	Cl	Cl	201	273	150	>300	120
12	3	H	CH_2CH_3	Cl	Cl	158	19	4	121	22
13	3	H	Cyclopropyl	Cl	Cl	651	36	2	32	12
14	3	CH_3	CH_3	Cl	Cl	19	4	1	25	10
15	3	CH_3	CH_2CH_3	Cl	Cl	156	9	1	66	17
16	3	CH_2CH_3	CH_2CH_3	Cl	Cl	2240	6	<1	35	21
17	3			Cl	Cl	8571	232	2		
18	3			Cl	Cl	9674	114	12		
19	3			Cl	Cl	7932	790	2		
20	3			Cl	Cl	299	39	<1		
21	4	H	H	Cl	Cl	637	2783	75	66	18
22	2	CH_3	CH_3	Cl	Cl	278	63	9	19	14
23	1	CH_3	CH_3	Cl	Cl	898	22	82	19	10
24	3	CH_3	CH_3	H	Cl	102	51	26	28	8
25	3	H	CH_3	H	Cl	1870	326	395	>300	51
26	3	CH_3	CH_3	Cl	H	55	9990	42	23	8
27	3	H	CH_3	Cl	H	767	269	884	208	40
28	3	CH_3	CH_3	F	Cl	145	26	17	21	16
29	3	CH_3	CH_3	Cl	F	31	10	11	19	13
30	3	H	CH_3	Cl	F	689	137	170	>300	>300
31	3	CH_3	CH_3	F	F	57	61	57	25	15
32	3	H	CH_3	F	F	2723	2188	3639	211	55
33	3	CH_3	CH_3	F	H	224	146	546		
34	3	CH_3	CH_3	H	F	33	206	125		
35	3	CH_3	CH_3	H	H	249	346	384		
36	3	CH_3	CH_3	CF_3	H	1530	28	625		
37	3	CH_3	CH_3	H	CF_3	96	529	268		

Table 2
IC_{50} values for inhibition of human recombinant SERT, NET, and DAT transporters and human and rat liver microsome metabolic stability ($t_{1/2}$, min) for aromatic analogs



Compd	R^1	R^2	Ar	IC_{50} (nM)			In vitro microsomal stability ($t_{1/2}$, min)	
				5-HT	NE	DA	Human	Rat
38	H	H	1-Naphthyl	1517	5761	6043	Not tested	Not tested
39	H	CH_3	1-Naphthyl	1079	3177	1777	>300	5
40	CH_3	CH_3	1-Naphthyl	<1	20	1	25	22
41	H	H	2-Naphthyl	305	232	83	>300	2
42	H	CH_3	2-Naphthyl	34	295	90	136	4
43	CH_3	CH_3	2-Naphthyl	3	7	3	20	5
44	H	H	4-Biphenyl	855	8733	996	91	29
45	H	CH_3	4-Biphenyl	286	9217	739	75	21
46	H	H	2-Thiophene	>10K	9987	>10K	265	215

including biphenyl, thiophene and 1- and 2-naphthyl systems. The 1-naphthyl analog **40** was among the most potent compounds at the three transporters, although the potency declined dramatically for 1-naphthyl *N*-methyl analog **39**. The 2-naphthyl analogs showed potent inhibition across all the analogs tested, with the *N*-CH_3 analog **42** showing the best combination of potency and in vitro metabolic stability.

The relative in vitro potencies at the three transporters and in vitro metabolic profile of dichlorophenyl *N*-Me amine **1** and 2-naphthyl cyclohexane *N*-Me amine **42** prompted us to evaluate them in the tail suspension test[15] in mice, an in vivo model predictive of antidepressant activity in humans. HCl salts of both **1** and **42** were prepared and dosed 3, 10, and 30 mg/kg po. A 30-min pre-treatment time was used based on exposure work

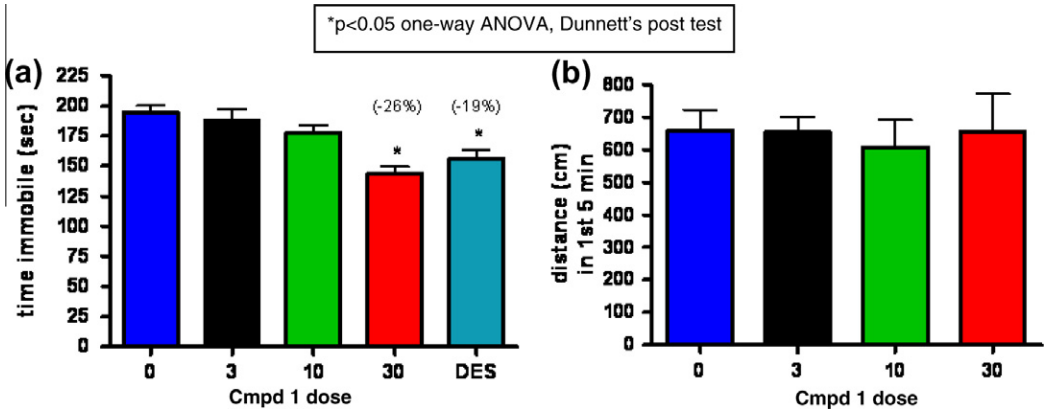


Figure 3. (a) Compound **1** in the mouse TST at 3, 10, and 30 mpk po. DES = desipramine 100 mpk po. (b) Compound **1** in mouse locomotor activity assay at 5-min time point, at 3, 10, and 30 min.

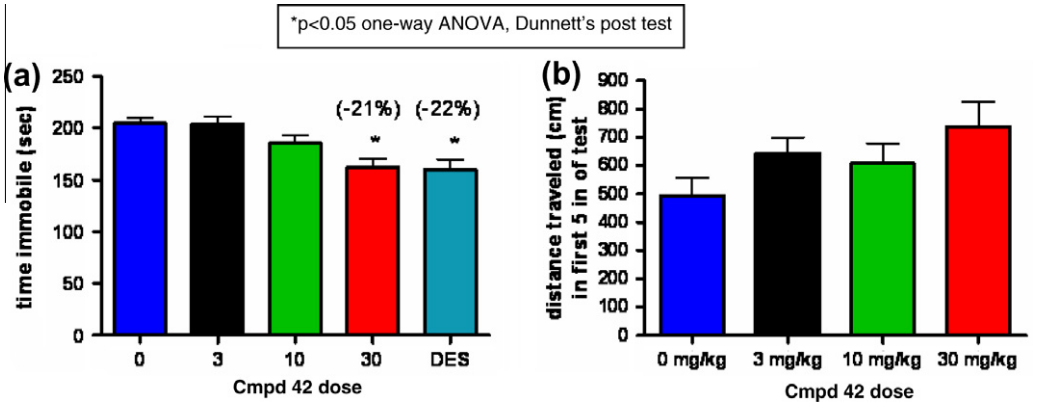


Figure 4. (a) Compound **42** in the mouse TST at 3, 10, and 30 mpk po. DES = desipramine 100 mpk po. (b) Compound **42** in mouse locomotor activity assay at 5 min time point, at 3, 10, and 30 min.

Table 3
Whole brain and plasma levels of compounds **1** and **42** from TST animals

Compd	Dose (mg/kg), po	Plasma levels (ng/ml)	Brain levels (ng/g)
1	3	11	546
	10	50	2166
	30	227	7464
42	3	8	123
	10	21	306
	30	120	2175

prior to the assay which showed maximal brain levels 30 min after po dosing. Compound **1** (Fig. 3a) and **42** (Fig. 4a) showed a dose-dependent reduction in immobility, which was statistically significant at the 30 mpk dose; the positive control desipramine also showed a reduction in immobility. Both compounds showed brain concentrations at the 30 mpk dose that were significantly higher than their IC_{50}'s (Table 3). The effect of compound **1** (Fig. 3b) and **42** (Fig. 4b) 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 30 mpk dose—the 5-min time point was significant because that is the amount of time the compounds were evaluated in the TST.

Our initial lead optimization efforts directed toward a novel triple reuptake inhibitor for depression achieved the goal of developing a novel cyclohexane alkyl amine chemotype. Exemplified by dichlorophenyl methylamine **1** and 2-naphthyl methylamine **42**, we developed compounds with good potency for all three monoamine transporters, excellent in vitro metabolic stability, brain penetration and, most importantly, efficacy in an in vivo model predictive of antidepressant activity in humans. Our future efforts will be targeted at fine tuning the potency and metabolic stability of the scaffold, with an emphasis on increasing potency at SERT and NET and finding the optimal potency at DAT.

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