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Discovery and optimization of substituted piperidines as potent, selective, CNS-penetrant α4β2 nicotinic acetylcholine receptor potentiators

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ABSTRACT

The discovery of a series of small molecule α4β2 nAChR potentiators is reported. The structure–activity relationship leads to potent compounds selective against nAChRs including α3β2 and α3β4 and optimized for CNS penetrance. Compounds increased currents through recombinant α4β2 nAChRs, yet did not compete for binding with the orthosteric ligand cytisine. High potency and efficacy on the rat channel combined with good PK properties will allow testing of the α4β2 potentiator mechanism in animal models of disease.

Expression of genes encoding nicotinic acetylcholine receptors (nAChRs) is widespread, particularly in the nervous system, where such receptors mediate the psychoactive effects of nicotine.[1] Nineteen separate genes encoding individual nAChR subunits are known, and individual subunits form acetylcholine- or nicotine-gated ion channels in combinations that are not fully understood. Genes α2–α10 and β2–β4 encode nicotinic receptor subunits expressed in the nervous system; the heteromeric α4β2 receptor is the subtype most widely expressed in brain.[2] Much effort has gone into making ligands that activate individual nicotinic receptor subtypes, particularly the α4β2 and α7 receptors, in an effort to increase the therapeutic window of nicotine for diseases including Parkinson's, chronic pain, attention deficit/hyperactivity disorder, and schizophrenia.[3] The most common off-target liability of nAChR agonists is the activity on α3-containing nicotinic receptors that govern synaptic transmission in autonomic ganglia that may mediate emesis.[4] It has been particularly difficult to engineer agonists with functional selectivity for α4β2, since the nicotinic receptor orthosteric site is highly conserved amongst the various subtypes.[5,6] Accordingly, we searched for positive modulators of α4β2, in hopes of finding compounds that would potentiate the receptor and be selective against off-target nAChRs. In theory, positive modulators should increase receptor opening only in the presence of acetylcholine, the physiological stimulus, and so avoid potential problems such as receptor up-regulation and/or desensitization associated with continual exposure to agonist.

Herein, we report the discovery and optimization of a series of selective CNS-penetrant piperidines useful for pharmacological profiling.[7]

A high-throughput screening effort was performed with recombinant human nAChRs[7] using a cell-based functional assay. Compounds were assessed at 10 μM for their ability to increase the fluorescent response to EC_{12} of nicotine. Potentiators were followed up with a dose–response analysis and both EC_{50} and the peak activity compared to the maximum possible response from nicotine (% efficacy) were recorded. From this campaign, piperidine amide **1** was identified as a moderate potentiator of α4β2 nAChR with exquisite selectivity relative to α3-containing nAChRs (Fig. 1).

The initial SAR studies were focused on simple modifications to piperidine amide **1**. We met little success on improving either the potency or efficacy of piperidine amide **1** by modifying the piperi-

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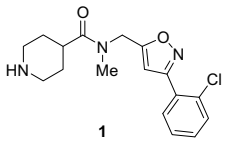


1

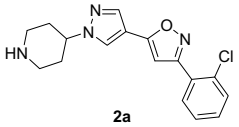
	EC_{50} [a] (μM)	% Efficacy [a, b]
hα4β2	0.35	41
hα3β2	>100	-
hα3β4	>100	-

Figure 1. Lead identified from HTS screen. [a]Measurement of test compound to potentiate a submaximal response to nicotine (EC_{12}). Average of $n \geqslant 2$. [b]As compared to maximal nicotine response.

dine ring, the isoxazole portion or the right-side aryl ring.[8] Next, we hypothesized that constraining the amide into a ring system might be advantageous. To this end, pyrazole piperidine **2a** maintained similar efficacy to our HTS hit with a 3-fold improvement in potency and a similar selectivity profile (Fig. 2). It was determined that pyrazole piperidine **2a** was effective at crossing the blood–brain barrier with a brain to plasma ratio of >10. In addition, **2a** had an efflux ratio in MDR1-LLC-PK1 cells of 3.[9] With our initial lead capable of entering the CNS, vida infra, it was decided to focus our efforts on lead optimization.

The synthesis of piperidine **2a** and similar analogs is outlined in Scheme 1. *N*-Boc-piperidine pyrazole bromides **3** were prepared according to the literature precedent (Scheme 1).[10] Attempts at installing an acetylenic group utilizing Sonogashira conditions were met with low yields. Alternatively, bromide **3** was treated with *n*-BuLi followed by a DMF quench to afford aldehyde **4**, which was converted to the corresponding alkyne **5** with the Bestmann–Ohira reagent.[11] Concurrently, aldehydes **6** was converted to the corresponding oximes and then treated with NCS to provide the requisite *N*-hydroxyimidoyl chlorides **7**. Finally, in situ formation of the nitrileoxides **7** and 1,3-dipolar cycloaddition onto alkynes **5** followed by deprotection provided analogs **2**.

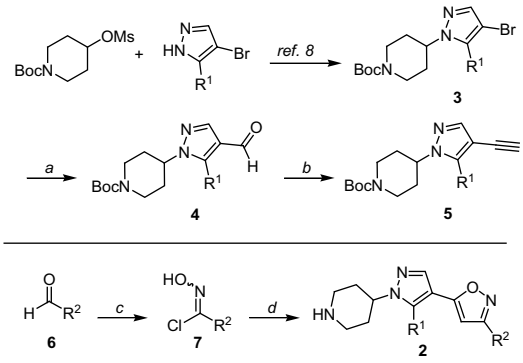
The structure–activity relationship of the right-hand aryl portion of **2** is outlined in Table 1. The ortho-chloro substituent greatly enhances α4β2 nAChR potentiation in this series of compounds, as the unsubstituted phenyl compounds **2c/d** lost significant activity. Although the combination of Cl and F substitution was tolerated (**2e–i**), the 2,6-relationship was ideal (**2e/f**). The phenyl bioisosteres 3-Cl-4-pyridyl **2j** and 2-Cl-3-thienyl **2k** have similar potencies to the corresponding 2-Cl phenyl analog **2a**. The 2,6-dichloro substitution had a detrimental effect (**2l** vs **2e**). Neither the aliphatic methyl **2o** nor cyclohexyl **2p** were tolerated by the receptor. Since the pyrazole ring mimicked the amide bond we hypothesized that adding a methyl group on the pyrazole ring might give a boost in potency. Much to our satisfaction a 6- to 8-fold increase in potency was observed upon installation of a methyl group onto the pyrazole as seen in examples **2b/d**.



2a

	EC_{50} [a] (μM)	% Efficacy [a, b]
hα4β2	0.12	38
hα3β2	>100	-
hα3β4	>100	-

Figure 2. Replacement of amide with pyrazole. [a,b]See Fig. 1 caption.



Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, 15 min; then DMF, 30 min; (b) Bestmann–Ohira Reagent, K_2CO_3, MeOH, 12 h; (c) i—hydroxylamine hydrochloride, KOAc, MeOH; ii—NCS, DMF, 40 °C, 30 min; (d) i—**5**, $KHCO_3$, EtOAc, 60 °C, 12h; ii—TFA, CH_2Cl_2, rt, 30 min.

Next, our efforts were focused on replacing the pyrazole with other heterocyles. This chemistry is outlined in Scheme 2. Treatment of heterocyclic aldehydes **8** under Bestmann–Ohira conditions afforded the corresponding alkynes **9** (Scheme 2). 1,3-Dipolar cycloaddition of benzonitrile oxides onto alkyne **9** yielded isoxazoles **10**. Either halogen–metal exchange or direct deprotonation with *n*-BuLi at the 2-position of the thiazole and imidazole, respectively, followed by quenching of the anion with *N*-Boc-4-piperidinone afforded tertiary alcohols **11**. The resulting alcohols could either be converted to a fluorine atom with DAST or eliminated with Martin sulfurane followed by reduction of the double bond to yield analogs **12**.

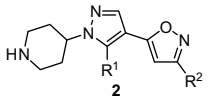
The SAR revealed that both thiazole and imidazole were competent replacements for the pyrazole portion of the molecule (**12a/b**; Table 2). When compared to **2a**, thiazole **12a** was 4-fold more potent and almost 2-fold more efficacious. In a manner similar to the pyrazole, addition of alkyl groups onto the imidazole gave a marked improvement in potency. Increasing the alkyl group size from methyl (**12b**) to ethyl (**12c**) resulted in a 4-fold increase in potency to low single digit nM. In the imidazole series, it was determined that analogs **12b/c** were substrates for Pgp (MDR1-LLC-PK1efflux ratios >10). In an effort to reduce amine basicity and thus decrease the efflux propensity, 4-F piperidines were prepared.[12] Both fluoropiperidines **12d/e** were potent analogs that had reduced Pgp liabilities (MDR1-LLC-PK1efflux ratios ∼1).

Analog **2b** was further evaluated in several in vitro assays. It was determined that **2b** at concentrations $\geqslant$30 μM did not displace the known agonist ^{3}H-cytisine from the orthosteric site of hα4β2 in a binding assay, thereby confirming a different binding mode than traditional agonists. Compound **2b** was highly selective for the hα4β2 nAChR, with EC_{50} > 100 μM against hα3β2, hα3β4, hα7, and the nAChR of embryonic human muscle receptor. Compound **2b** was tested against the rat α4β2 clone and shown to have an EC_{50} = 0.020 μM in a functional fluorescence assay.

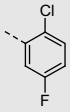
Finally, it was confirmed with whole-cell patch–clamp electrophysiology using a fast-flow perfusion system that **2b** is an effective potentiator of rα4β2 under physiological ionic conditions and with the physiological ligand acetylcholine (Fig. 3). Taken together with the above EC_{50} data, this activity on the rat receptor will allow for future pharmacological profiling.

Representative examples from the pyrazole, thiazole, and imidazole scaffolds were studied in a pharmacokinetic study to ensure CNS-penetration. Male Sprague–Dawley rats were treated

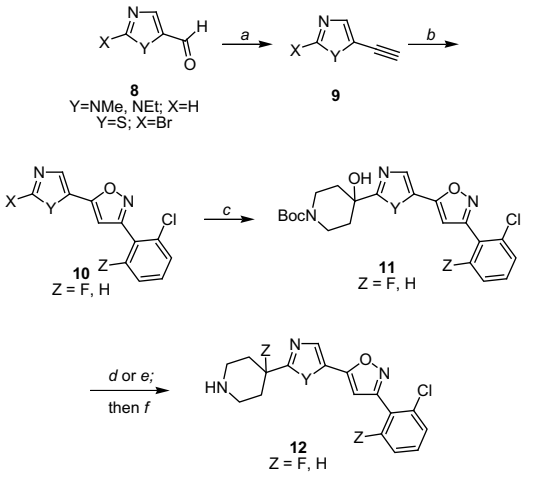
Table 1
Right-hand aryl portion SAR against hα4β2 nAChR



2

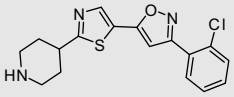
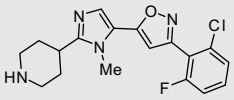
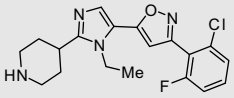
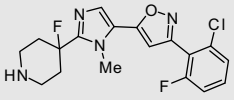
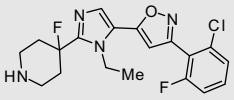
Compound	R^1	R^2	EC_{50}[a] (μM)	% Efficacy[a]
2a	H		0.12	38
2b	Me		0.018	71
2c	H		>100	—
2d	Me		1.2	31
2e	H		0.048	60
2f	Me		0.006	66
2g	H		0.27	29
2h	H		0.22	56
2i	H		0.23	47
2j	H		0.20	50
2k	H		0.13	42
2l	H		0.41	73
2o	H	Me	1.3	19
2p	H		4.6	16

[a] See Fig. 1 caption.



Scheme 2. Reagents and conditions: (a) Bestmann–Ohira Reagent, K_2CO_3, MeOH, 12 h; (b) benzoyl chloride oxime, $KHCO_3$, EtOAc, 60 °C, 12 h; (c) *n*-BuLi, THF, −78 °C, 15 min, *N*-Boc-4-piperidinone; (d) DAST, CH_2Cl_2, 0 °C; (e) i—Martin sulfurane, CH_2Cl_2, 0 °C; ii—PtO_2, 40 psi H_2, EtOAc/MeOH (4:1), rt; (f) TFA, CH_2Cl_2, rt, 30 min.

Table 2
SAR of pyrazole replacements

Compound	Structure	EC_{50}[a] (μM)	% Efficacy[a]
12a		0.033	63
12b		0.012	71
12c		0.003	87
12d		0.034	68
12e		0.003	74

[a] See Fig. 1 caption.

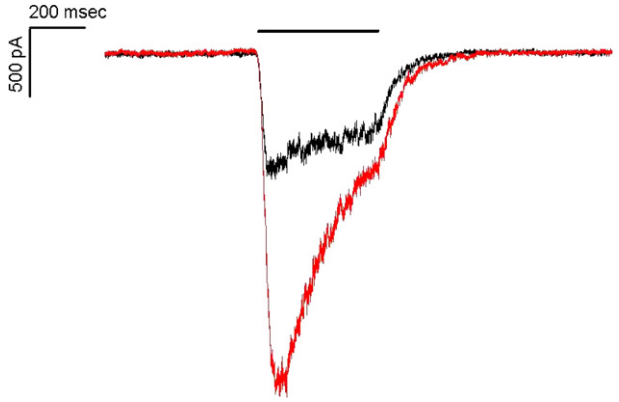


Figure 3. Currents evoked by 1 μM acetylcholine (black) and by 1 μM acetylcholine after a 10 s application of 0.1 μM **2b** (red), recorded with whole-cell patch–clamp electrophysiology from a HEK-293 cell expressing rα4β2 nAChRs. Holding voltage was −80 mV. Solid bar marks the time of application of acetylcholine. 0.1 μM **2b** alone evoked no inward currents.

Table 3
CNS-penetration of select compounds

Compound	Concentration (μM)		
	Plasma	Brain	CSF
2b	0.61	5.2	0.13
12a	0.30	8.5	0.021
12e	0.60	6.5	0.029

Concentrations assessed at 1 h post ip injection of 5 mg/kg test compound in DMSO.

with test compound at 5 mg/kg via ip injection. At one hour time point, animals were sacrificed. Plasma, brain, and CSF samples were collected and analyzed for drug concentrations (Table 3). Each of the compounds tested had significant CNS-penetration and CSF concentrations consistent with in vitro protein binding and free fraction.

In summary, a series of potent and selective small molecule hα4β2 nAChR potentiators have been reported. These compounds show excellent CNS-penetration and good activity against the human and rat α4β2 nAChR clone. A lead series with good CNS properties has allowed for testing of the potentiator mechanism in animal models of disease, studies that will be reported in due course.

Acknowledgments

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