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Design, synthesis, and pharmacological evaluation of phenoxy pyridyl derivatives as dual norepinephrine reuptake inhibitors and 5-HT$_{1A}$ partial agonists

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ABSTRACT

Preclinical studies suggest that compounds with dual norepinephrine reuptake inhibitor (NRI) and 5-HT$_{1A}$ partial agonist properties may provide an important new therapeutic approach to ADHD, depression, and anxiety. Reported herein is the discovery of a novel chemical series with a favorable NRI and 5-HT$_{1A}$ partial agonist pharmacological profile as well as excellent selectivity for the norepinephrine transporter over the dopamine transporter.

Drugs that increase monoamine neurotransmission are widely used to treat a variety of neuropsychiatric disorders. For example, stimulant medications such as methylphenidate and amphetamine, which increase norepinephrine and dopamine (DA) neurotransmission throughout the brain, are commonly prescribed to treat ADHD; however, these medications also carry a burden of abuse liability.[1] Alternatively, norepinephrine reuptake inhibitors (NRIs) such as atomoxetine, which more selectively increase norepinephrine neurotransmission with less impact on dopamine, are also used to treat ADHD. In addition, NRI agents are clinically efficacious in the treatment of depression[2] and anxiety.[3] Preclinical microdialysis studies suggest that 5-HT$_{1A}$ receptors play a crucial role in regulating DA transmission in the prefrontal cortex upon treatment with monoamine reuptake inhibitors.[4] Additional studies indicate that adding 5-HT$_{1A}$ partial agonist properties to a norepinephrine reuptake inhibitor may selectively enhance cortical DA neurotransmission.[5] Moreover, preclinical behavioral studies measuring antidepressant efficacy and cognitive function demonstrate that compounds possessing both NRI and 5-HT$_{1A}$ partial agonist properties are more effective than NRI agents alone.[5] Thus, identification of compounds with dual NRI and 5-HT$_{1A}$ partial agonist pharmacologies may provide an important new therapeutic approach to ADHD, depression, and anxiety.

As part of our efforts to identify novel agents with dual NRI and 5-HT$_{1A}$ partial agonist pharmacology, we recently reported the discovery of a diphenyl ether series exemplified by **1** (Fig. 1).[6] This compound showed excellent activity in our in vitro assays and in vivo efficacy models. During our optimization of compounds such as **1**, we investigated alternate templates in which one or more of the phenyl groups were replaced with a heterocyclic system. Our goal was to maintain the favorable NRI/5-HT$_{1A}$ profile of **1**, while improving upon its overall monoamine transporter selectivity profile. Additionally, we anticipated that introduction of a heterocycle would lower log *P*, thereby reducing microsomal

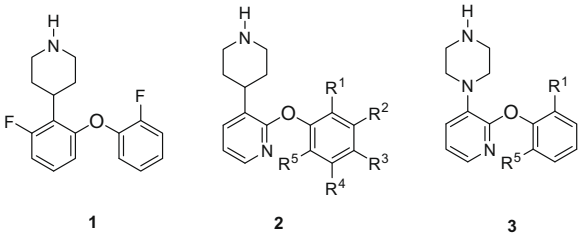

Figure 1. Exploration of the 2-phenoxypyridyl template.

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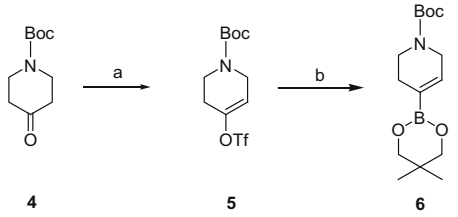
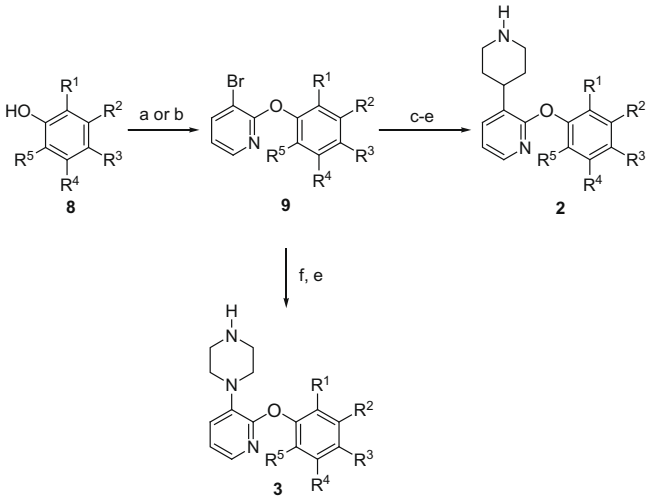


Scheme 1. Reagents and conditions: (a) LDA, $PhNTf_2$, THF, −78 °C to rt; (b) bis(neopentylglycolato) diboron, $Pd(dppf)Cl_2$, K_3PO_4, toluene/dioxane, 110 °C, 71% yield (two steps).

clearance and also potentially enhancing safety margins in the series.[7] Following a preliminary survey of heterocyclic replacements for the phenyl groups in **1**, we focused our medicinal chemistry efforts on the novel phenoxy pyridyl series **2** and **3**.

The synthesis of compounds **2a–p** was achieved in straightforward fashion beginning with commercially available *N*-Boc-piperidinone (**4**, Scheme 1). Treatment of **4** with LDA and *N*-phenyltriflimide provided the vinyl triflate **5**. Subsequent reaction of **5** with bis(neopentylglycolato)diboron in the presence of catalytic $Pd(dppf)Cl_2$ yielded vinyl boronate **6** as the key Suzuki coupling partner required for preparation of piperidine analogs **2a–p**. Commercially available 3-bromo-2-chloropyridine (**7**) also served as a key starting material in the synthetic route toward **2a–p** (Scheme 2). The addition of phenols **8** to **7** to form the 2-phenoxy pyridyl intermediate **9** was initially explored using K_2CO_3 in DMF at 150 °C. Although these reaction conditions were generally useful for the preparation of analogs in this series, sluggish reactions and low conversions were observed with some sterically hindered (e.g., 2,6-disubstituted) or electron-deficient phenols. In these cases, alternate reaction conditions employing Cs_2CO_3 in DMSO at 120 °C provided superior results (68–96% yield). The Suzuki coupling of boronate ester **6** and 3-bromo-2-phenoxypyridine **9** proceeded in good yields using $Pd(dppf)Cl_2$ and CsF in DMF at 100 °C. Finally, hydrogenation of the alkene and removal of the Boc group proceeded smoothly under standard conditions to provide analogs **2a–p**. Our synthetic route toward piperazine analogs 3a–**b** also took advantage of the key 3-bromo-2-phenoxy intermediate **9** (Scheme 2). In this case, palladium-mediated amination of **9** with *N*-Boc-piperazine was accomplished using Pd_2dba_3, Dave-Phos, and potassium *t*-butoxide in toluene at 60 °C.[8]



Scheme 2. Reagents and conditions: (a) 2-chloro-3-bromopyridine (**7**), K_2CO_3, DMF, 150 °C, 8–94%; (b)Cs_2CO_3, DMSO, 120 °C, 68–96%; (c) **6**, $Pd(dppf)Cl_2$, CsF, DMF, 100 °C, 33–85%; (d) Pd/C, H_2, EtOH, 68–98%; (e) AcCl/MeOH or HCl/MeOH, rt, 49–91%; (f) Pd_2dba_3, Dave-Phos, *t*-BuOK/toluene, 60 °C, *N*-Boc-piperazine, 60–82%.

Our medicinal chemistry strategy for the development of series **2** and **3** was designed with the following objectives: (a) optimization of the NET inhibitor potency; (b) optimization of selectivity for NET over the other monoamine transporters, with a primary focus on NET/DAT and a secondary focus on NET/SERT; (c) optimization of the 5-HT_{1A} binding potency and partial agonist profile. Our previous studies on compounds such as **1** suggested that a detailed survey of the SAR of phenyl ring substitutions in series **2** could be a fruitful approach toward achieving our objectives in this series.

Medicinal chemistry studies in this series commenced with compound **2a,** which bears an unsubstituted phenyl group. This compound is a very weak inhibitor of NET, but still maintains moderate 5-HT_{1A} binding activity. These results are consistent with our previous findings in series **1**, which suggested that the presence of a substituent at the 2-phenyl position (R^1) is important for potency at NET.[6] Addition of a fluoro group at the R^1 position provides a modest improvement in potency at NET (**2b**), but a slightly larger substituent imparts a more significant enhancement of NET potency, with chloro, methoxy, and trifluoromethoxy groups all being well tolerated by both NET and 5-HT_{1A} (**2c–f**). Compounds **2g–h** demonstrate that NET and 5-HT_{1A} affinities are not significantly affected by the 2,3-dihalo substitution pattern (R^1, R^2 = Cl, F); however, placement of a chloro group at the 3-position significantly enhanced potency at SERT. We noted that the 5-HT_{1A} affinity is not significantly affected by the addition of substituents at the 4-phenyl position (R^3), whereas the transporters are particularly sensitive to modifications at this position. Thus, compounds **2i–j** (R^3 = Me, Cl) are significantly less potent at NET and more potent at SERT than their respective parent compounds (**2b**, **2c**); comparison with **2k** confirms that the sensitivity of substitution at the R^3 position is primarily a steric effect. Substitution at the R^4 position with a fluorine atom (**2l**) results in a slightly weaker 5-HT_{1A} binding, but does not significantly affect potency at the transporters.Lipophilic efficiency (LipE) has been recently described as a parameter that incorporates both potency and lipophilicity (e.g., *c* log *P*, *c* log *D*, or measured log *D*).[16,17] For assessment of series **1** and **2**, we calculated lipophilic efficiency using the following equation:

$$\mathrm{LipE} = -\log K_i - c\log D^{12} \quad (1)$$

It is noteworthy that numerous compounds in series **2** achieve high NET and 5-HT_{1A} binding affinities while reducing *c* log *P* by 1–2 units compared to compound **1** (Table 1). Thus, many compounds in series **2** show significant improvement in LipE compared to **1**. It is also of note that compounds in phenoxy pyridyl series **2**, unlike their counterparts in phenoxy phenyl series **1**, are generally devoid of activity at DAT. The structural basis for selectivity over DAT in this series is not well understood. Nevertheless, the modifications in the substitution pattern on the phenyl ring that cause significant changes in NET and SERT affinities do not impact DAT affinity.

In the course of optimizing for NET potency in the phenoxy pyridyl series **2**, analogs were computationally investigated by analyzing their fit to the pharmacophore model previously built for the norepinephrine transporter.[18] This model consists of (1) a basic nitrogen which interacts with a receptor site point, likely an acidic hydrogen bond donating group; (2) a primary aromatic pocket flanked by receptor walls or excluded volumes; and (3) a second pocket that is presumed to be at least partially solvent-exposed, as the SAR in this area is relatively insensitive to small structural changes.

Mapping of series **2** analogs onto the model (Fig. 2a) indicates that the piperidine nitrogen maintains a good interaction with the receptor site point, the primary aromatic site is occupied by the phenyl group while the pyridine is directed into an area of

Table 1
Binding affinities at 5-HT_{1A} receptor, NET, DAT, and SERT

Compound	R^1	R^2	R^3	R^4	R^5	5-HT_{1A} K_i[a] (nM)	NET K_i[b] (nM)	DAT K_i[b] (nM)	SERT K_i[b] (nM)	*c* log *P*[c]	LipE[d] (5-HT_{1A})	LipE[d] (NET)
1						4.6	32	278	1260	4.1	6.6	5.8
2a	H	H	H	H	H	101	2440	4310	1890	2.5	7.1	5.7
2b	F	H	H	H	H	13	536	>6180	728	2.5	8.0	6.3
2c	Cl	H	H	H	H	16	74	5150	57	3.0	7.5	6.8
2d	OMe	H	H	H	H	92	292	6410	610	2.1	8.4	7.9
2e	Me	H	H	H	H	60	330	>6170	292	3.0	6.9	6.4
2f	CF_3	H	H	H	H	71	140	>6650	203	3.4	6.2	5.9
2g	Cl	Cl	H	H	H	37	25	310	2.0	3.6	6.5	6.7
2h	F	F	H	H	H	173	1810	>6650	109	2.6	6.7	5.7
2i	F	H	Me	H	H	5.5	921	>6140	36	3.0	7.9	5.7
2j	Cl	H	Cl	H	H	37	413	>5500	2.7	3.7	6.5	5.5
2k	F	H	F	H	H	29	143	2590	30	2.6	7.3	6.6
2l	Cl	H	H	F	H	17	247	>6220	251	3.2	7.3	6.2
2m	F	H	H	H	OMe	24	276	6410	887	2.2	8.9	7.9
2n	F	H	H	H	Cl	11	33	>6180	194	3.0	8.1	7.6
2o	F	H	H	H	F	7.2	181	>6630	1850	2.4	8.1	6.8
2p	F	H	H	H	Me	10	27	>6370	102	3.0	7.6	7.2
3a	Cl	H	H	H	H	4.5	747	>6320	>4710	3.2	7.2	5.0
3b	F	H	H	H	Cl	3.7	663	>6320	>4710	3.2	7.7	5.5

[a] Serotonin$_{1A}$ RBSHA binding assay; K_i values are the mean of at least two experiments carried out in duplicate.[9]
[b] Monoamine transporter binding scintillation proximity assay (SPA); K_i values are the mean of at least two experiments carried out in duplicate.[10]
[c] Calculated logarithm of octanol/water partition coefficient.[11]
[d] LipE = $-\log K_i - c \log D$.[12]

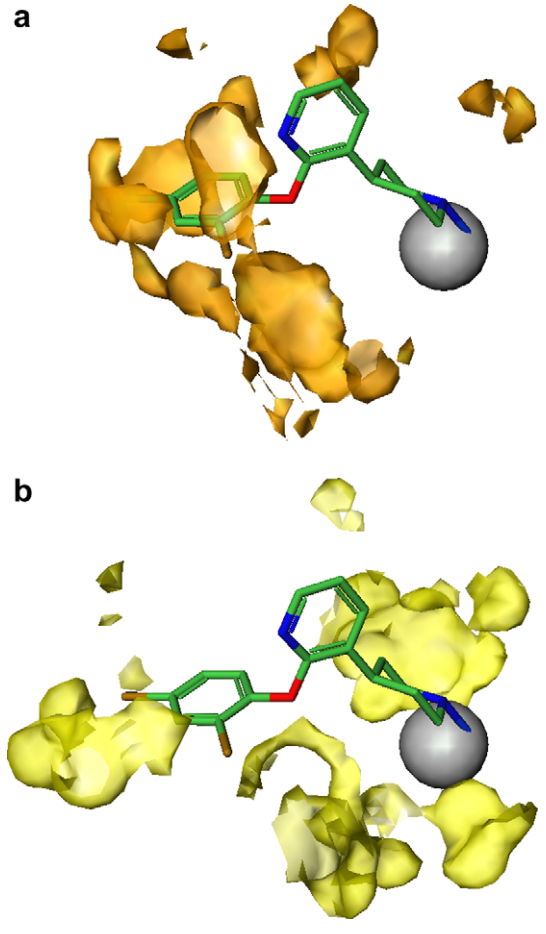


Figure 2. Mapping of compound **2j** onto the (a) NET and (b) SERT pharmacophores showing sterically disfavored areas (excluded volumes shown in orange and yellow for NET and SERT, respectively). A receptor interaction site point placed 2.8 Å (optimum hydrogen bonding distance) from the basic amine is shown in gray.

the model that is currently not well defined. The observed SAR trends with various substitutions on the phenyl are consistent with the steric restriction in the model. Phenyl is open to substitution in the R^1 position but R^3 is limited to small substitution, as bigger groups could project onto the sterically sensitive area and result in decrease in binding affinity. Hence, the introduction of a methyl and chlorine substituents at R^3 resulted in compounds with decreased NET activity (e.g., **2i–j**) as these substituents come close to the receptor wall and cause unfavorable steric interactions. On the contrary, the presence of a substituent at R^1 seems to be important for modulating NET activity. Hence **2c–d,** which are substituted with chlorine or methoxy groups, respectively, are well accommodated in the active region and account for the good NET activity generally associated with the presence of small substituents at this position. It is to be noted that while a methyl or chlorine at the 3-position reduced NET affinity, potency at SERT is enhanced. This can be explained by the spatial information provided by the SERT pharmacophore model.[18] As shown in Figure 2b, these R^3-groups are well accommodated in the primary aromatic pocket for SERT. Moreover, previous findings indicated that the presence of an electronegative functional group in this aromatic region is favorable for SERT binding affinity.[19]

In addition to our studies on series **2** compounds, we also investigated series **3** compounds in which the piperidine is replaced by a piperazine. Compounds **3a** and **3b** (Table 2) exemplify our findings in this series. Although the piperazine system provides a slight improvement in 5-HT_{1A} binding affinity, this structural modification also causes a dramatic decrease in NET binding affinity. These data are consistent with our pharmacophore model, in which a basic amine is a key element required for potency against NET. The reduced basicity of the piperazines (calculated pK_a = 7.9)[20] in comparison with the piperidine (calculated pK_a = 10.2) likely results in weakening of the key interaction between the amine moiety and the transporter.

Ultimately, the best overall pharmacological profile in series **2** is achieved with a 2,6-disubstitution pattern in which one of the substituents is either F or Cl (**2m–p**). This subset of compounds showed the greatest improvement in LipE in comparison to lead compound **1**. For example, compound **2n**, in which the *c* log *P* is reduced by one unit compared to **1**, achieves LipE of 8.1 and 7.6 for 5-HT_{1A} and NET, respectively. Compound **2n** was profiled in our panel of functional assays and compared favorably to compound **1** with respect to 5-HT_{1A} and NET functional activities and

Table 2
Functional activity at 5-HT_{1A} receptor, NET, DAT, and SERT

Compound	5-HT_{1A} EC_{50}	5-HT_{1A}% IA[a]	NET EC_{50}[b] (nM)	DAT EC_{50}[b] (nM)	SERT EC_{50}[b] (nM)	DAT EC_{50}/NET EC_{50}	SERT EC_{50}/NET EC_{50}	CL int, app[c] (μl/min/mg)
1	341	84	28	498	915	18	33	9.7
2n	89	37	9	6880	409	764	45	<8.0

[a] Serotonin$_{1A}$ GTPγS functional assay; EC_{50} and intrinsic activity (IA) values are the mean of at least two experiments carried out in duplicate.[13]
[b] Monoamine functional assays; EC_{50} and intrinsic activity (IA) values are the mean of at least two experiments carried out in duplicate.[14]
[c] Human liver microsome clearance.[15]

monoamine transporter selectivity (Table 2). This compound was further profiled using ex vivo receptor occupancy studies.[21] Use of this technique to study compound **2n** demonstrated that it is brain penetrant and binds to the target receptors in rats following a 10 mg/kg subcutaneous injection. Ex vivo NET and 5-HT_{1A} receptor occupancies were determined to be 75.3 ± 1.7% (n = 4) and 37.4 ± 5.1% (n = 4), respectively.[22] These values are consistent with NET and 5-HT_{1A} agonist occupancy values required to drive in vivo functional activity.[21,23–25]

In summary, our efforts on the synthesis and pharmacological evaluation of phenoxy pyridyl series **2** have led to the discovery of novel compounds with a favorable NRI and 5-HT_{1A} partial agonist pharmacological profile as well as excellent selectivity for the norepinephrine transporter over the dopamine transporter. SAR studies demonstrate a number of key features of this series: (a) The NET and SERT potencies are sensitive to electronic and steric modifications on the phenyl ring, and affinities toward these transporters can be tuned by careful choice of substituents; (b) 5-HT_{1A} partial agonist properties are relatively insensitive to substituent modifications on the phenyl ring; (c) Owing to the presence of the pyridyl nitrogen, compounds in this series are devoid of activity at DAT; (d) Incorporation of the pyridyl nitrogen lowers *c* log *P*, and thus affords improved LipE, while also reducing microsomal clearance in the series. Compound **2n**, a leading compound in the series achieves excellent potency at NET and 5-HT_{1A}, a 5-HT_{1A} partial agonist profile, and selectivity for NET over both DAT and SERT.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.023.

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