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Discovery and pharmacological characterization of aryl piperazine and piperidine ethers as dual acting norepinephrine reuptake inhibitors and 5-HT$_{1A}$ partial agonists

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ABSTRACT

Compounds that are both norepinephrine reuptake inhibitors (NRI) and 5-HT1$_A$ partial agonists may have the potential to treat neuropsychiatric disorders including attention deficit hyperactivity disorder (ADHD) and depression. Targeted screening of NRI-active compounds for binding to the 5-HT$_{1A}$ receptor provided a series of thiomorpholinone hits with this dual activity profile. Several iterations of design, synthesis, and testing led to substituted piperidine diphenyl ethers which are potent NRIs with 5-HT1$_A$ partial agonist properties. In addition, optimization of these molecules provided compounds which exhibit selectivity for NRI over the dopamine (DAT) and serotonin (SERT) reuptake transporters. Monoamine and 5-HT$_{1A}$ in vitro functional activities for select compounds from the developed piperidine diphenyl ether series are also presented.

A number of drugs that are effective in treating psychiatric illness are thought to act via modulation of monoamine neurotransmitter levels including dopamine (DA), norepinephrine (NE), and/or serotonin (5-HT).[1] As a result of commercial success and developing science, the discovery of novel compounds that enhance synaptic monoamine signaling remains an active area of pharmaceutical research.[2] Compounds approved for the treatment of ADHD include amphetamine, methylphenidate, and atomoxetine, which all elevate monoamine synaptic neurotransmitter levels (DA and NE in particular), albeit via distinct cellular pathways.[3] Targeting the dual mechanism of norepinephrine reuptake inhibition (NRI) and 5-HT$_{1A}$ partial agonism could effect significant DA elevation in key cortical regions.[4,5] While NRIs increase synaptic NE and DA concentration by slowing clearance of these compounds from the synaptic space, stimulation of 5-HT$_{1A}$ receptors within the prefrontal cortex has been shown to activate subcortical DA systems resulting in elevated cortical DA release via a reciprocal pathway.[6] This hypothesis is supported by an intriguing synergistic increase in extracellular DA levels within the prefrontal cortex when atomoxetine and buspirone are dosed simultaneously.[7] Moreover, preclinical behavioral studies measuring antidepressant efficacy and cognitive function suggest that compounds possessing both NRI and 5-HT$_{1A}$ partial agonist properties are more effective than NRI agents alone.[8] Thus, identification of agents with dual NRI and 5-HT$_{1A}$ partial agonist pharmacology may provide a new therapeutic approach for the treatment of ADHD, depression, and anxiety.

With these ideas in mind, we sought to identify a series of compounds that had the desired dual activity (NRI and 5-HT$_{1A}$ agonism) with minimal activity at homologous receptors, such as the dopamine reuptake transporter (DAT) or serotonin reuptake transporter (SERT). Historical broad panel screening data for ligands that are individually potent for either NRI or 5-HT$_{1A}$ indicated that it might be challenging to obtain compounds that are <50 nM at both of the desired pharmacologies, while also displaying general selectivity.[9,10] We used computational techniques to query our extensive broad panel screening database and define the ligand properties and broad panel profiles of ligands which strongly interacted with NRI, 5-HT$_{1A}$, and SRI. In this analysis, there was a meaningful relationship between SRI and 5-HT$_{1A}$ ligands, but a very weak correlation between the properties and overall selectivity profiles of NRI ligands and 5-HT$_{1A}$ ligands. The concern from the outset was that any compound with potency at both NRI and

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5-HT_{1A} might contain a promiscuous basic amine which would interact with many G-protein coupled receptor (GPCR) targets. Consequently, we incorporated upfront selectivity screening versus SERT and DAT to get an early read on 'promiscuity' and placed an emphasis on this selectivity (vs potency at either target receptor) when deciding on which SAR directions to pursue.

We screened our internal collection of NRIs for compounds that also had binding affinity for 5-HT_{1A} receptors. Among other hits, we identified aryl piperazine thiomorpholinone structures **1** and **2** (Fig. 1) as promising dual activity molecules.[11] The aryl piperazine moiety is a recurring motif in many potent 5-HT_{1A} agonists.[12,13] Computational analysis of **1** and **2** in an empirical NRI pharmacophore model guided early follow-up design in this series.[14] This modeling suggested that initial hits such as **1** might be unnecessarily large, with the halogenated ring potentially contributing little to binding affinity at NET.

A number of truncated variants of **1** and **2** were prepared, and among these, a phenoxymethyl piperazine (**7a**, Table 1) was found to retain much of the desired activity when tested for binding to the transfected human NET transporter and 5-HT_{1A} receptor.[15,16]

The synthesis of analogous compounds (e.g., **7b–d**, **8a–b**, Table 1) was straightforward and could be executed using parallel chemistry techniques from common intermediates as outlined in Scheme 1. When the benzylic alcohols **3**[17] or **4**[18] were treated with methanesulfonyl chloride at room temperature, a mixture of the expected mesylate and the benzylic chloride was obtained in each case. The mixtures (**5** or **6**) were used directly in phenoxide substitution reactions to afford piperazine (**7**) and piperidine (**8**) benzyl phenyl ethers after carbamate deprotection. Not surprisingly, these compounds had generally modest selectivity for NET versus DAT and SERT, however SERT displayed a particular sensitivity to aryl substitution pattern (e.g., **7b** and **7c**, Table 1). Piperidine compounds in this set (**8a**, **8b**) had inferior NET and 5-HT_{1A} activity relative to piperazine-based congeners.

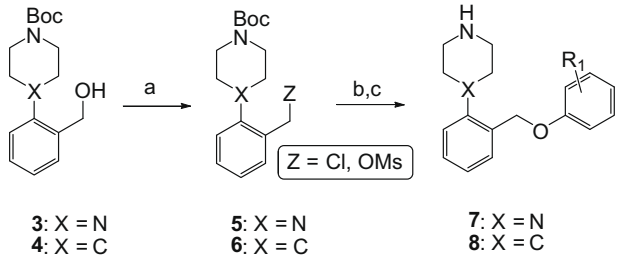
A variation of this series was obtained by reversing the benzylic ether linkage such that the oxygen atom is adjacent to the central aryl ring. These analogs were assembled from key intermediates **12** and **14**[20] via phenol alkylation (Scheme 2). Suzuki–Miyura cross coupling between 4-piperidine vinyl boronate **9** and various bromophenols (**10**) followed by subsequent hydrogenation of the dehydropiperidines **11** provided target phenols **12**.[21,22] Despite the modest yields of the Pd^0 coupling, we favored this method for installation of the piperidine by virtue of its generality and simplicity. We used TES protection of the phenols (**10**) with the assumption that this would improve overall handling and coupling yields, and nearly all of the phenolic piperidine templates **12** were prepared with this protection scheme.

The employed Suzuki conditions caused complete removal of the TES protecting group; however, subsequent experimentation showed that at least in one test case (R_1 = 3-F leading to **13d**, Table 2), the protection was unnecessary and overall yield for the transformation of **14** to **16** was actually improved by performing the Pd-mediated cross coupling with **9** on the unprotected phenol.

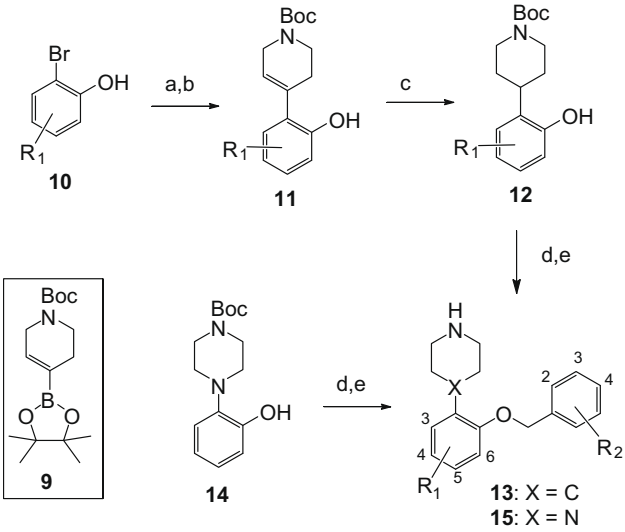
Reversing the ether linkage afforded additional active leads with general structures **13** and **15** including the compounds in Table 2. Efforts to optimize the targeted activities while prospectively designing for minimized in vitro microsomal turnover led us to frequently incorporate fluoro substitution on either or both of the aryl rings.[23] As summarized in Table 2, compounds with the reversed benzyl ether linkages often had both NET and 5-HT_{1A} binding activity, as well as meaningful selectivity versus DAT. Remarkably, the modified connectivity caused the piperidine analogs (**13**) to exhibit the superior profile. The majority of the analogs in this series

Table 1
5-HT_{1A}, NET, DAT, and SERT binding affinities for simplified aryl piperazine ethers[15,16]

ID	R_1	5-HT_{1A} K_i (nM)	NET K_i (nM)	DAT K_i (nM)	SERT K_i (nM)
7a	H	189	183	701	780
7b	3-F	116	104	>2000	17
7c	2,3-F	101	80	470	781
7d	2-*c*-Propyl	466	192	>6000	53
8a	H	>1000	1710	396	364
8b	3-F	>1000	1940	461	49



Scheme 1. Reagents and conditions: (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2, rt, 71–89% combined yield of Z = Cl and Z = OMs; (b) ROH (2 equiv), Cs_2CO_3, toluene/*t*BuOH, 110 °C; (c) 4 M HCl/dioxane, rt, then 'catch-and-release' (MP-TsOH resin) 49–86% yield (two steps).[19]



Scheme 2. Reagents and conditions: (a) Et_3SiCl, Et_3N, CH_2Cl_2, rt, 71–89% yield; (b) **9** (1.3 equiv), Pd(dppf)Cl_2 (0.15 equiv), CsF, DMF, 110 °C, 27–65% yield; (c) H_2 (3 atm), Pd/C, MeOH, rt, 49–94% yield; (d) $BrCH_2Ar$ (2 equiv), K_2CO_3, acetone, rt, 45–86% yield; (e) 4 M HCl/dioxane, rt, then 'catch-and-release' (MP-TsOH resin), 45–76% yield.[19]

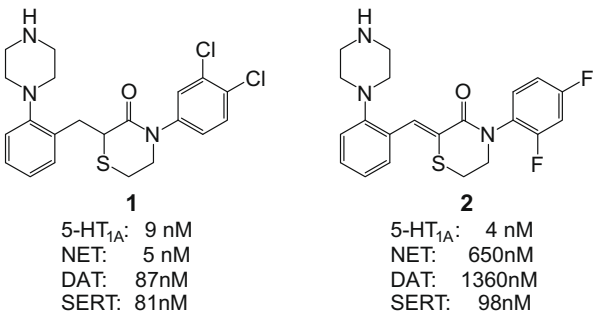


Figure 1. Structures and binding K_i's of initial NRI/5-HT_{1A} dual activity leads.

Table 2
Binding activity profiles of aryl piperidines and piperazines reversed benzyl ethers[15,16]

ID	R_1	R_2	5-HT_{1A} K_i (nM)	NET K_i (nM)	DAT K_i (nM)	SERT K_i (nM)
13a	H	H	121	76	1000	74
13b	H	2-F	47	34	1810	56
13c	6-F	H	606	9	7370	2970
13d	3-F	H	364	9	1030	28
13e	H	4-F	77	162	9120	274
13f	4-F	4-F	2210	2	659	2
13g	4-Cl	4-F	>1000	6	5530	40
13h	4-F	3,4-Cl	1160	20	452	2
15a	H	H	28	168	>8720	1140
15b	H	2-F	31	526	>10,000	978

were nearly equipotent at NET and SERT, although a few notable exceptions to this trend were identified (e.g., **13c**, Table 2). The SAR at the 4-position of the central ring was sensitive (e.g., **13e–f**), but fluorine atoms on other positions on either ring were tolerated.

Despite the promising activity profile of compounds in both ether variants, there were several immediate medicinal chemistry challenges to solve including general in vitro microsomal instability and a notable lack of functional agonism in our FLIPR-based 5-HT_{1A} functional activity screen (vide infra).[10a] With a desire to eliminate a potential benzylic metabolic 'soft-spot' and perhaps introduce functional agonist properties at the 5-HT_{1A} receptor, we targeted a set of analogous diphenyl ethers (**16** and **17**) starting from **12** or **14** according to the route in Scheme 3.

In order to enable parallel synthesis of diphenyl ethers **16** and **17**, a known Ullman procedure was adapted into a very effective one-pot coupling-deprotection sequence.[24] The crude Ullman coupling mixture (NMP, CuI, etc.) was 'quenched' with an excess of 4 M HCl in dioxane solution, which removed the Boc protecting group. The resulting reaction mixtures were diluted with MeOH and directly poured onto commercial MP-TsOH resin columns, washed with portions of MeOH, and eluted from the resin with 2 N NH_3 in MeOH.[19] This initial 'catch-and-release' isolation was followed by automated, mass-guided HPLC purification. The same procedure was successfully scaled to multi-gram quantities using sealed reaction vessels and larger amount of resin, followed by silica-gel based final purification.

Gratifyingly, compounds in the diphenyl ether series displayed NET and 5-HT_{1A} binding affinity (Table 3). Importantly, within this series, compounds such as **16e–g** had meaningful selectivity for NET binding versus the other two monoamine transporters while also retaining activity at 5-HT_{1A}. Significant activity changes occurred with minor variation in substitutions within this series. For instance, compound **16a** is a modest inhibitor of NET, but addition of an *ortho*-fluoro substituent to this compound (**16e**) dramatically enhances NET affinity. In agreement with previous SAR (see Table 2), certain central-ring substitutions were detrimental to 5-HT_{1A} activity (see **16h**, **j**); however, central-ring-fluoro substitution at the 3- and 6-positions (e.g., **16c**, **d**, **f**, **i**) preserved and even enhanced this desired pharmacology. Within the diphenyl ethers, the aryl piperazines (**17a**, **b**, **m**) were weaker inhibitors of NET than their piperidine counterparts (**16a**, **b**).

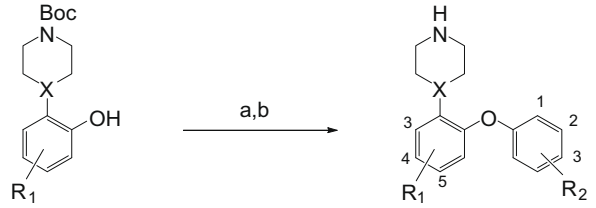
Table 3
5-HT_{1A} and monoamine binding affinities of piperidines and piperazine biaryl ethers[15,16]

ID	R_1	R_2	5-HT_{1A} K_i (nM)	NET K_i (nM)	DAT K_i (nM)	SERT K_i (nM)
16a	H	H	63	416	284	1760
16b	H	3-F	117	21	13	319
16c	3-F	H	128	170	45	706
16d	6-F	H	20	1370	239	2330
16e	H	2-F	11	11	166	901
16f	3-F	2-F	21	18	139	1040
16g	H	2-Cl	5	13	310	211
16h	5-Me	3-F	>4570	115	1680	427
16i	3-F	3-F	156	97	6	157
16j	4,5-F	3-F	>4570	1	1	260
16k	5-Me	4-F	>4570	170	1450	2
16l	H	2-Me, 5-F	57	3	1080	26
17a	H	H	128	554	4730	1880
17b	H	3-F	8	1710	4420	994
17m	H	4-F	9	1720	>7000	3100

The identification of selective and potent piperidine diphenyl ether hits such as **16e–g** (Table 3) was a breakthrough on the primary pharmacology and a few analogs from this series were selected for further profiling in functional assays for 5-HT_{1A} and the monoamine transporters. These data, along with early in vitro metabolic stability data are presented in Table 4.[25] The initially promising leads **16d** and **16g** had disappointing functional 5-HT_{1A} potency and eroded functional monoamine selectivity. Considering functional potency measures, the *ortho*-fluoro substituted compounds **16e** and **16f** emerged as superior because they combine into a single compound 5-HT_{1A} partial agonism and functional norepinephrine reuptake inhibition. These molecules also satisfied other important requirements for advancing chemical matter such as low microsomal clearance, functional selectivity versus DAT and SERT, and straightforward synthetic preparation.

Given the location of these targets in the CNS, we felt it was important to confirm that our lead compound **16f** was capable of interacting with central NET and 5-HT_{1A} receptors in vivo after systemic administration. Ex vivo receptor occupancy measurements can be a powerful tool for developing and understanding the relationship between pharmacodynamic effects and pharmacokinetic parameters.[26] In the case of central targets, this methodology can also provide confidence that molecules of interest reach an acceptable brain/plasma equilibrium concentration at a given dose. Using this technique, we demonstrated that compound **16f** was brain penetrant and binds to both target receptors in Sprauge-Dawley rats following a 10 mg/kg subcutaneous injection. Specifically, at 30 min post-dose in prefrontal cortex, ex vivo NET and 5-HT_{1A} receptor occupancies were determined to be 80% and 77%, respectively.[27] This level of occupancy was in line with targeted values at both receptors and consistent with expectations based on the compound's pharmacokinetic parameters.

In conclusion, rational application of NRI pharmacophore information to an initial thiomorpholinone aryl piperazine hit provided a lead series of aryl piperazine and aryl piperidines ethers that display both NET inhibition and 5-HT_{1A} binding activity. An iterative screening and re-design process that simultaneously considered potency and selectivity criteria led to some piperidine diphenyl ethers (e.g., **16e** and **16f**, Table 3) with the targeted activity as well



12: X = C
14: X = N

16: X = C
17: X = N

Scheme 3. Reagents and conditions: (a) ArI (2 equiv), CuI, 2,2,6,6-tetramethylheptane-3,5-dione, NMP, 140 °C; (b) 4 M HCl/dioxane, rt, then 'catch-and-release' (MP-TsOH resin), 25–46% yield (two steps).[19]

Table 4
5-HT_{1A}, NET, DAT, and SERT functional activities for select analogs[25]

ID	5-HT_{1A} EC_{50} (nM)	5-HT_{1A} E_{max} (%)	NE uptake EC_{50} (nM)	DA uptake EC_{50} (nM)	5-HT uptake EC_{50} (nM)	HLMCL (μL/min/mg)[a]
15a	>10,000	N/A	83	5210	2930	32
16d	877	35	358	982	1760	15
16e	219	51	21	657	1020	18
16f	137	74	28	498	915	10
16g	1150	44	49	1880	186	17

[a] In vitro turnover in human liver microsomes.

as selectivity versus DAT and SERT. As anticipated, subtle structural changes within this series sometimes resulted in distinct binding profiles across the four reported assays. Compounds with promising overall profiles were further characterized by screening in in vitro functional assays. Finally, ex vivo receptor occupancy was used to demonstrate CNS penetration and target binding for a prototype lead compound from this series. Additional results describing further refinement and characterization of these dual activity biaryl ether piperidines will be reported in due course.

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16. (a) *Monoamine binding assay*: Homogenized paste prepared from HEK293 cell lines expressing human clones for NE, DA, and 5HT transporters was incubated with drug and 50 pM [125I]-RTI-55 for 90 min at rt in PEI coated FlashPlates. Binding was terminated by assay volume withdraw with plates subsequently counted for beta emissions. Nonspecific binding was determined in the presence of selective inhibitors desipramine, GBR12909 and citalopram.; 5-HT_{1A} binding assay according to published protocol: (b) Graham, J. M.; Coughenour, L. L.; Barr, B. M.; Rock, D. L.; Nikam, S. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 489.
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