ChemPubSoc Europe

DOI: 10.1002/cmdc.201500073

CHEMMEDCHEM Full Papers

Synthesis and Pharmacological Evaluation of $\alpha_4\beta_2$ Nicotinic Ligands with a 3-Fluoropyrrolidine Nucleus

Lucia Tamborini,[a] Andrea Pinto,*[a] Roberta Ettari,[b] Cecilia Gotti,[c] Francesca Fasoli,[c] Paola Conti,[a] and Carlo De Micheli[a]

Nicotinic acetylcholine receptors (nAChRs) play an important role in many central nervous system disorders such as Alzheimer's and Parkinson's diseases, schizophrenia, and mood disorders. The $\alpha_4\beta_2$ subtype has emerged as an important target for the early diagnosis and amelioration of Alzheimer's disease symptoms. Herein we report a new class of $\alpha_4\beta_2$ receptor ligands characterized by a basic pyrrolidine nucleus, the basicity of which was properly decreased through the insertion of a fluorine atom at the 3-position, and a pyridine ring carrying at the 3-position substituents known to positively affect affinity and selectivity toward the $\alpha_4\beta_2$ subtype. Derivatives 3-(((2*S*,4*R*)-4-fluoropyrrolidin-2-yl)methoxy)-5-(phenylethynyl)pyridine (**11**) and 3-((4-fluorophenyl)ethynyl)-5-(((2*S*,4*R*)-4-fluoropyrrolidin-2-yl)methoxy)pyridine (**12**) were found to be the most promising ligands identified in this study, showing good affinity and selectivity for the $\alpha_4\beta_2$ subtype and physicochemical properties predictive of a relevant central nervous system penetration.

Introduction

Nicotinic acetylcholine receptors (nAChRs) play an important role in many central nervous system (CNS) disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), schizophrenia, mood disorders, and nicotine dependence. The predominant nAChRs in the CNS are the heteromeric $\alpha_4\beta_2$ subtype and the homomeric α_7 subtype. The $\alpha_3\beta_4$ nAChR subtype is the predominant subtype in restricted brain areas and in several autonomic nervous system ganglia. The $\alpha_4\beta_2$ subtype, which in brain constitutes about 90% of the high-affinity heteromeric receptors, has emerged as an important target for the early diagnosis and amelioration of AD symptoms.[1]

3-Pyridyl ether compounds (e.g., A-84543 and A-85380) are potent nAChR ligands that possess sub-nanomolar affinity for brain nAChRs and differentially activate subtypes of neuronal nAChRs.[2] Based on the structure of the azetidine derivative, the radiolabeled high-affinity $\alpha_4\beta_2$ agonist 5-[^{123}I]A-85380 has been developed for noninvasive imaging of nAChRs, using single-photon emission computed tomography (SPECT).[3–9] Unfortunately, 5-[^{123}I]A-85380 is characterized by slow imaging kinetics with scan times exceeding several hours, which causes patient discomfort.[10] It has been reported that decreasing the basicity of the secondary amine is an efficient strategy to obtain ligands with faster brain imaging kinetics, as is the case for [^{123}I]niodene,[11] in which a five-membered 2,5-dihydro-1*H*-pyrrole ring was introduced in place of the azetidine ring of the model compound 5-[^{123}I]A-85380 (Figure 1). Inspired by this work, we designed new niodene analogues characterized by a basic pyrrolidine nucleus. To attain optimal pK_a, which guarantees a correct balance between protonated and non-protonated forms, respectively necessary for receptor interac-

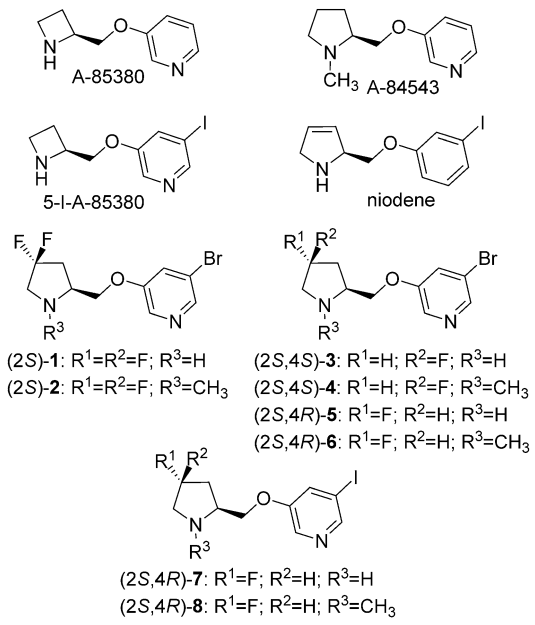


Figure 1. Structure of model compounds and target compounds **1**–**8**.

[a] *Dr. L. Tamborini, Dr. A. Pinto, Prof. P. Conti, Prof. C. De Micheli*
Dipartimento di Scienze Farmaceutiche
Università degli Studi di Milano
Via Mangiagalli 25, 20133 Milano (Italy)
E-mail: andrea.pinto@unimi.it

[b] *Dr. R. Ettari*
Dipartimento di Scienze del Farmaco e Prodotti per la Salute
Università degli Studi di Messina
Viale Annunziata 98168, Messina (Italy)

[c] *Dr. C. Gotti, Dr. F. Fasoli*
Dipartimento di Biotecnologie Mediche e Medicina Traslazionale
CNR, Istituto di Neuroscienze, Università degli Studi di Milano
Via Vanvitelli 32, 20129 Milan (Italy)

tion and blood–brain barrier permeability, we investigated the possibility of tuning the basicity of the amine by the insertion of electron-withdrawing groups at the 3-position of the pyrrolidine ring (Figure 1). As reported by Morgenthaler et al., the introduction of a fluorine atom at the β-position to an amine should produce a pK_a shift of ~1.7 units for each fluorine atom.[12] Therefore, we designed compounds **1**–**6**, structurally related to model compounds A-85380, A-84543, and niodene, in which the basic nucleus is represented by a secondary or tertiary amino group installed into a pyrrolidine nucleus, which was differently functionalized in order to tune its basicity; in particular, we inserted one or two fluorine atoms at the 3-position of the pyrrolidine nucleus (Figure 1).

Notably, the presence of a single fluorine atom at the 3-position generated a second stereogenic center; consequently, both stereoisomers were prepared. On the basis of preliminary binding assays, derivative **5** was selected as a model compound for an in-depth structure–activity relationship (SAR) investigation. Modifications with groups known to influence affinity and selectivity at the $\alpha_4\beta_2$ subtype were introduced at the 3-position of the pyridine ring to generate derivatives **7**–**13**. As reported for model compound A-85380,[13] we replaced the 3-bromo function with an iodo group (compounds **7**–**8**, Figure 1), as in niodene, or with a phenyl, phenylacetylene or 5-hexyn-1-ol groups, as in model compounds **A**, **B**, and sazetine-A (compounds **9**–**13**, Figure 2).

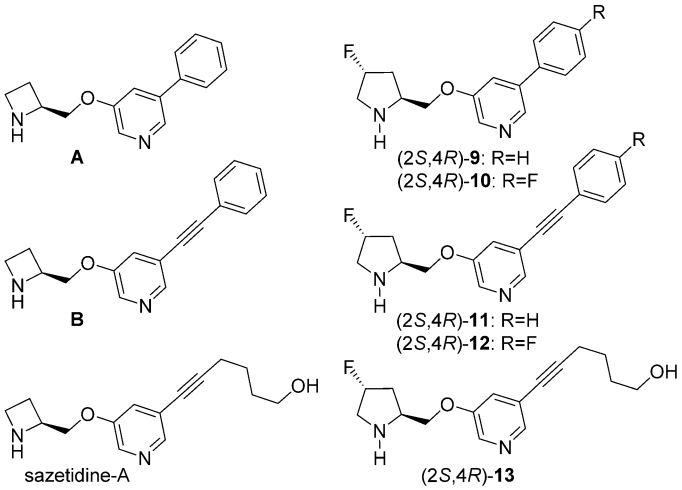
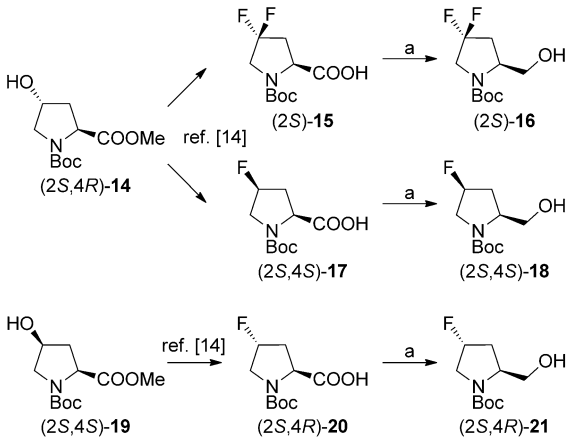


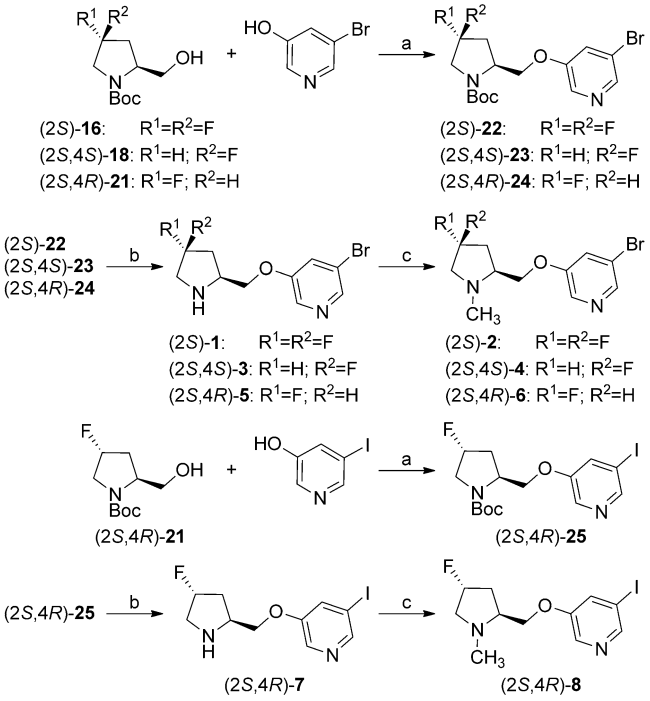
Figure 2. Structure of model compounds and target compounds **9**–**13**.



Scheme 1. *Reagents and conditions*: a) 1 M BH_3/THF, Δ, 4 h.

sponding alcohols (2*S*)-**16**, (2*S*,4*S*)-**18**, and (2*S*,4*R*)-**21**, respectively (Scheme 1).

Mitsunobu coupling of the properly functionalized alcohols (2*S*)-**16**, (2*S*,4*S*)-**18**, and (2*S*,4*R*)-**21** with 3-bromo-5-pyridinol provided the desired ethers (2*S*)-**22**, (2*S*,4*S*)-**23**, and (2*S*,4*R*)-**24** in very good yields (83–92%; Scheme 2). Compounds (2*S*)-**22**, (2*S*,4*S*)-**23**, and (2*S*,4*R*)-**24** were treated with a 30% solution of trifluoroacetic acid in dichloromethane to afford (2*S*)-**1**, (2*S*,4*S*)-**3**, and (2*S*,4*R*)-**5**, respectively. Finally, the *N*-methylpyrrolidine derivatives were prepared by reductive amination, using formaldehyde and sodium cyanoborohydride, of secondary amines (2*S*)-**1**, (2*S*,4*S*)-**3**, and (2*S*,4*R*)-**5** to give (2*S*)-**2**, (2*S*,4*S*)-**4**, and (2*S*,4*R*)-**6** in 34–80% yields, respectively (Scheme 2). Using the



Scheme 2. *Reagents and conditions*: a) DIAD, PPh_3, THF, 24 h; b) TFA, CH_2Cl_2, 4 h; c) HCHO (37 wt.% in H_2O), $NaBH_3CN$, CH_3CN, 2 h.

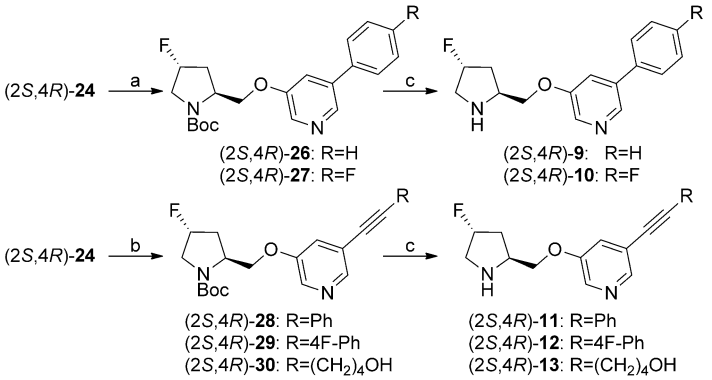
Results and Discussion

Chemistry

Synthesis of the target derivatives was performed starting from enantiomerically pure acids (2*S*)-**15**, (2*S*,4*S*)-**17**, and (2*S*,4*R*)-**20**, which were conveniently prepared by starting from protected *trans*-4-hydroxy-L-proline (2*S*,4*R*)-**14** or *cis*-4-hydroxy-L-proline (2*S*,4*S*)-**19**, following reported procedures (Scheme 1).[14] Reduction of the carboxylic acid function of (2*S*)-**15**, (2*S*,4*S*)-**17**, and (2*S*,4*R*)-**20** with 1 N BH_3/THF gave the corre-

same synthetic strategy, alcohol (2*S*,4*R*)-**21** was submitted to Mitsunobu reaction with 3-iodo-5-pyridinol to afford intermediate (2*S*,4*R*)-**25**, which was deprotected at the nitrogen atom to give compound (2*S*,4*R*)-**7**, which, in turn, was converted into the corresponding tertiary amine (2*S*,4*R*)-**8**, through reductive amination (Scheme 2).

Compounds (2*S*,4*R*)-**9** and (2*S*,4*R*)-**10** were synthesized by applying the Suzuki reaction to the key intermediate (2*S*,4*R*)-**24** and phenylboronic acid or 4-fluorophenylboronic acid, respectively, followed by Boc deprotection (Scheme 3). On the other hand, phenylacetylene, 4-fluorophenylacetylene, or 5-hexyn-1-ol were added to key intermediate (2*S*,4*R*)-**24** under Sonogashira reaction conditions to obtain derivatives (2*S*,4*R*)-**28**, (2*S*,4*R*)-**29**, and (2*S*,4*R*)-**30**, respectively, which were finally treated with a 30% solution of trifluoroacetic acid in dichloromethane to afford the desired compounds (2*S*)-**11**, (2*S*,4*R*)-**12**, and (2*S*,4*R*)-**13** (Scheme 3).



Scheme 3. *Reagents and conditions*: a) $Pd(PPh_3)_4$, 2 M Na_2CO_3, $ArB(OH)_2$, toluene/EtOH (3:1), 90 °C, 18 h; b) CuI, $Pd(PPh_3)_2Cl_2$, Et_3N, 90 °C, 18 h; c) TFA, CH_2Cl_2, 4 h.

Biology

The binding affinities of all new derivatives were determined in binding competition studies using rat cortical membranes labeled with [^{3}H]epibatidine for the $\alpha_4\beta_2$ receptors, and rat hippocampal membranes labeled with [^{125}I]α-bungarotoxin for the α_7 subtype.[15] The compounds were also tested for their affinity at human $\alpha_3\beta_4$ subunits transfected in HEK 293 cells, using [^{3}H]epibatidine as radioligand.[15] All the results are reported in Table 1. Interestingly, all the compounds show negligible affinity for α_7 nAChRs. Whereas the difluoro derivatives (2*S*)-**1** and (2*S*)-**2** show only micromolar affinity for both the $\alpha_4\beta_2$ and $\alpha_3\beta_4$ receptors, the monofluoro derivatives (2*S*,4*R*)-**3**–**6** display nanomolar affinities for the $\alpha_4\beta_2$ receptor subtype and notable selectivity (one or two orders of magnitude) over the $\alpha_3\beta_4$ subtype. The modest affinity displayed by (2*S*)-**1** and (2*S*)-**2** could be explained by the presence of two fluorine atoms on the pyrrolidine ring which may not be well tolerated in the binding pocket; moreover, these may decrease the basicity of the amine too much (i.e., compound (2*S*)-**2**, $pK_a = 5.15$). Conversely, the insertion of only one fluorine atom seems to be better tolerated and confers a pK_a value in the range 6.98–8.80, which guarantees a correct balance between the protonated and non-protonated forms, necessary for both receptor interaction and membrane permeability. Considering the first set of derivatives **1**–**6**, derivative (2*S*,4*R*)-**5** displayed the best affinity/selectivity profile, and therefore, it was selected for further SAR studies involving modifications at the pyridine nucleus, generating compounds **7**–**13**. Within this second set of derivatives, (2*S*,4*R*)-**11** and (2*S*,4*R*)-**12** showed higher

Table 1. Binding affinities, ligand efficiencies, and predicted physicochemical properties of compounds **1**–**13**.

Compd	K_i [nM][a] $\alpha_4\beta_2$[b]	$\alpha_3\beta_4$[c]	α_7[d]	LE[e] [kcal mol^{-1}]	M_r [Da][f]	clog P[g]	PSA [Å^2][h]	pK_{a1}/pK_{a2}[i]	log BB[j]
Nicotine[15b]	10.0	256	234	0.91	162.23	0.883	16.13	8.86/2.70	−0.234
Niodene[11]	0.27	ND	ND	0.94	302.11	2.164	34.15	9.77/3.05	−0.037
(2*S*)-**1**	1160 ± 500	34 000 ± 18 700	$>50\times10^3$	0.51	293.11	2.474	34.15	7.02/3.01	+0.010
(2*S*)-**2**	26 500 ± 14 000	11 2000 ± 57 120	$>50\times10^3$	0.39	307.13	3.019	25.36	5.18/2.73	+0.223
(2*S*,4*S*)-**3**	22.7 ± 7.9	383 ± 137.8	$>50\times10^3$	0.70	275.12	2.181	34.15	8.79/3.00	−0.035
(2*S*,4*S*)-**4**	34.7 ± 12.4	1080 ± 450	$>50\times10^3$	0.68	289.14	2.726	25.36	6.98/2.72	+0.178
(2*S*,4*R*)-**5**	10.9 ± 3.5	1430 ± 800	$>50\times10^3$	0.73	275.12	2.181	34.15	8.79/3.00	−0.035
(2*S*,4*R*)-**6**	12.1 ± 4.1	143 ± 61.4	$>50\times10^3$	0.72	289.14	2.726	25.36	6.98/2.72	+0.178
(2*S*,4*R*)-**7**	76.3 ± 29.7	126 ± 49.1	$>50\times10^3$	0.65	322.12	2.441	34.15	8.79/3.06	+0.005
(2*S*,4*R*)-**8**	29.0 ± 8.4	53.3 ± 18.1	$>50\times10^3$	0.69	336.14	2.986	25.36	6.99/2.77	+0.218
(2*S*,4*R*)-**9**	32.5 ± 18.2	497 ± 114.3	1800 ± 684	0.51	272.32	3.165	34.15	8.80/4.34	+0.115
(2*S*,4*R*)-**10**	14.1 ± 8	95 ± 38	3600 ± 2600	0.51	290.31	3.320	34.15	8.80/4.35	+0.138
(2*S*,4*R*)-**11**	7.4 ± 2.8	306 ± 125.5	16 000 ± 1400	0.51	296.34	3.915	34.15	8.79/2.63	+0.229
(2*S*,4*R*)-**12**	7.5 ± 3.6	475 ± 156.7	36 000 ± 2880	0.49	314.33	4.058	34.15	8.79/2.63	+0.251
(2*S*,4*R*)-**13**	24.1 ± 13.5	430 ± 141.9	34 000 ± 3000	0.50	292.35	1.01	54.38	8.79/3.77	−0.112

[a] Values are the mean ± SEM, and were calculated with the LIGAND program to fit the data obtained from three independent saturation and competition binding experiments for each compound that, for each dilution, was tested in triplicate. All the compounds on each subtype have been tested in three separate experiments. ND: not determined. [b] Determined in binding competition studies using rat cortical membranes labeled with [^{3}H]epibatidine. [c] Determined with human $\alpha_3\beta_4$ subunits transfected in HEK 293 cells, using [^{3}H]epibatidine as radioligand. [d] Determined with rat hippocampal membranes labeled with [^{125}I]α-bungarotoxin.[15] [e] Ligand binding efficiency was calculated according to the Hopkins equation: $LE = 1.372\times[-\log K_i(\text{moles})]/N$. [f,g] Molecular weight and clog P were calculated from ChemBioDraw Ultra 11.0. [h,i] Polar surface area and pK_a were calculated from www.chemicalize.org. [j] log BB was calculated from Clark's equation: $\log BB = -0.0148\,PSA + 0.152\log P + 0.139$.

affinity for the $\alpha_4\beta_2$ receptor subtype ($K_i = 7.4$ and 7.5 nM, respectively) and good selectivity over $\alpha_3\beta_4$ and α_7 subtypes.

For all the tested derivatives, we calculated the ligand efficiency (LE), which is an important metric in drug discovery and has been used to measure the relationship between ligand affinity and molecular size.[16] LE is a useful optimization tool to evaluate a ligand's ability to bind effectively the target protein. Considering the binding affinity (K_i) of the compounds, we calculated the LE using the Hopkins equation.[17] LE $\geq$ 0.3 is considered favorable and suggests that a compound is optimized for receptor occupancy. All the new derivatives possess LE values in the range of 0.4–0.7 kcal mol^{-1}.

To identify the most promising derivatives in terms of good CNS penetration, we evaluated a series of physicochemical properties that influence blood–brain barrier (BBB) penetration,[18,19] such as molecular weight (M_r), polar surface area (PSA), basicity (pK_a), and lipophilicity (clog P). These parameters were calculated for all new compounds and are listed in Table 1. All the compounds in this report, with the exception of (2*S*,4*R*)-**13**, have a $M_r < 350$ Da, clog $P > 2$, and PSA < 40 Å^2, which taken together are predictive of good BBB penetration. Using Clark's equation, we also calculated the log BB, a model for the prediction of blood–brain partitioning.[20–22] From this analysis, we highlighted derivatives (2*S*,4*R*)-**11** and (2*S*,4*R*)-**12** as the most promising ligands identified in this study, since beside their good affinity and selectivity profile for the $\alpha_4\beta_2$ subtype, they showed improved physicochemical properties over compound (2*S*,4*R*)-**5**. In particular, whereas compound (2*S*,4*R*)-**5** was characterized by a negative log BB value (−0.035), the insertion of the additional phenyl acetylene moiety conferred to derivatives (2*S*,4*R*)-**11** and (2*S*,4*R*)-**12** an increased lipophilicity, resulting in positive log BB values (i.e., +0.229 and +0.225, respectively).

Conclusions

In summary, we have developed a novel series of $\alpha_4\beta_2$ ligands characterized by the 3-fluoropyrrolidine moiety, endowed with nanomolar receptor affinity. Further decoration of the pyridine nucleus has allowed significant improvements in physicochemical properties important for BBB penetration, leading to derivatives (2*S*,4*R*)-**11** and (2*S*,4*R*)-**12**, which display single-digit nanomolar affinity for the $\alpha_4\beta_2$ receptor. Considering that compound (2*S*,4*R*)-**12** is characterized by the presence of a fluorine atom at the *para* position of the aromatic ring, it is easy to foresee the development of [^{18}F](2*S*,4*R*)-**12** as a radiolabeled ligand for PET imaging applications.

Experimental Section

Materials and methods: All reagents were purchased from Sigma. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm, and coupling constants (J) are expressed in Hz. Optical rotation determinations were carried out using a Jasco P-1010 spectropolarimeter, coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Melting points were determined on a model B540 Büchi apparatus and are uncorrected. MS analyses were performed on a Varian 320-MS triple quadrupole mass spectrometer with ESI source. Microanalyses (C, H, N) of new compounds were within ±0.4% of theoretical values.

General procedure for the synthesis of *N*-Boc-protected alcohols: To a stirred solution of the *N*-Boc-protected carboxylic acid derivative (1.5 mmol) in THF (10 mL) was added 1 M BH_3 solution in THF (3.2 mL). The resulting mixture was stirred at 70 °C for 4 h and then AcOH (1 mL) was carefully added. EtOAc (50 mL) was added and the organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (cyclohexane/EtOAc).

(*S*)-*tert*-Butyl 4,4-difluoro-2-(hydroxymethyl)pyrrolidine-1-carboxylate [(2*S*)-16]: Yield: 84% (colorless oil); $R_f = 0.70$ (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20} = -34.0$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.26$–4.02 (m, 1H), 3.90–3.52 (m, 4H), 2.55–2.38 (m, 1H), 2.30–2.04 (m, 1H), 1.48 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 156.0, 126.5 (t, $J = 249.1$), 81.7, 65.6, 58.5, 54.2 (t, $J = 30.9$), 36.7 (t, $J = 23.8$), 28.5 ppm; MS: 238.2 $[M+H]^+$; Anal. calcd for $C_{10}H_{17}F_2NO_3$: C 50.63, H 7.22, N 5.90, found: C 50.95, H 7.44, N 5.70.

(2*S*,4*S*)-*tert*-Butyl 4-fluoro-2-(hydroxymethyl)pyrrolidine-1-carboxylate [(2*S*,4*S*)-18]: Yield: 80% (colorless oil); $R_f = 0.40$ (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20} = -22.6$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.30$–5.00 (m, 1H), 4.20–4.00 (m, 1H), 3.85–4.75 (m, 1H), 3.70–3.40 (m, 3H), 2.40–1.95 (m, 2H), 1.40 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.8$, 91.4 (d, $J = 176.3$), 81.0, 67.6, 59.3, 54.2 (d, $J = 23.8$), 35.2 (d, $J = 21.2$), 28.5 ppm; MS: 220.1 $[M+H]^+$; Anal. calcd for $C_{10}H_{18}FNO_3$: C 54.78, H 8.27, N 6.39, found: C 54.98, H 8.47, N 6.15.

(2*S*,4*R*)-*tert*-Butyl 4-fluoro-2-(hydroxymethyl)pyrrolidine-1-carboxylate [(2*S*,4*R*)-21]: Yield: 80% (colorless oil); $R_f = 0.40$ (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20} = -47.6$ ($c = 1.4$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.22$–5.00 (m, 1H), 4.22–4.05 (m, 1H), 3.95–3.70 (m, 2H), 3.60–3.32 (m, 2H), 2.40–2.23 (m, 1H), 1.90–1.58 (m, 1H), 1.45 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 157.0$, 91.4 (d, $J = 176.6$), 81.2, 66.7, 59.1, 54.4 (d, $J = 23.2$), 35.7 (d, $J = 22.0$), 28.6 ppm; MS: 220.2 $[M+H]^+$; Anal. calcd for $C_{10}H_{18}FNO_3$: C 54.78, H 8.27, N 6.39, found: C 54.70, H 8.42, N 6.20.

General procedure for the Mitsunobu reaction: To a solution of the *N*-Boc-protected alcohol (1.5 mmol), 3-bromo-5-hydroxypyridine (1.7 mmol) and PPh_3 (2.3 mmol) in dry THF (20 mL), stirred under argon at 0 °C, diisopropyl azodicarboxylate (DIAD; 2.3 mmol) was added. The reaction mixture was allowed to warm to room temperature over a period of 2 h and was stirred for 24 h. The reaction mixture was evaporated to dryness and the residue was purified by flash chromatography (cyclohexane/EtOAc).

(*S*)-*tert*-Butyl 2-((5-bromopyridin-3-yloxy)methyl)-4,4-difluoropyrrolidine-1-carboxylate [(2*S*)-22]: Yield: 83% (thick colorless oil); $R_f = 0.60$ (cyclohexane/EtOAc 7:3); $[\alpha]_D^{20} = -42.85$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.26$ (bs, 1H), 8.22 (d, $J = 2.7$, 1H), 7.38 (bs, 1H), 4.46–4.26 (m, 1H), 4.26–3.55 (m, 4H), 2.66–2.42 (m, 2H), 1.44 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 155.1$, 154.1, 143.6, 136.7, 127.1 (t, $J = 250.8$), 124.2, 120.6, 81.3, 68.2, 54.6, 54.1 (t, $J = 32.1$), 36.5 (t, $J = 24.9$), 28.5 ppm; MS: 393.1 $[M+H]^+$; Anal. calcd for $C_{15}H_{19}BrF_2N_2O_3$: C 45.82, H 4.87, N 7.12, found: C 45.70, H 4.97, N 7.03.

(2*S*,4*S*)-*tert*-Butyl 2-((5-bromopyridin-3-yloxy)methyl)-4-fluoropyrrolidine-1-carboxylate [(2*S*,4*S*)-23]: Yield: 91%; (colorless

solid); R_f = 0.45 (cyclohexane/EtOAc 7:3); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: >118 °C (dec.); $[\alpha]_D^{20}$ = −53.60 (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.35–8.25 (m, 2H), 7.50–7.40 (m, 1H), 5.25 (bd, *J* = 52.3, 1H), 4.44–4.22 (m, 2H), 4.00–3.85 (m, 1H), 3.80–3.50 (m, 2H), 2.56–2.38 (m, 1H), 2.34–2.04 (m, 1H), 1.50 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.4, 154.4, 143.3, 136.9, 124.1, 120.6, 93.1 (d, *J* = 174.9), 80.8, 68.8, 55.3, 53.8 (d, *J* = 23.8), 34.8 (d, *J* = 19.5), 28.7 ppm; MS: 375.1 $[M+H]^+$; Anal. calcd for $C_{15}H_{20}BrFN_2O_3$: C 48.01, H 5.37, N 7.47, found: C 47.84, H 5.49, N 7.34.

(2*S*,4*R*)-*tert*-Butyl 2-((5-bromopyridin-3-yloxy)methyl)-4-fluoropyrrolidine-1-carboxylate [(2*S*,4*R*)-24]: Yield: 92%; (colorless solid); R_f = 0.45 (cyclohexane/EtOAc 7:3); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: 62–64 °C; $[\alpha]_D^{20}$ = −39.00 (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.25 (bs, 1H), 8.20 (d, *J* = 2.7, 1H), 7.36 (bs, 1H), 5.20 (bd, *J* = 53.0, 1H), 4.44–4.22 (m, 2H), 4.18–4.10 (m, 1H), 4.08–3.76 (m, 1H), 3.43 (ddd, *J* = 35.7, 13.2, 3.6, 1H), 2.56–2.35 (m, 1H), 2.23 (dddd, *J* = 37.7, 14.6, 7.7, 4.7, 1H), 1.45 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 154.8, 143.4, 137.0, 124.3, 120.6, 91.9 (d, *J* = 172.3), 80.6, 69.8, 55.2, 54.1 (d, *J* = 22.9), 35.3 (d, *J* = 20.6), 28.7 ppm; MS: 375.1 $[M+H]^+$; Anal. calcd for $C_{15}H_{20}BrFN_2O_3$: C 48.01, H 5.37, N 7.47, found: C 47.94, H 5.36, N 7.44.

(2*S*,4*R*)-*tert*-Butyl 2-((5-iodopyridin-3-yloxy)methyl)-4-fluoropyrrolidine-1-carboxylate [(2*S*,4*R*)-25]: Yield: 86% (colorless solid); R_f = 0.40 (cyclohexane/EtOAc 75:25); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: 41–43 °C; $[\alpha]_D^{20}$ = −40.15 (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.40 (bs, 1H), 8.21 (d, *J* = 2.5, 1H), 7.36 (bs, 1H), 5.20 (bd, *J* = 52.5, 1H), 4.42–4.22 (m, 2H), 4.18–4.08 (m, 1H), 4.08–3.76 (m, 1H), 3.43 (ddd, *J* = 35.7, 13.2, 3.3, 1H), 2.56–2.35 (m, 1H), 2.21 (dddd, *J* = 37.7, 14.3, 7.7, 4.1, 1H), 1.44 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 154.8, 148.4, 137.0, 129.8, 93.1, 91.0 (d, *J* = 174.1), 80.5, 69.1, 55.2, 54.1 (d, *J* = 22.9), 36.0 (d, *J* = 21.3), 28.7 ppm; MS: 423.1 $[M+H]^+$; Anal. calcd for $C_{15}H_{20}BrFN_2O_3$: C 42.67, H 4.77, N 6.63, found: C 42.57, H 4.92, N 6.51.

General procedure for *N*-Boc deprotection: Mitsunobu adduct (1.0 mmol) was treated with a 30% CH_2Cl_2 solution of trifluoroacetic acid (TFA; 10.0 mmol) at 0 °C, and the solution was stirred at room temperature for 4 h. The volatiles were removed under vacuum, aqueous 1 N NaOH (5 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, evaporated to dryness and the residue was purified by flash chromatography (EtOAc or CH_2Cl_2/MeOH).

3-(((*S*)-4,4-Difluoropyrrolidin-2-yl)methoxy)-5-bromopyridine [(2*S*)-1]: Yield: 75% (white solid); R_f = 0.40 (EtOAc); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: 52–54 °C; $[\alpha]_D^{20}$ = + 8.83 (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.30 (d, *J* = 1.9, 1H), 8.23 (d, *J* = 2.5, 1H), 7.37 (dd, *J* = 1.9, 2.5, 1H), 4.03 (dd, *J* = 5.0, 9.4, 1H), 3.98 (dd, *J* = 6.1, 9.4, 1H), 3.82–3.72 (m, 1H), 3.34 (ddd, *J* = 12.4, 12.4, 12.4, 1H), 3.23 (ddd, *J* = 12.4, 12.4, 12.4, 1H), 2.52–2.34 (m, 1H), 2.24 (bs, 1H), 2.22–2.00 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.2, 143.7, 136.5, 131.08 (t, *J* = 250.8), 124.3, 120.6, 70.8, 56.1 (t, *J* = 4.5), 54.5 (t, *J* = 29.2), 38.3 ppm (t, *J* = 25.0); MS: 293.0 $[M+H]^+$; Anal. calcd for $C_{10}H_{11}BrF_2N_2O$: C 40.98, H 3.78, N 9.56, found: C 40.90, H 3.97, N 9.35.

3-(((2*S*,4*S*)-4-Fluoropyrrolidin-2-yl)methoxy)-5-bromopyridine [(2*S*,4*S*)-3]: Yield: 82% (white solid); R_f = 0.30 (CH_2Cl_2/MeOH 9:1); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: 69–71 °C; $[\alpha]_D^{20}$ = + 6.70 (*c* = 0.7 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.25 (d, *J* = 1.7, 1H), 8.20 (d, *J* = 2.7, 1H), 7.34 (dd, *J* = 2.7, 1.7, 1H), 5.22 (ddd, *J* = 54.5, 4.4, 4.4, 1H), 4.12–3.94 (m, 2H), 3.62–3.50 (m, 1H), 3.34 (dd, *J* = 21.2, 13.0, 1H), 2.95 (ddd, *J* = 35.5, 13.0, 4.4, 1H), 2.34–2.10 (m, 1H), 2.22 (bs, 1H), 1.93 ppm (ddd, *J* = 28.3, 14.6, 4.4, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.4, 143.4, 136.7, 124.2, 120.6, 94.7 (d, *J* = 175.2), 71.5, 56.9, 54.1 (d, *J* = 22.9), 36.1 ppm (d, *J* = 21.2); MS: 275.0 $[M+H]^+$; Anal. calcd for $C_{10}H_{12}BrFN_2O$: C 43.66, H 4.40, N 10.18, found: C 43.52, H 4.58, N 9.95.

3-(((2*S*,4*R*)-4-Fluoropyrrolidin-2-yl)methoxy)-5-bromopyridine [(2*S*,4*R*)-5]: Yield: 85% (thick colorless oil); R_f = 0.40 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{20}$ = + 21.60 (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.24 (d, *J* = 1.7, 1H), 8.20 (d, *J* = 2.7, 1H), 7.34 (dd, *J* = 2.7, 1.7, 1H), 5.22 (ddd, *J* = 53.5, 3.9, 3.9, 1H), 3.96–3.85 (m, 2H), 3.85–3.75 (m, 1H), 3.30–3.15 (m, 1H), 3.05 (ddd, *J* = 36.0, 12.6, 3.9, 1H), 2.35–2.16 (m, 1H), 2.34 (bs, 1H), 1.73 ppm (dddd, *J* = 39.3, 14.6, 8.5, 3.9, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 143.3, 136.6, 124.2, 120.5, 95.2 (d, *J* = 174.6), 71.9, 55.9, 53.6 (d, *J* = 22.6), 36.5 ppm (d, *J* = 21.1); MS: 275.0 $[M+H]^+$; Anal. calcd for $C_{10}H_{12}BrFN_2O$: C 43.66, H 4.40, N 10.18, found: C 43.62, H 4.48, N 10.02.

3-(((2*S*,4*R*)-4-Fluoropyrrolidin-2-yl)methoxy)-5-iodopyridine [(2*S*,4*R*)-7]: Yield: 85% (thick colorless oil); R_f: 0.30 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{20}$ = + 20.80 (*c* = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.38 (d, *J* = 1.6, 1H), 8.22 (d, *J* = 2.5, 1H), 7.52 (dd, *J* = 2.5, 1.6, 1H), 5.22 (ddd, *J* = 53.9, 3.9, 3.9, 1H), 3.96–3.85 (m, 2H), 3.85–3.75 (m, 1H), 3.32–3.16 (m, 1H), 3.05 (ddd, *J* = 36.0, 13.6, 3.9, 1H), 2.34–2.18 (m, 1H), 2.28 (bs, 1H), 1.73 ppm (dddd, *J* = 39.3, 14.3, 8.5, 3.9, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 148.3, 137.0, 129.7, 95.3 (d, *J* = 174.1), 93.0, 71.9, 55.9, 53.6 (d, *J* = 22.9), 36.5 ppm (d, *J* = 21.3); MS: 323.0 $[M+H]^+$; Anal. calcd for $C_{10}H_{12}FIN_2O$: C 37.29, H 3.75, N 8.70, found: C 37.39, H 3.97, N 8.49.

3-(((2*S*,4*R*)-4-Fluoropyrrolidin-2-yl)methoxy)-5-phenylpyridine [(2*S*,4*R*)-9]: Yield: 82%; (colorless oil); R_f = 0.40 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{20}$ = + 19.10 (*c* = 1.05 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (d, *J* = 1.9, 1H), 8.28 (d, *J* = 2.7, 1H), 7.56–7.50 (m, 2H), 7.48–7.32 (m, 4H), 5.25 (ddd, *J* = 54.2, 4.0, 4.0, 1H), 4.03 (dd, *J* = 9.1, 5.5, 1H), 3.98 (dd, *J* = 9.1, 5.5, 1H), 3.90–3.80 (m, 1H), 3.28 (dd, *J* = 24.5, 13.2, 1H), 3.12 (ddd, *J* = 35.7, 13.2, 4.0, 1H), 2.64 (bs, 1H), 2.29 (ddd, *J* = 23.1, 14.6, 7.1, 1H), 1.81 ppm (dddd, *J* = 39.0, 14.6, 8.8, 4.0, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.3, 141.0, 137.7, 137.6, 136.5, 129.3, 128.5, 127.4, 120.1, 95.2 (d, *J* = 174.3), 71.5, 56.1, 53.6 (d, *J* = 22.6), 36.6 ppm (d, *J* = 21.2); MS: 273.1 $[M+H]^+$; Anal. calcd for $C_{16}H_{17}FN_2O$: C 70.57, H 6.29, N 10.29, found: C 70.35, H 6.57, N 9.90.

3-(4-Fluorophenyl)-5-(((2*S*,4*R*)-4-fluoropyrrolidin-2-yl)methoxy)-pyridine [(2*S*,4*R*)-10]: Yield: 84%; (colorless oil); R_f = 0.40 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{20}$ = + 17.30 (*c* = 1.10 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.39 (d, *J* = 1.9, 1H), 8.26 (d, *J* = 2.7, 1H), 7.52–7.45 (m, 2H), 7.31 (dd, *J* = 2.7, 1.9, 1H), 7.16–7.08 (m, 2H), 5.25 (ddd, *J* = 54.2, 4.1, 4.1, 1H), 4.03 (dd, *J* = 9.1, 5.2, 1H), 3.98 (dd, *J* = 9.1, 5.8, 1H), 3.92–3.80 (m, 1H), 3.28 (dd, *J* = 23.9, 13.2, 1H), 3.13 (ddd, *J* = 35.2, 13.2, 4.1, 1H), 2.77 (bs, 1H), 2.29 (dddd, *J* = 22.8, 14.6, 6.9, 1.1, 1H), 1.81 ppm (dddd, *J* = 39.0, 14.6, 8.8, 4.1, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.2 (d, *J* = 248.2), 155.3, 140.8, 136.7, 136.5, 133.8 (d, *J* = 3.2), 129.1 (d, *J* = 8.3), 120.0, 116.3 (d, *J* = 21.7), 95.1 (d, *J* = 175.2), 71.4, 56.1, 53.6 (d, *J* = 23.2), 36.5 ppm (d, *J* = 21.5); MS: 291.1 $[M+H]^+$; Anal. calcd for $C_{16}H_{16}F_2N_2O$: C 66.20, H 5.56, N 9.65, found: C 66.15, H 5.80, N 9.35.

3-(((2*S*,4*R*)-4-Fluoropyrrolidin-2-yl)methoxy)-5-(phenylethynyl)-pyridine [(2*S*,4*R*)-11]: Yield: 87%; (colorless solid); R_f = 0.50 (CH_2Cl_2/MeOH 9:1); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: 85–88 °C; $[\alpha]_D^{20}$ = + 23.30 (*c* = 1.10 in $CHCl_3$); ^{1}H NMR (300 MHz,

$CDCl_3$): δ = 8.37 (d, J = 1.1, 1H), 8.22 (d, J = 2.7, 1H), 7.58–7.50 (m, 2H), 7.40–7.34 (m, 3H), 7.34–7.30 (m, 1H), 5.22 (ddd, J = 54.2, 4.0, 4.0, 1H), 4.02–3.94 (m, 2H), 3.90–3.80 (m, 1H), 3.28 (dd, J = 24.2, 13.2, 1H), 3.12 (ddd, J = 35.5, 13.2, 4.0, 1H), 2.38–2.22 (m, 1H), 2.30 (bs, 1H), 1.79 ppm (dddd, J = 39.0, 14.3, 8.5, 4.0, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.6, 145.0, 137.9, 131.9, 129.1, 128.7, 123.2, 122.7, 120.8, 95.2 (d, J = 174.7), 92.7, 86.0, 71.6, 56.0, 53.6 (d, J = 22.9), 36.5 ppm (d, J = 21.5); MS: 297.1 $[M+H]^+$; Anal. calcd for $C_{18}H_{17}FN_2O$: C 72.95, H 5.78, N 9.45, found: C 72.63, H 6.10, N 9.07.

3-((4-Fluorophenyl)ethynyl)-5-(((2*S*,4*R*)-4-fluoropyrrolidin-2-yl)-methoxy)pyridine [(2*S*,4*R*)-12]: Yield: 88%; (colorless solid); R_f = 0.60 (CH_2Cl_2/MeOH 9:1); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: 102–105 °C; $[\alpha]_D^{20} = +20.60$ (c = 1.10 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.36 (d, J = 1.4, 1H), 8.26 (d, J = 2.7, 1H), 7.55–7.48 (m, 2H), 7.30 (dd, J = 2.7, 1.4, 1H), 7.10–7.03 (m, 2H), 5.28 (ddd, J = 53.9, 4.0, 4.0, 1H), 4.05–3.95 (m, 2H), 3.92–3.82 (m, 1H), 3.30 (dd, J = 25.0, 13.7, 1H), 3.14 (ddd, J = 35.5, 13.7, 4.0, 1H), 2.30 (ddd, J = 22.9, 14.3, 7.7, 1H), 2.08 (bs, 1H), 1.80 ppm (dddd, J = 39.0, 14.3, 8.8, 4.0, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.1 (d, J = 250.5), 154.5, 145.0, 137.9, 133.9 (d, J = 8.3), 123.2, 120.7, 118.8 (d, J = 3.5), 116.3 (d, J = 22.3), 95.0 (d, J = 178.4), 91.7, 85.7, 71.3, 56.1, 53.5 (d, J = 22.9), 36.5 ppm (d, J = 21.2); MS: 315.1 $[M+H]^+$; Anal. calcd for $C_{18}H_{16}F_2N_2O$: C 68.78, H 5.13, N 8.91, found: C 68.63, H 5.45, N 8.58.

6-(5-(((2*S*,4*R*)-4-Fluoropyrrolidin-2-yl)methoxy)pyridin-3-yl)hex-5-yn-1-ol [(2*S*,4*R*)-13]: Yield: 74%; (colorless oil); R_f = 0.20 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D^{20} = +6.70$ (c = 1.20 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.20 (d, J = 1.4, 1H), 8.16 (d, J = 2.7, 1H), 7.17 (dd, J = 2.7, 1.4, 1H), 5.25 (ddd, J = 53.9, 4.0, 4.0, 1H), 3.98 (dd, J = 9.0, 5.2, 1H), 3.94 (dd, J = 9.0, 5.8, 1H), 3.90–3.80 (m, 1H), 3.74–3.66 (m, 2H), 3.28 (ddd, J = 25.0, 13.7, 3.6, 1H), 3.15 (ddd, J = 25.5, 13.7, 4.0, 1H), 2.78 (bs, 2H), 2.50–2.42 (m, 2H), 2.30 (ddd, J = 22.6, 14.6, 6.9, 1H), 1.81 (dddd, J = 39.0, 14.6, 8.8, 4.0, 1H), 1.74–1.62 ppm (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.4, 145.1, 137.1, 123.5, 121.5, 94.9 (d, J = 174.6), 93.9, 77.4, 71.0, 62.4, 56.2, 53.4 (d, J = 22.9), 36.3 (d, J = 20.6), 32.1, 25.1, 19.4 ppm; MS: 293.1 $[M+H]^+$; Anal. calcd for $C_{16}H_{21}FN_2O_2$: C 65.73, H 7.24, N 9.58, found: C 65.70, H 7.50, N 9.19.

General procedure for reductive amination: To a solution of the secondary amine (0.5 mmol) in CH_3CN (3 mL) was added 37% formaldehyde solution (2.5 mmol), and the mixture was stirred for 20 min at room temperature, then $NaBH_3CN$ (0.8 mmol) was added in small portions. After 2 h, the solvent was evaporated at reduced pressure, and 1 N NaOH (5 mL) was added to the residue. The resulting mixture was extracted with CH_2Cl_2 (3×20 mL). The combined organic extracts were washed with water (10 mL), brine (10 mL), dried with anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (cyclohexane/EtOAc).

3-(((*S*)-4,4-Difluoro-1-methylpyrrolidin-2-yl)methoxy)-5-bromopyridine [(2*S*)-2]: Yield: 34% (white solid); R_f = 0.50 (cyclohexane/EtOAc 7:3); crystallized form *n*-hexane/EtOAc as colorless prisms; mp: 41–43 °C; $[\alpha]_D^{20} = -36.01$ (c = 0.7 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.31 (d, J = 1.6, 1H), 8.25 (d, J = 2.5, 1H), 7.38 (dd, J = 2.5, 1.6, 1H), 4.09 (dd, J = 9.6, 5.0, 1H), 4.00 (dd, J = 9.6, 5.2, 1H), 3.50–3.40 (m, 1H), 3.08–2.96 (m, 1H), 2.75 (ddd, J = 16.0, 16.0, 11.0, 1H), 2.60–2.42 (m, 1H), 2.47 (s, 3H), 2.35–2.16 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.3, 143.5, 136.3, 127.8 (dd, J = 246.0, 249.4), 124.6, 120.7, 69.8, 64.2 (t, J = 29.0), 63.0, 41.0, 39.4 ppm (t, J = 24.9); MS: 307.0 $[M+H]^+$; Anal. calcd for $C_{11}H_{13}BrF_2N_2O$: C 43.02, H 4.27, N 9.12, found: C 42.90, H 4.44, N 8.93.

3-(((2*S*,4*S*)-4-Fluoro-1-methylpyrrolidin-2-yl)methoxy)-5-bromopyridine [(2*S*,4*S*)-4]: Yield: 79% (colorless oil); R_f = 0.40 (EtOAc); $[\alpha]_D^{20} = -36.75$ (c = 0.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.26 (d, J = 2.0, 1H), 8.23 (d, J = 2.5, 1H), 7.36 (dd, J = 2.5, 2.0, 1H), 5.11 (ddd, J = 53.9, 4.4, 4.1 1H), 4.06 (dd, J = 9.3, 5.2, 1H), 3.97 (dd, J = 9.3, 5.8, 1H), 3.35 (ddd, J = 17.9, 11.8, 2.2, 1H), 2.80–2.68 (m, 1H), 2.56–2.32 (m, 2H), 2.47 (s, 3H), 2.10–1.90 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 143.3, 136.6, 124.2, 120.6, 92.6 (d, J = 176.6), 71.5, 63.7 (d, J = 21.2), 63.4, 41.6, 36.8 ppm (d, J = 22.3); MS: 289.0 $[M+H]^+$; Anal. calcd for $C_{11}H_{14}BrFN_2O$: C 45.69, H 4.88, N 9.69, found: C 45.54, H 5.02, N 9.51.

3-(((2*S*,4*R*)-4-Fluoro-1-methylpyrrolidin-2-yl)methoxy)-5-bromopyridine [(2*S*,4*R*)-6]: Yield: 80% (colorless oil); R_f = 0.40 (EtOAc); $[\alpha]_D^{20} = -36.55$ (c = 0.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.27 (d, J = 1.7, 1H), 8.23 (d, J = 2.5, 1H), 7.36 (dd, J = 2.5, 1.7, 1H), 5.30–5.04 (m, 1H), 4.01 (dd, J = 9.6, 5.0, 1H), 3.97 (dd, J = 9.6, 5.0, 1H), 3.54 (ddd, J = 26.1, 11.8, 5.8, 1H), 3.10–3.00 (m, 1H), 2.64 (dddd, J = 31.9, 11.8, 3.0, 1.1, 1H), 2.49 (s, 3H), 2.35–2.17 (m, 1H), 1.95 ppm (dddd, J = 33.6, 16.0, 9.9, 5.8, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 143.4, 136.6, 124.3, 120.6, 92.4 (d, J = 174.6), 70.4, 63.3 (d, J = 22.9), 62.9, 41.8, 37.3 ppm (d, J = 21.5); MS: 289.0 $[M+H]^+$; Anal. calcd for $C_{11}H_{14}BrFN_2O$: C 45.69, H 4.88, N 9.69, found: C 45.58, H 4.95, N 9.61.

3-(((2*S*,4*R*)-4-Fluoro-1-methylpyrrolidin-2-yl)methoxy)-5-iodopyridine [(2*S*,4*R*)-8]: Yield: 51% (colorless oil); R_f = 0.20 (EtOAc); $[\alpha]_D^{20} = -35.78$ (c = 1.10 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.27 (d, J = 1.7, 1H), 8.23 (d, J = 2.5, 1H), 7.36 (dd, J = 2.2, 1.7, 1H), 5.30–5.04 (m, 1H), 4.04–3.92 (m, 2H), 3.54 (ddd, J = 26.1, 11.8, 5.8, 1H), 3.10–2.98 (m, 1H), 2.74–2.56 (m, 1H), 2.49 (s, 3H), 2.34–2.18 (m, 1H), 1.95 ppm (dddd, J = 33.6, 15.6, 10.1, 5.8, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 148.4, 137.0, 129.8, 93.0, 92.4 (d, J = 175.5), 70.3, 63.3 (d, J = 23.2), 62.9, 41.8, 37.4 ppm (d, J = 21.5); MS: 337.0 $[M+H]^+$; Anal. calcd for $C_{11}H_{14}FIN_2O$: C 39.30, H 4.20, N 8.33, found: C 39.26, H 4.39, N 8.14.

General procedure for Suzuki coupling reaction: To a solution of (2*S*,4*R*)-**24** (0.40 mmol) in toluene (6 mL) and EtOH (2 mL) was added phenylboronic acid (0.47 mmol), followed by 1.4 mL of 2 M Na_2CO_3 and tetrakis(triphenylphosphine)palladium (0.02 mmol). The reaction was stirred for 18 h at 90 °C under nitrogen. Then, the reaction was cooled to room temperature, diluted with water (10 mL), and extracted with EtOAc (3×5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (cyclohexane/EtOAc).

(2*S*,4*R*)-*tert*-Butyl 4-fluoro-2-((5-phenylpyridin-3-yloxy)methyl)-pyrrolidine-1-carboxylate [(2*S*,4*R*)-26]: Yield: 98%; (colorless oil); R_f = 0.15 (cyclohexane/EtOAc 8:2); $[\alpha]_D^{20} = -32.20$ (c = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.47 (bs, 1H), 8.28 (d, J = 2.5, 1H), 7.58–7.52 (m, 2H), 7.50–7.34 (m, 4H), 5.22 (bd, J = 53.1, 1H), 4.50–4.26 (m, 2H), 4.24–4.16 (m, 1H), 4.08–3.76 (m, 1H), 3.46 (ddd, J = 36.2, 12.9, 3.6, 1H), 2.58–2.14 (m, 2H), 1.44 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5, 154.8, 140.6, 137.8, 137.6, 136.4, 129.3, 128.6, 127.6, 120.2, 92.0 (d, J = 177.8), 80.4, 69.2, 55.3, 53.9 (d, J = 23.2), 35.9 (d, J = 20.9), 28.6 ppm; MS: 373.2 $[M+H]^+$; Anal. calcd for $C_{21}H_{25}FN_2O_3$: C 67.72, H 6.77, N 7.52, found: C 67.61, H 7.03, N 7.23.

(2*S*,4*R*)-*tert*-Butyl 4-fluoro-2-((5-(4-fluorophenyl)pyridin-3-yloxy)-methyl)pyrrolidine-1-carboxylate [(2*S*,4*R*)-27]: Yield: 98%; (colorless oil); R_f = 0.25 (cyclohexane/EtOAc 7:3); $[\alpha]_D^{20} = -32.00$ (c = 1.00 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.42 (bs, 1H), 8.28 (d, J = 2.5, 1H), 7.56–7.48 (m, 2H), 7.44–7.28 (m, 1H), 7.20–7.10 (m, 2H),

5.24 (bd, $J=52.8$, 1H), 4.50–4.28 (m, 2H), 4.28–4.16 (m, 1H), 4.14–3.78 (m, 1H), 3.46 (ddd, $J=35.5$, 13.2, 3.6, 1H), 2.58–2.38 (m, 1H), 2.28 (ddd, $J=37.4$, 14.6, 4.4, 1H), 1.44 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=163.2$ (d, $J=248.2$), 155.5, 154.8, 140.6, 136.8, 136.7, 133.6 (d, $J=3.2$), 129.2 (d, $J=8.3$), 120.2, 116.3 (d, $J=21.8$), 91.9 (d, $J=175.7$), 80.5, 69.2, 55.2, 54.0 (d, $J=23.2$), 36.0 (d, $J=21.5$), 28.6 ppm; MS: 391.2 $[M+H]^+$; Anal. calcd for $C_{21}H_{24}F_2N_2O_3$: C 64.60, H 6.20, N 7.18, found: C 64.61, H 6.47, N 6.91.

General procedure for Sonogashira coupling reaction: The Mitsunobu adduct (2*S*,4*R*)-**24** (0.27 mmol), $Pd(PPh_3)_2Cl_2$ (0.014 mmol), and CuI (0.27 mmol) were placed in an oven-dried round-bottom flask with nitrogen. After addition of Et_3N (4 mL), the mixture was stirred at room temperature for 5 min, the desired alkyne (0.74 mmol) was added, and the resulting mixture was stirred at room temperature for 10 min. Then, the mixture was heated at 90 °C for 18 h. After cooling to room temperature, the solution was diluted with water (10 mL), and extracted with EtOAc (3×5 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (cyclohexane/EtOAc).

(2*S*,4*R*)-*tert*-Butyl 4-fluoro-2-((5-(phenylethynyl)pyridin-3-yloxy)methyl)pyrrolidine-1-carboxylate [(2*S*,4*R*)-28]: Yield: 98%; (colorless solid); $R_f=0.33$ (cyclohexane/EtOAc 8:2); crystallized from *n*-hexane/EtOAc as colorless prisms; mp: 92–94 °C; $[\alpha]_D^{20}=-16.53$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.38$ (bs, 1H), 8.22 (bs, 1H), 7.58–7.48 (m, 2H), 7.40–7.26 (m, 4H), 5.20 (bd, $J=53.1$, 1H), 4.48–4.24 (m, 2H), 4.18–4.08 (m, 1H), 4.08–3.76 (m, 1H), 3.43 (ddd, $J=35.5$, 13.2, 3.3, 1H), 2.56–2.12 (m, 2H), 1.45 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=154.5$, 154.4, 145.0, 138.2, 131.9, 129.1, 128.7, 123.2, 122.7, 121.1, 92.8, 91.9 (d, $J=178.1$), 86.0, 80.5, 69.0, 55.1, 54.0 (d, $J=23.5$), 35.7 (d, $J=20.7$), 28.7 ppm; MS: 397.2 $[M+H]^+$; Anal. calcd for $C_{23}H_{25}FN_2O_3$: C 69.68, H 6.36, N 7.07, found: C 69.46, H 6.68, N 6.75.

(2*S*,4*R*)-*tert*-Butyl 4-fluoro-2-((5-((4-fluorophenyl)ethynyl)pyridin-3-yloxy)methyl)pyrrolidine-1-carboxylate [(2*S*,4*R*)-29]: Yield: 98%; (colorless oil); $R_f=0.35$ (cyclohexane/EtOAc 8:2); $[\alpha]_D^{20}=-15.16$ ($c=0.95$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.32$ (bs, 1H), 8.20 (d, $J=2.5$, 1H), 7.54–7.44 (m, 2H), 7.30 (bs, 1H), 7.06–6.98 (m, 2H), 5.20 (bd, $J=53.1$, 1H), 4.45–4.22 (m, 2H), 4.20–4.10 (m, 1H), 4.05–3.75 (m, 1H), 3.42 (ddd, $J=35.7$, 13.2, 3.3, 1H), 2.56–2.12 (m, 2H), 1.43 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=163.0$ (d, $J=250.5$), 154.8, 154.6, 144.9, 138.0, 133.8 (d, $J=8.3$), 123.1, 120.7, 118.8, 116.0 (d, $J=22.0$), 91.9 (d, $J=176.6$), 91.7, 85.7, 80.5, 69.0, 55.1, 54.0 (d, $J=23.7$), 35.0 (d, $J=21.8$), 28.6 ppm; MS: 415.2 $[M+H]^+$; Anal. calcd for $C_{23}H_{24}F_2N_2O_3$: C 66.65, H 5.84, N 6.76, found: C 66.58, H 6.17, N 6.47.

(2*S*,4*R*)-*tert*-Butyl 4-fluoro-2-((5-(6-hydroxyhex-1-ynyl)pyridin-3-yloxy)methyl)pyrrolidine-1-carboxylate [(2*S*,4*R*)-30]: Yield: 95%; (colorless oil); $R_f=0.25$ (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20}=-25.70$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.14$ (bs, 1H), 8.08 (d, $J=2.5$, 1H), 7.14 (bs, 1H), 5.16 (bd, $J=52.8$, 1H), 4.36–4.16 (m, 2H), 4.14–4.02 (m, 1H), 4.00–3.70 (m, 1H), 3.66–3.52 (m, 2H), 3.38 (ddd, $J=35.5$, 13.2, 3.6, 1H), 2.86 (bs, 1H), 2.48–2.36 (m, 2H), 2.30–2.18 (m, 1H), 2.18–2.06 (m, 1H), 1.72–1.48 (m, 4H), 1.45 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=154.8$, 154.6, 144.9, 137.0, 132.2, 123.6, 121.7, 94.0, 91.9 (d, $J=176.0$), 80.5, 77.4, 69.0, 62.1, 55.2, 54.0 (d, $J=22.9$), 36.0 (d, $J=20.6$), 32.0, 28.6, 25.1, 19.2 ppm; MS: 393.2 $[M+H]^+$; Anal. calcd for $C_{21}H_{29}FN_2O_4$: C 64.27, H 7.45, N 7.14, found: C 64.29, H 7.67, N 6.88.

Nicotinic receptor subtype binding: Frozen cortex and hippocampus specimens were taken from adult male Sprague–Dawley rats (Charles River, Calco, Italy). Tissue preparations as well as [^{3}H]epibatidine and [^{125}I]α-bungarotoxin binding to $\alpha_4\beta_2$, $\alpha_3\beta_4$, and α_7 membrane subtypes were performed as previously described.[15] For each compound, the experimental data obtained from the three saturation and three competition binding experiments were analyzed by a nonlinear least-squares procedure using the LIGAND program as described by Tasso et al.[15a] When final compound concentrations up to 200 μM did not inhibit [^{125}I]α-bungarotoxin binding, the K_i value was defined as being >50 μM based on the Cheng–Prusoff equation.

Acknowledgements

Financial support of this research by the Italian Ministry of Education and Research (MIUR—Rome) and by the CNR Research Project on Aging, Regione Lombardia Projects NUTEC ID 30263049 and MbMM-convenzione no. 18099/RCC (CG) is acknowledged.

Keywords: 3-fluoropyrrolidines · nicotinic receptors · structure–activity relationships · $\alpha_4\beta_2$ ligands · selectivity

[1] a) C. Gotti, F. Clementi, *Prog. Neurobiol.* **2004**, *74*, 363–396; b) R. Hurst, H. Rollema, D. Bertrand, *Pharmacol. Ther.* **2013**, *137*, 22–54.

[2] a) M. A. Abreo, N. Lin, D. S. Garvey, D. E. Gunn, A. Hettinger, J. T. Wasicak, P. A. Pavlik, Y. C. Martin, D. L. Donnelly-Roberts, D. J. Anderson, J. P. Sullivan, M. Williams, S. P. Arneric, M. W. Holladay, *J. Med. Chem.* **1996**, *39*, 817–825; b) N. Lin, D. E. Gunn, Y. Li, Y. He, H. Bai, K. B. Ryther, T. Kuntzweiler, D. L. Donnelly-Roberts, D. J. Anderson, J. E. Campbell, J. P. Sullivan, S. P. Arneric, M. W. Holladay, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 249–254.

[3] J. L. Musachio, V. L. Villemagne, U. A. Scheffel, R. F. Dannals, A. S. Dogan, F. Yokoi, D. F. Wong, *Nucl. Med. Biol.* **1999**, *26*, 201–207.

[4] H. Fan, U. A. Scheffel, P. Rauseo, Y. Xiao, A. S. Dogan, F. Yokoi, J. Hilton, K. J. Kellar, D. F. Wong, J. L. Musachio, *Nucl. Med. Biol.* **2001**, *28*, 911–921.

[5] E. Terrière, M. Sharman, C. Donaghey, L. Herrmann, J. Lonie, M. Strachan, N. Dougall, J. Best, K. P. Ebmeier, S. Pimlott, J. Patterson, D. Wyper, *Neurochem. Res.* **2008**, *33*, 643–651.

[6] J. R. Brašić, Y. Zhou, J. L. Musachio, J. Hilton, H. Fan, A. Crabb, C. J. Endres, M. J. Reinhardt, A. S. Dogan, M. Alexander, O. Rousset, M. A. Maris, J. Galecki, A. Nandi, D. F. Wong, *Synapse* **2009**, *63*, 339–358.

[7] K. P. Cosgrove, E. M. Mitsis, F. Bois, E. Frohlich, G. D. Tamagnan, E. Krantzler, E. Perry, P. K. Maciejewski, C. N. Epperson, S. Allen, S. O'Malley, C. M. Mazure, J. P. Seibyl, C. H. van Dyck, J. K. Staley, *J. Nucl. Med.* **2007**, *48*, 1633–1640.

[8] E. M. Mitsis, K. P. Cosgrove, J. K. Staley, F. Bois, E. B. Frohlich, G. D. Tamagnan, K. M. Estok, J. P. Seibyl, C. H. van Dyck, *Neurobiol. Aging* **2009**, *30*, 1490–1497.

[9] B. J. Caldarone, D. Wang, N. E. Paterson, M. Manzano, A. Fedolak, K. Cavino, M. Kwan, T. Hanania, S. K. Chella-pan, A. P. Kozikowski, B. Olivier, M. R. Picciotto, A. Ghavami, *Psychopharmacology* **2011**, *217*, 199–210.

[10] a) H. Saji, M. Ogawa, M. Ueda, Y. Iida, Y. Magata, A. Tominaga, H. Kawashima, Y. Kitamura, M. Nakagawa, Y. Kiyono, T. Mukai, *Ann. Nucl. Med.* **2002**, *16*, 189–200; b) M. Fujita, J. P. Seibyl, D. B. Vaupel, G. Tamagnan, M. Early, S. S. Zoghbi, R. M. Baldwin, A. G. Horti, A. O. Koren, A. G. Mukhin, S. Khan, A. Bozkurt, A. S. Kimes, E. D. London, R. B. Innis, *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 183–190.

[11] S. K. Pandey, S. Pan, R. Kant, S. A. Kuruvilla, M. Pan, J. Mukherjee, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7610–7614.

[12] M. Morgenthaler, E. Schweizer, A. Hoffmann-Röder, F. Benini, R. E. Martin, G. Jaeschke, B. Wagner, H. Fischer, S. Bendels, D. Zimmerli, J.

Schneider, F. Diederich, M. Kansy, K. Müller, *ChemMedChem* **2007**, *2*, 1100 – 1115.

[13] a) A. P. Kozikowski, J. Brek Eaton, K. Mohan Bajjuri, S. K. Chellappan, Y. Chen, S. Karadi, R. He, B. Caldarone, M. Manzano, P. Yuen, R. J. Lukas, *ChemMedChem* **2009**, *4*, 1279 – 1291; b) V. M. Yenugonda, Y. Xiao, E. D. Levin, A. H. Rezvani, T. Tran, N. Al-Muhtasib, N. Sahibzada, T. Xie, C. Wells, S. Slade, J. E. Johnson, S. Dakshanamurthy, H. Kong, Y. Tomita, Y. Liu, M. Paige, K. J. Kellar, M. L. Brown, *J. Med. Chem.* **2013**, *56*, 8404 – 8421.

[14] J. Chiba, G. Takayama, T. Takashi, M. Yokoyama, A. Nakayama, J. J. Baldwin, E. McDonald, K. J. Moriarty, C. R. Sarko, K. W. Saionz, R. Swanson, Z. Hussain, A. Wong, N. Machinaga, *Bioorg. Med. Chem.* **2006**, *14*, 2725 – 2746.

[15] a) B. Tasso, C. Canu Boido, E. Terranova, C. Gotti, L. Riganti, F. Clementi, R. Artali, G. Bombieri, F. Meneghetti, F. J. Sparatore, *Med. Chem.* **2009**, *52*, 4345 – 4357; b) M. Sala, D. Braida, L. Pucci, I. Manfredi, M. J. Marks, C. R. Wageman, S. R. Grady, B. Loi, S. Fucile, F. Fasoli, M. Zoli, B. Tasso, F. Sparatore, F. Clementi, C. Gotti, *Br. J. Pharmacol.* **2013**, *168*, 835 – 849.

[16] C. Abad-Zapatero, O. Perisic, J. Wass, A. P. Bento, J. Overington, B. Al-Lazikani, M. E. Johnson, *Drug Discovery Today* **2010**, *15*, 804 – 811.

[17] A. L. Hopkins, C. R. Groom, A. Alex, *Drug Discovery Today* **2004**, *9*, 430 – 431.

[18] L. K. Chico, L. J. Van Eldik, D. M. Watterson, *Nat. Rev. Drug Discovery* **2009**, *8*, 892 – 909.

[19] H. Pajouhesh, G. R. Lenz, *NeuroRx* **2005**, *2*, 541 – 553.

[20] A. K. Ghose, T. Herbertz, R. L. Hudkins, B. D. Dorsey, J. P. Mallamo, *ACS Chem. Neurosci.* **2012**, *3*, 50 – 68.

[21] J. T. Goodwin, D. E. Clark, *J. Pharmacol. Exp. Ther.* **2005**, *315*, 477 – 483.

[22] D. E. Clark, *Pharm. Sci.* **1999**, *88*, 815 – 821.

Received: February 17, 2015
Revised: March 10, 2015
Published online on April 16, 2015