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Research paper

Synthesis and biological evaluation of novel hybrids of highly potent and selective α4β2-Nicotinic acetylcholine receptor (nAChR) partial agonists

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ABSTRACT

We previously reported the cyclopropylpyridine and isoxazolylpyridine ether scaffolds to be versatile building blocks for creating potent α4β2 nicotinic acetylcholine receptor (nAChR) partial agonists with excellent selectivity over the α3β4 subtype. In our continued efforts to develop therapeutic nicotinic ligands, seven novel hybrid compounds were rationally designed, synthesized, and evaluated in [^{3}H] epibatidine binding competition studies. Incorporation of a cyclopropane- or isoxazole-containing side chain onto the 5-position of 1-(pyridin-3-yl)-1,4-diazepane or 2-(pyridin-3-yl)-2,5-diazabicyclo[2.2.1] heptane led to highly potent and selective α4β2* nAChR partial agonists with K_i values of 0.5–51.4 nM for α4β2 and negligible affinities for α3β4 and α7. Moreover, compounds **21**, **25**, and **30** maintained the functional profiles (EC_{50} and IC_{50} values of 15–50 nM) of the parent azetidine-containing compounds **3** and **4** in the $^{86}Rb^+$ ion flux assays. *In vivo* efficacy of the most promising compound **21** was confirmed in the mouse SmartCube® platform and classical forced swim tests, supporting the potential use of α4β2 partial agonists for treatment of depression.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) belong to the cys-loop superfamily of ligand-gated ion channels, which are widely distributed in the central and peripheral nervous systems. Common nAChRs are homomeric or heteromeric pentamers assembled from a varying combination of subunits (α2–α10, β2–β4, γ, and δ), with α4, β2, and α7 as the most widespread in mammalian brain. The heteromeric α4β2*–nAChR (the asterisk denotes the possible integration of other subunits into the pentamer) complexes are the predominant form of nAChRs in the central nervous system (CNS) and have been pursued as drug targets for various CNS disorders including, but not limited to, nicotine addiction, Parkinson's disease, depression, and pain [1].

The best-known drug targeting the α4β2* receptors is the partial agonist varenicline (**1**) (Fig. 1) [2], which is considered to be the

Abbreviations: CNS, central nervous system; AD, Alzheimer's disease; ADHD, attention deficit hyperactivity disorder; NIMH-PDSP, National Institute of Mental Health Psychoactive Drug Screening Program; nAChR(s), nicotinic acetylcholine receptor(s); TCA, tricyclic antidepressant; FST, forced swim test; SAR, structure-activity relationship; SSRI, selective serotonin reuptake inhibitor; CC, column chromatography; rt, room temperature; TFA, trifluoroacetic acid; HS, high-sensitivity; LS, low-sensitivity.

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treatment of choice for long-term smoking cessation. This is consistent with the well-established role of α4β2*-nAChR mediation of the reinforcing properties of nicotine [3,4]. Of particular interest for the clinical use of varenicline is the observation of improved mood and cognition both in non-depressed and depressed smokers attempting to quit [5]. Another α4β2*−nAChR partial agonist, CP-601,297 (**2**), was also recently shown to possess clinical antidepressant efficacy in non-obese subjects [6]. The use of varenicline, however, is also associated with unwanted side effects, such as nausea, constipation, and dizziness, which may be due to its activity at the α3β4*-nAChRs [7]. As such, potent partial agonists of α4β2*-nAChRs that are highly selective over the α3β4* subtypes are expected to exert higher efficacy and likely fewer side effects in rodent behavioral models of mood disorders.

Previously our group reported the discovery of highly potent and selective α4β2*-nAChR partial agonists containing a cyclopropyl- or isoxazolylpyridine ether scaffold as versatile building blocks for achieving selectivity for α4β2-over α3β4-nAChRs [8–11]. Compounds **3** and **4** bind potently to α4β2-nAChRs (K_i < 1 nM) but not to α3β4*- (K_i > 10^4 nM) and α7-nAChRs (K_i > 10^4 nM) or other neurotransmitter receptors and transporters widely distributed throughout the CNS, while possessing favorable ADMET profiles. During our lead optimization campaign, however, the hydrochloride or trifluoroacetate salts of compound **3** or **4** were found to be hygroscopic and gradually decomposed to form a dimer of the parent structure. This was consistent with the literature findings in the case of the related compound ABT-594 (**5**), which was reported to dimerize to compound **6** in the presence of HCl or TFA following exposure to water [12]. Synthetic efforts were thus made to overcome this issue by utilizing an *N*-methylpyrrolidine or diazabicyclo [3.3.0]octane moiety to replace the azetidine group present in compound **3** [13,14].

N-Pyridyldiamine compounds exemplified by 1-(pyridin-3-yl)-1,4-diazepane (NS3531, **7**) and 2-(pyridin-3-yl)-2,5-diazabicyclo [2.2.1]heptane (**8**), were reported in the literature as potent α4β2-nAChR agonists with K_i values of 0.7 nM and 0.15 nM, respectively, in [^{3}H]cytisine binding competition assays [15,16]. In oocytes expressing human nAChRs, the functional EC_{50} value of compound **7** is 21 nM at the high sensitivity (HS) α4β2-nAChR with an efficacy of 41% relative to that of acetylcholine. While the potency at α4β2 subtypes is excellent, compound **7** also behaved as an agonist at the α7 subtype with a K_i value of 136 nM. Substituents at the 5-position of the pyridine core were found to be beneficial for the selectivity for α4β2 over α7 and ganglionic nAChRs, consistent with our own findings employing sazetidine-A analogs [8–11,13]. In our continued efforts to develop highly selective α4β2-nAChR partial agonists with improved stability, we herein report preliminary SAR findings focused on the incorporation of the diazepane and diazabicycloheptane moieties into our cyclopropyl- or isoxazolylpyridine ether scaffolds.

2. Chemistry

[a] **Reagents and conditions:** (a) BnOH, NaH, DMF; (b) *n*-butyl acrylate, 1% $Pd(OAc)_2$, 2% $PhNHCONH_2$, K_2CO_3, 130 °C; (c) 2 N NaOH, MeOH/THF (1:1); (d) $MeNH_2^{\ddagger}OMeCl^-$, EDCI, DMAP, CH_2Cl_2; (e) $Me_3S(O)^+I^-$, NaH, DMSO; (f) (I) DIBAL-H, THF, -78 °C to −20 °C; (II) $NaBH_4$, MeOH; (g) ChiralPak AD, EtOH; (h) (I) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C; (II) $Ph_3P=CHOCH_3$, THF, 0 °C; (i) PtO_2, H_2, CH_2Cl_2; (j) 10% Pd/C, H_2; (k) $(CF_3SO_2)_2O$, pyridine; (l) *tert*-butyl 1,4-diazepane-1-carboxylate or 1-methyl-1,4-diazepane or *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate, tris-(dibenzylideneacetone)dipalladium, 2-(dicyclohexylphosphino)-2′,4′,6′-triisopropylbiphenyl, K_3PO_4, 1,4-dioxane, microwave, 160 °C, 10 min; (m) CF_3CO_2H, CH_2Cl_2; (n) (I) ethynyl(trimethyl)silane, CuI, $PdCl_2(PPh_3)_2$, PPh_3, Et_3N, 60 °C, (II) TBAF, THF; (o) $O_2N(CH_2)_2OTBS$, PhNCO, Et_3N, PhMe.

The syntheses of the hybrid compounds **21**–**23**, **25**, and **26** are depicted in Scheme 1. 1,3-Dibromopyridine (**9**) was advanced to the optically pure chiral intermediates **16** and **17** according to published methods [9]. Thus, one of the bromine atoms was replaced by a benzyloxy group to obtain compound **10** which underwent a Heck reaction with *n*-butyl acrylate to afford the α,β-unsaturated ester **11**. Following ester hydrolysis, the Weinreb amide **13** of carboxylic acid **12** was formed by reaction with *N*,*O*-dimethylhydroxylamine hydrochloride. This functional group interchange was found to be necessary since the α,β-unsaturated Weinreb amide **13** gave a much superior yield in the subsequent Corey-Chaykowsky cyclopropanation compared to the ester **11**. The cyclopropanecarboxylate **14** was sequentially reduced with DIBAL-H and $NaBH_4$ to furnish the primary alcohol **15**. Both enantiomers, **16** and **17**, were acquired in essentially 100% enantiomeric excess and high recovery through resolution of the racemate **15** by HPLC on a chiral stationary phase, ChiralPak AD® (Chiral Technologies, Inc.). The optically pure alcohol **17** was subjected to Swern oxidation followed by Wittig reaction to obtain the chain-extended olefin **18**. Sequential hydrogenolysis in the presence of PtO_2 and 10% Pd/C yielded the saturated ether **19**, which was transformed to triflate **20** and subsequently reacted with *tert*-butyl 1,4-diazepane-1-carboxylate, 1-methyl-1,4-diazepane, or *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate under modified Buchwald–Hartwig conditions followed by treatment with trifluoroacetic acid to afford the corresponding trifluoroacetates **21**, **22**, and **23**. The diastereoisomers **25** and **26** were synthesized from intermediates **16** and **24** by employing the same sequence of steps.

3-(Benzyloxy)-5-ethynylpyridine (**27**) was prepared via the Sonogashira coupling of **10** and ethynyl(trimethyl)silane followed by removal of the TMS group with tetrabutylammonium fluoride (TBAF). Compound **27** then underwent 1,3-dipolar cycloaddition with propionitrile oxide generated from 1-nitropropane to form isoxazole **28**, which was subsequently subjected to hydrogenolysis to give hydroxyisoxazole **29**. Successive triflation and amination afforded the trifluoroacetates **30** and **31** after removal of the Boc group under acidic conditions.

3. Results and discussion

3.1. In vitro characterization—radioligand Binding Studies

The K_i values of all the synthesized hybrid compounds were evaluated by [^{3}H]epibatidine binding competition assays at eight heterologously expressed rat nAChR subtypes (Table 1) [17]. Compound **21** bearing a (1*S*,2*R*)-configured cyclopropane side chain exhibited high binding affinities at both α4β2-and α4β2*-nAChRs, which are comparable to those found for parent compound **7**. Its diastereoisomer **25** with a (1*R*,2*S*)-configured cyclopropane side chain displayed a similar binding profile. The presence of an *N*-methyl group (compound **22**) caused more than 10-fold drop in binding affinities at α4β2 and α4β2*-nAChRs. Importantly, all three of these compounds (**21**, **22**, and **25**) were confirmed to show improved selectivity for nAChRs containing β2 subunits (α2β2-, α3β2-, α4β2-, and α4β2*-nAChRs) over nAChRs containing β4 subunits (α2β4-, α3β4-, and α4β4-nAChRs) when compared to parent compound **5**, consistent with our previous findings. In particular, the selectivity for α4β2-over α3β4-nAChRs of these three compounds was >10,000-fold, essentially abolishing the potential peripheral side effects thought to be associated with α3β4-nAChRs. Similarly, incorporation of an isoxazole-containing side chain to compound **7**, as exemplified in compounds **30** and **31**, resulted in retention of the activity at both α4β2-and α4β2*-nAChRs and