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Synthesis and activity of substituted carbamates as potentiators of the α4β2 nicotinic acetylcholine receptor

Stephanie K. Springer [a,†,*], Katrina S. Woodin [a,†], Virginia Berry [c], Alessandro A. Boezio [a], Lei Cao [c], Kristie Clarkin [d], Jean-Christophe Harmange [a], Markus Hierl [b], Johannes Knop [e], Annika B. Malmberg [b], Jeff S. McDermott [b], Hung Q. Nguyen [d], Daniel Waldon [c], Brian K. Albrecht [a], Stefan I. McDonough [b]

[a] Department of Medicinal Chemistry, Amgen Inc., 1 Kendall Square, Building 1000, Cambridge, MA, USA
[b] Department of Neuroscience, Amgen Inc., 1 Kendall Square, Building 1000, Cambridge, MA, USA
[c] Department of Pharmacokinetics and Drug Metabolism, Amgen Inc., 1 Kendall Square, Building 1000, Cambridge, MA, USA
[d] Department of Protein Sciences, Amgen Inc., 1 Kendall Square, Building 1000, Cambridge, MA, USA
[e] HTS/Molecular Pharmacology, Amgen Research GmbH, Josef-Engert-Str. 11, Regensburg, Germany

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ABSTRACT

The synthesis and structure–activity relationship of a series of carbamate potentiators of α4β2 nAChR is reported herein. These compounds were highly selective for α4β2 over other nAChR subtypes. In addition, compounds increased the response of α4β2 nAChRs to acetylcholine, as measured with patch–clamp electrophysiology.

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated cation-permeable ion channels with pentameric structure.[1] Genes α2–α10 and β2–β4 encode nicotinic receptor subunits expressed in the nervous system; the heteromeric α4β2 channel is the nAChR most widely expressed in brain. Other prominent nAChRs include the neuronal α3β4, α3β2, and homomeric α7 channels, as well as the α1β1δγ/ε nAChR expressed in skeletal muscle. To date there are few pharmacological tools that distinguish among these and the many additional nAChR subtypes that likely exist in vivo.[2] Agonism of the α4β2 neuronal nAChR is the basis for current or potential treatments of several neurological disorders, including chronic pain, impaired cognition, and nicotine addiction. Circuitry responsible for therapeutic benefit is generally unclear, but Chantix® (varenicline) likely aids in smoking cessation by providing an nAChR-mediated increase in dopamine levels in the brain's reward centers.[3,4] Historically, a major challenge to developing α4β2-targeted agonists that have a wide therapeutic window has been sufficient selectivity against other nAChR subtypes. Of particular concern is agonism of α3-containing receptors, which may be responsible for undesirable side effects including emesis.[5] Since the orthosteric site is highly conserved among nAChR subunits,[6,7] we searched for potentiator ligands in hopes of finding α4β2-selective compounds that retained the efficacy of the nonselective α4β2 agonists. A potentiator could have the additional advantages of activating the nAChR only during physiological stimulus, and of avoiding the receptor desensitization seen with prolonged agonist application. We recently screened our sample collection for α4β2 nAChR potentiators. Herein we report the discovery and the structure–activity relationship of a series of selective carbamate derivatives as positive modulators of α4β2 nAChR.

A high-throughput screening effort was performed on recombinant human nAChRs using a cell-based fluorescent Ca^{2+} flux assay in which compounds were assessed at 10 μM for their ability to increase the fluorescent response to 1/8 EC_{50} of nicotine.[8] Hits were further confirmed by recording the EC_{50} and peak activity compared to the maximum possible response from nicotine (% efficacy). From this campaign, urea **1*R*** was identified as a moderate potentiator with selectivity against off target nAChRs (Fig. 1). Its enantiomer **1*S*** had no measurable activity against α4β2.

As it was clear that one enantiomer was preferred over the other, we desired an efficient, enantioselective synthetic route. While the asymmetric synthesis of aziridines is not trivial, enantiomerically enriched epoxides are commercially available

* Corresponding author. Tel.: +1 617 444 5096; fax: +1 617 577 9822.
E-mail address: stephanie.springer@amgen.com (S.K. Springer).
† These authors contributed equally to this work.

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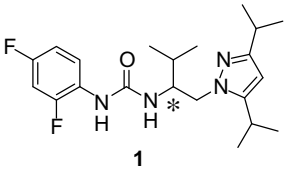


1

		EC_{12}[a] (μM)	% Efficacy[a]
	hα4β2	0.31	25
1*R*	hα3β2	>100	-
	hα3β4	>100	-
1*S*	hα4β2	>10	19

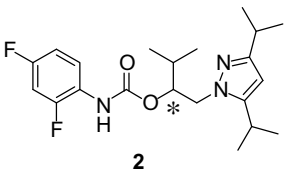
[a] Average of n ≥2.

Figure 1. Lead identification from HTS screen.

or readily accessed via hydrolytic kinetic resolution. Therefore our initial goal was to replace the urea functionality with a carbamate to expedite SAR development. Much to our satisfaction it was determined that carbamate **2*R*** maintained a similar potency as urea **1*R*** with increased efficacy (Fig. 2). As was the case with the *S* enantiomer of **1**, the carbamate analog **2*S*** was less active.

Lead carbamate **2*R*** was further evaluated to better understand its pharmacokinetic properties and its ability to cross the blood–brain barrier, as CNS penetration is thought to be required for pharmacological activity. Neither the urea **1*R*** nor the carbamate **2*R*** were P-glycoprotein substrates, each having an efflux ratio of 1.1 as measured in a porcine kidney cell line transfected with human MDR1 gene.[9] Both **1*R*** and **2*R*** had brain to plasma ratios of ~1 in Sprague–Dawley rats. Pharmacokinetic profiling in rats showed that rat in vivo clearance was similar for both carbamate **2*R*** and **1*R*** (4.9 and 3.0 L/h/kg, respectively); hence, the urea to carbamate modification did not seem to play a role in the observed high clearance, validating our decision to develop SAR using the carbamate analogues. When incubated with rat and human liver microsomes, it was found that **2*R*** was unstable (>399 and 644 μL/min/mg, respectively). Metabolic identification studies revealed that all three isopropyl groups were highly susceptible to metabolic oxidation. Thus, our medicinal chemistry strategy was to focus on improving the metabolic stability of **2*R*** while increasing the in vitro potency and efficacy, with the aim of obtaining an improved overall pharmacokinetic profile for these nAChR potentiators.

The analogues of **2*R*** reported herein were synthesized by the procedure outlined in Scheme 1.[10] The synthesis commenced with

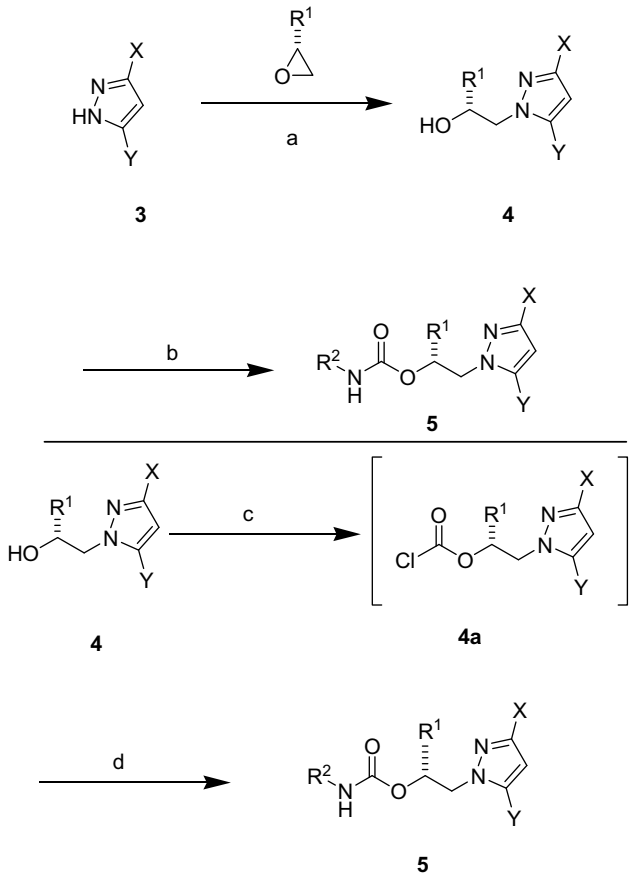


2

		EC_{12}[a] (μM)	% Efficacy[a]
	hα4β2	0.25	76
2*R*	hα3β2	>100	-
	hα3β4	>100	-
2*S*	hα4β2	>50	9

[a] Average of n ≥2.

Figure 2. Carbamate replacement of urea **1**.



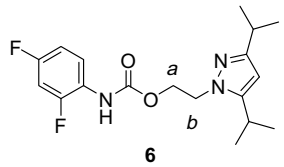
Scheme 1. Reagents and conditions: (a) Cs_2CO_3, *i*-PrOH, 80 °C, 16 h; (b) R_2-NCO, PhMe, 80 °C, 16 h; (c) $CO(OCCl_3)$, DIEA, CH_2Cl_2, 30 min; (d) R_2-NH_2, CH_2Cl_2, 50 °C.

the opening of (*R*)-3-methyl-1,2-epoxybutane[11] with 3,5-diisopropylpyrazole **3** (X = Y = *i*-Pr) exclusively at the terminal position. The resulting alcohol **4** (R_1 = *i*-Pr) could then be treated with isocyanates to form the desired carbamates. To explore beyond readily available isocyanates, **4** was treated with triphosgene followed by an amine to form the carbamate. In addition, the chloroformate intermediate **4a** could be dispensed in a parallel fashion to facilitate rapid analoging.

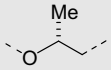
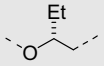
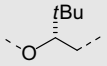
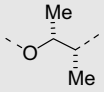
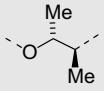
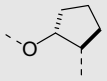
We first turned our attention to exploring alternatives to the isopropyl moiety of the oxy-ethyl linker of **2** to improve the metabolic stability. A variety of epoxides allowed us access to a number of new linkers (Table 1). Replacing the isopropyl group with methyl reduced both potency and efficacy, as observed with **6a**. The less drastic truncation to ethyl (**6b**) provided similar activity as the isopropyl, and was an attractive alternative due to the commercial availability of (*R*)-2-ethyl oxirane. Moving in the opposite direction by increasing steric hindrance was less successful; our attempt to block oxidation by replacing the isopropyl with a *tert*-butyl group resulted in a loss of both potency and efficacy (**6c**). The presence of methyl substituents at both ethylene positions *a* and *b* did not provide any advantage, in either the cis or the trans arrangement (**6d**,**e**). However, when the ethyl linker was constricted within a ring (**6f**), the potency and efficacy were restored. Unfortunately, **6f** suffered from clearance which was greater than liver blood flow in rats (8.7 L/h/kg).

As the replacement of the isopropyl group at the chiral center did not improve the pharmacokinetic profile of **2*R***, we turned our attention to the pyrazole ring. We began by modify-

Table 1
Chiral center modifications of the linker

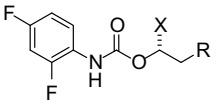


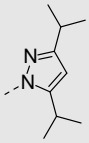
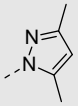
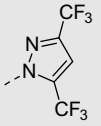
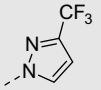
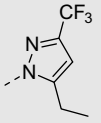
6

Compound		EC_{12} (μM)	% Efficacy
			
6a		0.70	15
6b		0.38	83
6c[a]		3.7	11
6d[a]		1.3	12
6e[a]		>100	10
6f		0.25	73

[a] Racemic.

Table 2
Effect of substitution on pyrazole



Compound	X	R	EC_{12}	% Efficacy
2	*i*-Pr		0.25	76
6b	Et		0.38	83
7a	*i*-Pr		>50	6
7b	Et		1.18	15
7c	*i*-Pr		3.9	16
7d	*i*-Pr		2.1	30
7e	Et		0.89	15
7f	*i*-Pr		0.21	50

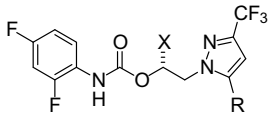
ing the alkyl substituents in an attempt to maintain potency and remove the metabolically labile isopropyl groups (Table 2). Removing the isopropyl groups altogether led to loss of activity, as seen in **7a**. Since all activity was lost by complete removal of the isopropyl groups, we sought to improve the potency by gradually increasing the size of the pyrazole substituents. The dimethyl pyrazole **7b** showed weak potency and efficacy, as did the di-trifluoromethyl pyrazole **7c**. A trifluoromethyl group paired with an alkyl group proved to be promising. Comparing the regioisomers **7d** and **7e**, the trifluoromethyl group was preferred at the 3-position of the pyrazole. Efficacy was boosted with the reintroduction of substitution at the 5-position (**7f**).

We next explored substituents at the 5-position of the 3-trifluoromethyl pyrazoles (Table 3). The substituted pyrazoles were accessed through palladium catalyzed cross coupling reactions using the common intermediate **8** (Scheme 2). We hypothesized that by constraining the isopropyl group into a ring system, we might be able to improve the pharmacokinetic profile while maintaining potency. Unsaturated or partially unsaturated lipophilic groups were less potent than the corresponding cycloalkanes, indicating a preference for sp^3 substitution (**7g** and **7h** vs **7k** and **7i**). However, lipophilic substituents seem to be required for both potency and efficacy. This observation is illustrated in the comparison of **7i** and **7k** to **7m** and **7l**, respectively. While **7i** was potent, it suffered from the same high in vivo clearance in rat observed in the lead compounds (4.23 L/h/kg). The SAR of the pyrazole was determined to be relatively flat and thus efforts were focused elsewhere.

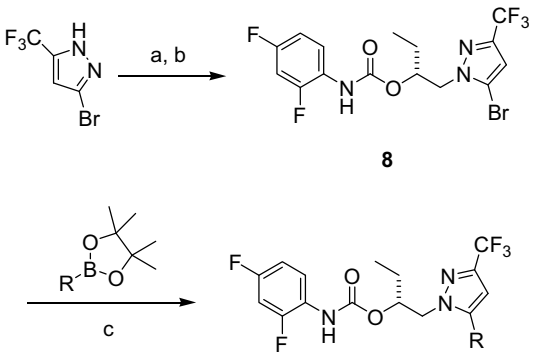
Although replacement of the isopropyl groups on the linker and on the pyrazole did not provide significant improvement, exploration of the aryl portion of the molecule might lead to more potent compounds (Table 4). Replacement of the aryl group by its saturated equivalent resulted in a loss of potency (**9a**). The unsubstituted phenyl ring **9b** also lead to a decrease in efficacy. As shown in examples **9c** and **9d**, replacement with the phenyl group isosteres thiophene and pyridine resulted in a decline in both potency and efficacy towards α4β2. To identify the optimal substitution pattern of the phenyl group, a methyl group was placed at the ortho-, meta-, and para-positions. Methylation at the para- position of the aryl ring increased the activity, whilst substituents at the other positions proved detrimental (**9e**–**g**). Having identified the para position as optimal for substitution, we then looked at the effect of electron-donating and -withdrawing groups on potency and efficacy (**9h**–**i**). Gratifyingly, when electron-donating groups such as ethers were incorporated into the para- position, an increase in both potency and efficacy was achieved (**9j**–**m**). Replacing the aliphatic group with an aromatic substituent provided the equipotent compound **9j**; the best potency was achieved with the *p*-ethyl-ether aryl moiety (**9m**).

After extensive SAR studies it was determined that carbamate **9m** was optimal for both potency and efficacy against α4β2 nAChR

Table 3
Effect of substitution at C5 of 3-trifluoromethyl pyrazole

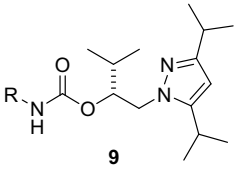


Compound	X	R	EC_{12}	% Efficacy
7e	Et	H	0.89	15
7g	*i*-Pr		2.4	10
7h	Et		0.46	20
7i	Et		0.57	95
7j	Et		0.70	54
7k	Et		0.38	88
7l	Et		>100	−3
7m	Et		>100	3

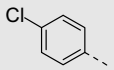
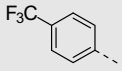
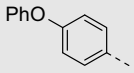
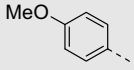
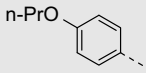
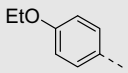


Scheme 2. Reagents and conditions: (a) (*R*)-2-ethyl oxirane, Cs_2CO_3, *i*-PrOH, 80 °C, 16 h; (b) 2,6-difluorophenyl isocyanate, PhMe, 80 °C, 16 h, regioisomers separated via flash chromatography; (c) $Pd_2(dba)_3$, P^tBu_3, Cs_2CO_3, dioxane, water; followed by Pd/C, H_2 (as needed).

Table 4
Aryl derivatives



9

Compound	R	EC_{12} (μM)	% Efficacy
9a		2.7	13
9b[a]		0.38	18
9c[a]		0.70	12
9d		>100	7
9e		0.13	59
9f		0.90	25
9g		>100	9
9h		0.33	91
9i		0.40	106
9j		0.34	89
9k		0.14	77
9l		0.33	107
9m		0.11	100

[a] Racemic.

and was further evaluated. Analog **9m** was confirmed to potentiate currents through hα4β2 nAChRs evoked by the physiological ligand acetylcholine under physiological ionic conditions. Application of 1 μM **9m** itself evoked no currents, but increased the response to 3 μM acetylcholine (Fig. 3). Compound **9m** had a significantly lower plasma clearance (1.8 L/h/kg) as compared to our initial lead **2*R***. In addition, **9m** at concentrations ⩾30 μM did not displace ^{3}H-cytisine binding to α4β2 from rat cerebral cortical membranes, suggesting that **9m** is not binding as a traditional agonist.

In summary, the optimization of a new series of pyrazole carbamates as selective potentiators of α4β2 has been described. These compounds were readily synthesized via common intermediates, allowing for rapid diversification over each region of the molecule. Lead optimization resulted in a series of compounds with enhanced potency and efficacy. Our findings lead to compound **9m**, which was an efficacious potentiator of α4β2 nAChRs.

Acknowledgements

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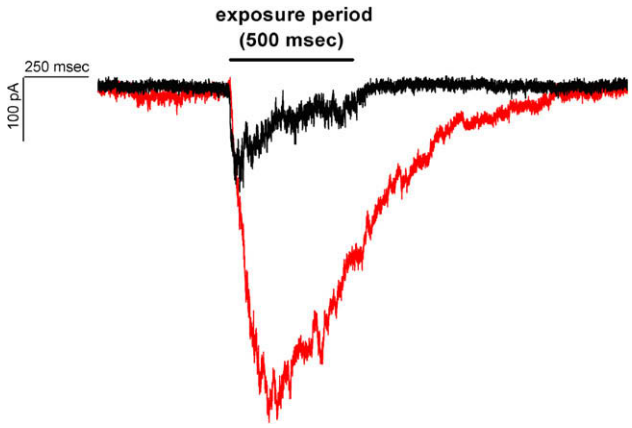

Figure 3. Currents in response to 3 μM acetylcholine (black) and to 3 μM acetylcholine plus 1 μM **9m** (red) immediately following a ten second application of 1 μM **9m** (not shown). 1 μM **9m** alone evoked no inward currents. Currents were recorded with whole-cell patch–clamp electrophysiology from a HEK-293 cell expressing hα4β2 nAChRs and voltage-clamped to −80 mV.

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10. All compounds were tested as the free base. Structure and purity of each compound was determined by HPLC, LC/MS, and ^{1}H NMR. Additional NMR studies were employed as necessary.
11. Enantiomerically enriched epoxide obtained from HKR.