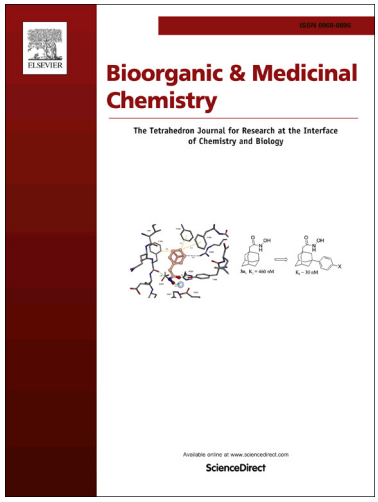

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The twin drug approach for novel nicotinic acetylcholine receptor ligands

Isabelle Tomassoli and Daniela Gündisch*

Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI, U.S.A.

ABSTRACT

The association of two pharmacophoric entities generates so-called "twin drugs" or dimer derivatives. We applied this approach for the design of a small compound library for the interaction with α4β2* nicotinic acetylcholine receptors (nAChRs). In this compound series, the nAChR ligand *N,N*-dimethyl-2-(pyridin-3-yloxy)ethan-1-amine **9** served as one pharmacological entity and it was initially kept constant as one part of the "twin" compound. "Twin" compounds with identical or non-identical entities using the "no linker mode" or "overlap" mode were synthesized and evaluated for their nAChR affinities. Compound **17a** showed the highest affinity for the α4β2* nAChR subtype (K_i = 0.188 nM) and its (di)fluoro analogs could retain nanomolar affinities, when replacing pyridine as the hydrogen bond acceptor system by mono- or difluoro-phenyls. The "twin drug" approach proved to provide compounds with high affinity and subtype selectivity for α4β2* nAChRs.

Keywords:
Twin drugs
Nicotinic acetylcholine receptor
nAChR
Structure-activity relationship
3D QSAR pharmacophore

Abbreviations: ADME, absorption/distribution/metabolism/excretion; BBB, blood-brain barrier; CD_3OD, tetradeuteromethanol; $CDCl_3$, deuterochloroform; CH_2Cl_2, dichloromethane; ClogD, distribution coefficient; ClogP, calculated partition coefficient; CNS, central nervous system; DAT, dopamine transporter; DCC, N,N'-dicyclohexylcarbodiimide; DIAD, diisopropyl

azodicarboxylate; DMAP, 4-(Dimethylamino)pyridine; DMF, N,N-dimethylformamide; Et_2O, diethyl ether; Et_3N, triethylamine; EtOAc, ethyl acetate; HA, heavy atom; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; HCHO, formaldehyde; HCOOH, formic acid; HCl, hydrogen chloride; HEPES, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; HSS, HEPES-buffered salt solution; K_2CO_3, potassium carbonate; KBr, potassium bromide; KOH, potassium hydroxide; LE, ligand efficiency; LLE, lipophilic ligand efficiency; MeCN, acetonitrile; MeI, iodomethane; MeOH, methanol; $MgSO_4$, magnesium sulfate; MW microwave or molecular weight; nAChR, nicotinic acetylcholine receptor; $NaHCO_3$, sodium hydrogen carbonate; NaOH, sodium hydroxide; NaO*t*Bu, sodium *tert*-butoxide; *n*Bu_4NBr, tetra-n-butylammonium bromide; NHA, number of hydrogen bond acceptors; NHD number of hydrogen bond donors; Pd/C, palladium on activated charcoal; PEI, poly(ethyleneimine); PPh_3, triphenylphosphine; RMS, root mean square; Ro5, rule of five; SerT, serotonin transporter; *t*BuOH, *tert*-butanol; THF, tetrahydrofuran; TPSA, topological polar surface area; TRIS, Tri(hydroxymethyl)-aminomethane; VMAT2, vesicular monoamine transporter 2; $ZnBr_2$, zinc bromide.

*Corresponding author. Tel.: +1-808-933-2943; fax: +1-808-933-2974. E-mail: danielag@hawaii.edu

1. Introduction

"Twin drugs", which can be also defined as hybrids, can be found among natural products with potent bioactivity.[1-3] Combining two active compounds in a single molecule increases interaction points with biological target(s) and can provide desired multiple or complementary modes of action. Such compounds are new entities with their own pharmacokinetics and pharmacodynamic properties.[1-4] The combination of two (identical or non-identical) biologically active compounds can be classified by the type of connection: "linker mode" – "no linker mode" – "overlap mode" (Fig. 1).

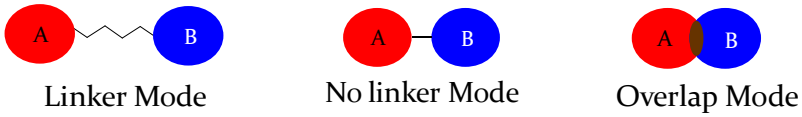


Figure 1: Different connection types for twin drugs/hybrids. Compounds A and B can be structurally identical or non-identical. They can interact with the same or with different biological targets.

Some natural products active on nAChRs are displaying a "twin" structure (Fig. 2). There is e.g. lobeline **1** found in *Lobelia inflata (Indian tobacco)*.[5] Lobeline **1** can be considered as a non-identical twin composed of sedamine and its carbonyl analog overlapped at the "cation part" (*N*-methyl piperidine). The "cation part" is an important pharmacophoric element for the interaction with nAChRs.[6,7] Lobeline **1** interacts with α4β2* (K_i = 4-30 nM) and α7 (K_i = 6.6 µM) nAChRs, VMAT2, DAT, SerT, and opioid receptors.[8] Anatalline **2** and 3,5-bis-(1-methyl-pyrrolidin-2-yl)-pyridine **3** are minor alkaloids found in tobacco roots.[9] Anatalline **2** has its overlap at the "cation part" (piperidine moiety) like lobeline and it can be considered as a twin of tobacco alkaloid anabasine with 4-phenylpiperidine, a privileged scaffold of a variety of bioactive compounds. Anatalline **2** has been identified in tobacco smoke, but no pharmacological studies have been reported so far.[10,11] Alkaloid **3** is a merged compound of two nicotine molecules overlapped at the pyridyl part displaying a hydrogen acceptor system.[12] A hydrogen acceptor system at a defined distance to the "cation part" is the second important pharmacophoric element for the interaction with nAChRs.[6,7] Compound **3** shows micromolar affinity for α4β2* (K_i = 1.18 µM).[12] Further, curare alkaloids are prominent examples of twin drugs which interact with muscle type nAChRs (Fig. 2; tubocurarine **4**).[13] A prototype of a

synthetically derived "twin" is suxamethonium (succinylcholine) **5**, a duplication of the neurotransmitter acetylcholine used as a depolarizing muscle relaxant.[13] Also dimers of the highly potent nAChR frog alkaloid epibatidine **6** and of the plant alkaloid cytisine, e.g. 1,2-bis-*N*-cytisinylethane (CC4) **7**, have been synthesized and pharmacologically evaluated in the past.[14-16] Both series combine the single molecules at their basic nitrogen motif. Recently, the interferon inducer tilorone **8** developed in the 1970s, was discovered as a selective α7 nAChR agonist (IC_{50} = 110 nM).[17] Two identical diethylaminoethanol moieties, which can be considered as choline-like motifs, are attached symmetrically to a fluorenone core. In general, the twin drug or hybrid approach has been rarely used in the development of CNS-active nAChR compounds.

NAChRs are ligand-gated cation channels assembled as homo- or heteropentamers and permeable for Na^+, K^+ and Ca^{2+}.[18,19] They are of great interest as therapeutic targets for a variety of central nervous system (CNS) diseases, e.g. β2-containing subtypes, which seem to play a major role in mood disorders and addiction.[20,21]

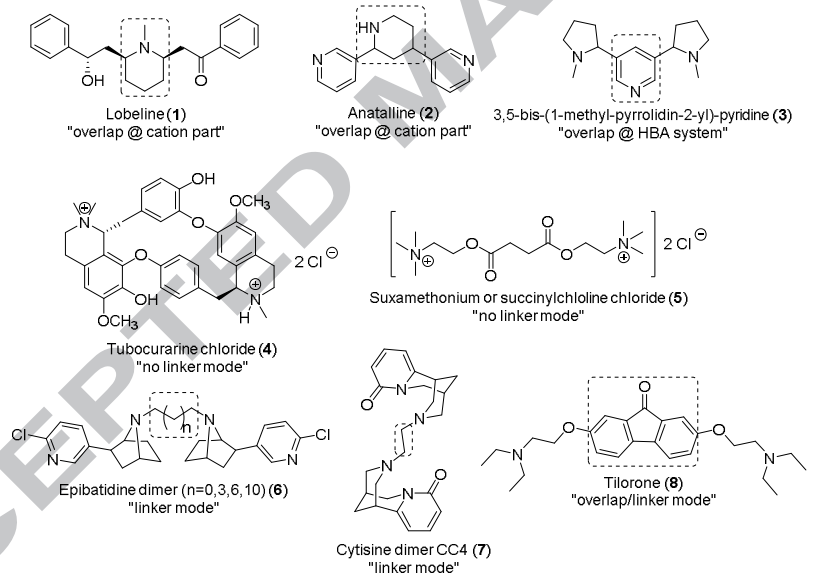


Figure 2: Structures of some natural product "twins" (**1**-**4**) active on nAChRs or/and found in tobacco, "twins" synthetically derived from natural products (**5**-**7**), and tilorone (**8**).

Recently, we applied the "twin drug" concept for the development of a novel nAChR ligand, BPC **17a** (Fig. 3A).[22] BPC **17a** is a "non-identical twin drug" using the overlap mode in its design (Fig. 3) and has partial agonist activity for α4β2* nAChRs, improved selectivity profiles over varenicline and cytisine, and efficacy in the mouse tail suspension test.[22] The design

strategy for BPC **17a** has not been developed previously for α4β2* nAChR ligands, especially using the hydrogen bond acceptor (HBA) moiety for the overlap mode. Here, we report on the design, synthesis, and in vitro evaluation of "twin" compounds based on the "overlap" mode as pharmacological tools and as new lead compounds for β2-containing nAChRs.

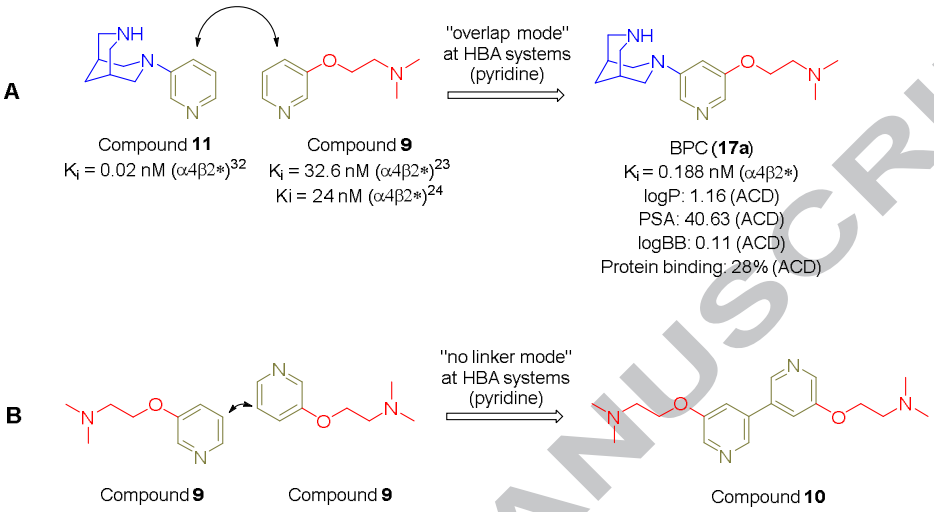


Figure 3: Design of BPC (**17a**) and compound **10**

2. Design

In our search for α4β2* nAChR ligands with a desirable selectivity and functionality profile, we used the "twin drug" approach as an alternative strategy in the optimization process of biologically active compounds.[1-4] Like mentioned earlier, the combination of two active ligands in a single molecule increases interaction points with its biological target(s), which can lead to a compound with a better on-/off-target profile.[1-4] In addition, we took into consideration, that substitution at position 5 of the pyridine moiety is mostly tolerated for nAChR activity including the possibility of enhancing α4β2* subtype selectivity in contrast to non-substituted analogs.[7]

The design for the compound series reported here is based on *N,N*-dimethyl-2-(pyridin-3-yloxy)ethan-1-amine **9** displaying a choline-like motif attached to a pyridyl moiety. Compound **9** is a non-azacyclic pyridyl ether nAChR ligand prototype developed by Abbott Laboratories and has a K_i value of 32.6 nM for α4β2* nAChRs.[23] Compound **9** was also described by Simsek et al. as active on α4β2* nAChRs (K_i = 24 ± 2.4 nM) and an ED_{50} value of 15 mg/kg in the mice tail-flick assay.[24] Firstly, we attached two molecules of *N,N*-dimethyl-2-(pyridin-3-yloxy)ethan-1-amine **9** via "no linker mode" to each other applying the simplest "twin drug" design apparent

in suxamethonium's (**5**) structure to obtain the "twin" compound **10.** We were interested to investigate, if the interaction with α4β2* could be retained (Fig. 3B). To enhance affinity for α4β2* nAChR and to reduce the number of rotatable bonds, we designed a small "twin drug" library based on two non-identical structural entities (Fig. 4 and Fig. 5). We chose the "overlap mode" to keep compounds' physicochemical parameters in CNS drug-like ranges.[25] *N,N*-dimethyl-2-(pyridin-3-yloxy)ethan-1-amine **9** and diverse diaza(bi)cyclic pyridines (**11, 12a/b, 13a/b)** (Fig. 4; Table 1) were overlapped at their pyridyl moieties to obtain the "twins" (Fig. 5A). The diaza(bi)cyclic pyridines (**11, 12a/b, 13a/b**) used in this design were developed by Romanelli et al., NeuroSearch A/S and Abbott Laboratories (Fig. 4; Table 1).[26-32] Since compound **3** has reduced affinity for α4β2* comparing with nicotine, we did not use the "identical twin drug" approach in the overlap mode for the diaza(bi)cyclic pyridine series. In addition, we were interested by introducing several azacyclic systems at position 5 at the pyridyl moiety of compound **9** to investigate the influence of such moieties on nAChRs' affinities (Fig. 5B). Here, the dimethylamine part of **9** could function as the "cation part" (Fig. 5B). In the diaza(bi)cyclic pyridine series, we kept the acyclic "cation part" (choline like substructure) constant (Fig. 5A). In addition, we replaced the pyridine by mono- or di-fluoro phenyl to keep HBA properties (Fig. 5A). The fluorine substitution will enhance lipophilicity properties and favorise BBB penetration, but will alter the hydrogen bond acceptor property.

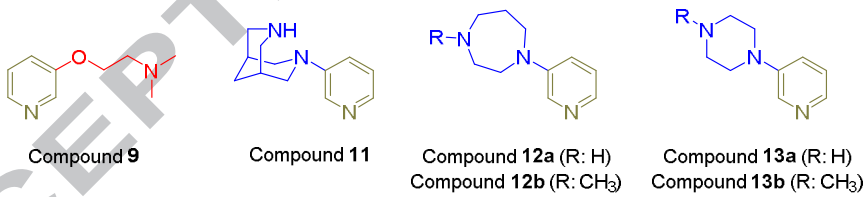


Figure 4: Structures of nAChR ligands (**9**, **11**, **12a/b**, **13a/b**) used as pharmacological entities for the twin drug approach.

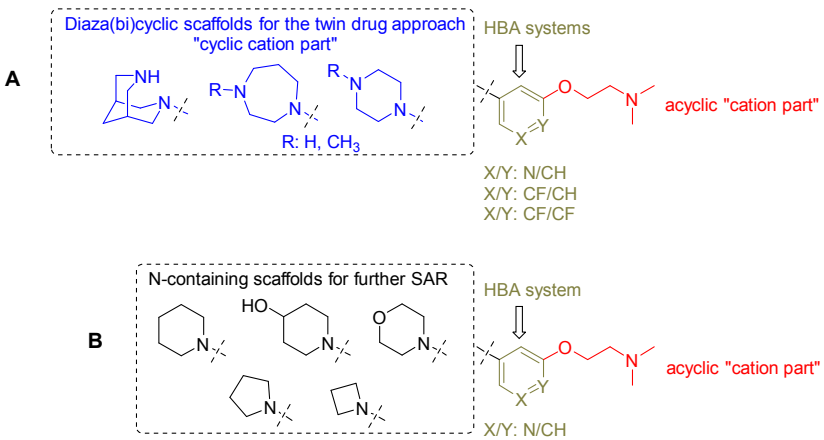
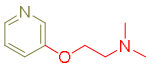
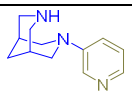
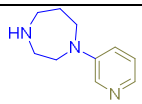
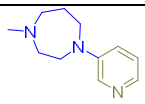
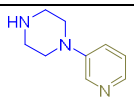
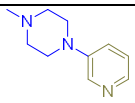


Figure 5: Design of nAChR ligands (**17a-26**). A: "Twin drugs"; B: N-containing scaffolds as substituents at position 5 at the pyridyl moiety.

Table 1. Affinities, *in vivo* activity and calculated physicochemical parameters of known nAChR ligands ("single entities")

Cpd #	Structure	α4β2* K_i [nM][a]	α7* K_i [nM][b]	*In vivo* activity	M_r	ClogP[d]	TPSA[d]	logBB[d]
9		32.6 [23], 24 ± 2.4 [24]		ED_{50} (mg/kg) = 15 (mouse tail-flick test) [24]	165.21	0.93	25.36	0.09
11		0.02 [32]		MED[c] = 1.9 µmol/kg (mouse hot plate test) [32]	202.27	0.97	28.16	-0.09
12a		1.9 (IC_{50}) [28,31]	190 (IC_{50}) [31]		176.23	0.65	28.16	0.04
12b		150 (IC_{50}) [31]	4,600 (IC_{50}) [31]		190.26	1.19	19.37	0.03
13a		90 [26]			162.21	0.19	28.16	-0.03
13b		90 [26]			176.23	0.88	19.37	0.07

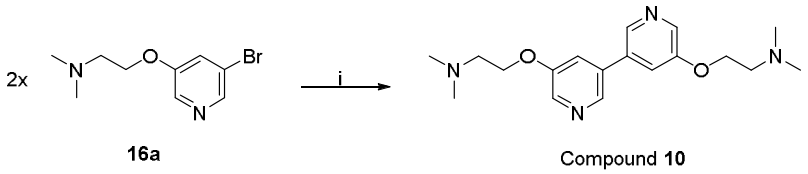
[a][^{3}H]cytisine; [b][^{3}H]alpha-bungarotoxin; [c]MED: minimally effective dose; [d]ClogP, TPSA, and logBB values were calculated using ACD/ADME Suite 5.0.

3. Results

3.1. Chemistry

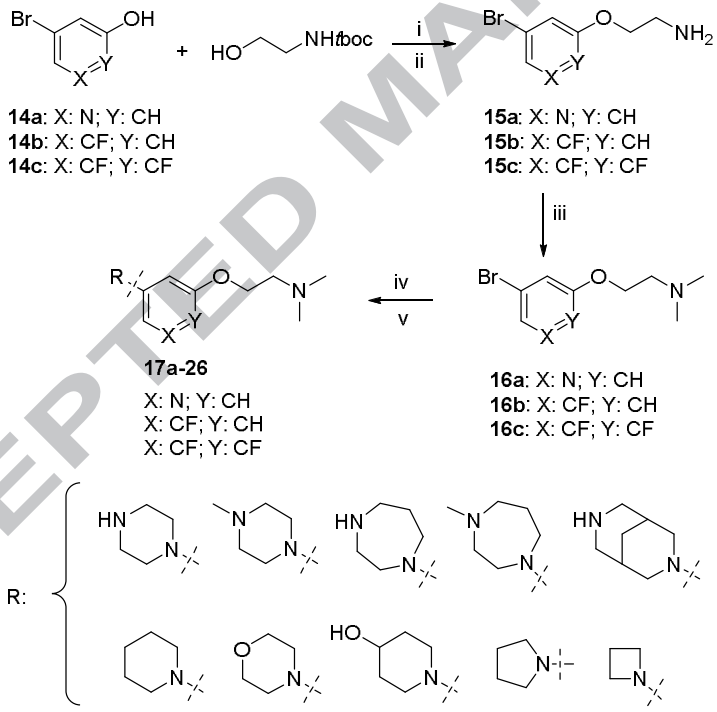
The twin compound **10** was prepared in one step (Scheme 1). Various methods can be used to form a biaryl system. A general method is the Ullmann reaction which requires the use of copper salts.[33,34] To avoid possible complex formation of copper (II) ions with compound **16a**, alternative synthetic pathways were considered. Other methods to form biaryl systems use aryboronic acid, arylzinc and arylstannane derivatives.[34] In our case, we adapted an effective catalytic alternative of the Ullmann reaction.[35,36] This reaction uses $Pd(OAc)_2$ as the catalyst, tert-butylammonium bromide as base in DMF/H_2O. To reduce the reaction time, the synthesis

was conducted in a Q-tube™. Compound **10** was obtained in a moderate yield and 96 % purity after purification by preparative HPLC.



Scheme 1. i) $Pd(OAc)_2$, nBu_4NBr, K_2CO_3, DMF/H_2O, Q-tube™, 115°C, 4h.

The "twin compounds" (**17a-21c**) and compound **9** analogs (**22-26**) were synthesized using the procedure described in Scheme 2. The key intermediates **15a-c** were synthesized in two steps with 23-54% overall yield. The first step is a Mitsunobu reaction of N-*t*boc protected aminoethanol and the appropriate bromo-substituted hydroxypyridine or fluorophenol (**14a-c**).[37]



Scheme 2. i) PPh_3, DIAD, THF, rt, 5-6 h. ii) HCl/1,4dioxane, rt, 20h. iii) HCHO, HCOOH, reflux 6h, rt 20h. iv) $Pd(OAc)_2$, R,S-BINAP, NaO*t*Bu, *t*BuOH 1% in toluene, Q-tube, 160°C, 2h [N_2] or MW, 140°C, 1h20. v) N-deprotection for mono N-*t*boc protected piperazine, homopiperazine and bispidine analogs: HCl/1,4dioxane, rt, 20h.

The reaction has been optimized by evaluating different reagents and conditions.[38-41] The highest yields were obtained by using triphenylphosphine. Product formation was not observed with tri-n-butylphosphine. 1,1'-(Azodicarbonyl)-dipiperidine and diethyl azodicarboxylate gave low yield whereas di-isopropyl azodicarboxylate (DIAD) produced the highest yields. Conducting the reaction under nitrogen atmosphere had no influence on the product yields. The optimal reaction time was 5-6 h, when using triphenylphosphine and DIAD. For 3-bromo-5-hydroxypyridine **14a** and 3-bromo-5-fluorophenol **14b**, the highest yields were obtained when reagents are introduced in the following sequence: DIAD and N-*t*boc ethanolamine were dissolved in THF; then, the 3-bromo-5-hydroxypyridine **14a** or 3-bromo-5-fluorophenol **14b** and triphenylphosphine were added. This sequence was changed for the reaction with 5-bromo-2,3-difluorophenol **14c** due to a lower yield first obtained. DIAD, triphenylphosphine and 5-bromo-2,3-difluorophenol **14c** were dissolved in THF and stirred at room temperature for 1 h, then N-*t*Boc ethanolamine was added. Since there was no benefit in an intermediate purification step, the obtained crude products were directly used for the N-deprotection step. The N-*t*boc group was cleaved under mild conditions using HCl in 1,4 dioxane. This reaction is achieved in good yield (95 %) and gives pure products (> 90 %) by precipitation. The N-methylation of the primary amine was carried out in presence of formaldehyde and formic acid (Eschweiler-Clarke reaction) and gave **16a-c** in low to moderate yields (23% - 62%).[42] Purification was done by flash chromatography using CH_2Cl_2/MeOH as eluent. The Buchwald-Hartwig coupling reaction was applied to attach diverse (di)aza(bi)cyclic scaffolds to compounds **16a-c** (Scheme 2).[43] Various ligands, bases, catalysts and solvents are described for this coupling in the literature.[44-49] For our reactions, highest yields were achieved using R,S-BINAP and XPhos. PPh_3 and 1,1'-bis(diphenylphosphino) ferrocene were ineffective. Among the bases used (NaO*t*Bu, Cs_2CO_3 and K_2CO_3) NaO*t*Bu produced the best results. Furthermore, we evaluated different solvents like toluene, toluene/*tert*-butanol (5/1) and DMF/H_2O for our reactions. Toluene and toluene/*tert*-butanol gave the best results. The mixture toluene/*tert*-butanol was used for complete dissolution of reagents when necessary. Finally, a number of catalysts have been tested such as $Pd(OAC)_2$, $Pd_2(dba)_3$, and $Pd(PPh_3)_4$. The best product formation was observed using $Pd(OAc)_2$. The reaction can be achieved either by microwave irradiation or in a pressure reactor (Q-tube™). For microwave assisted reactions palladium acetate, R,S-BINAP or XPhos and sodium *tert*-

butoxide were used. The reaction was heated 1 h 20 min at 140°C. Using the Q-tube™, temperature and reaction time had to be adapted, so, 2 h at 160°C gave equal results to the microwave alternative. In addition, one protecting group is necessary to perform the coupling of diaza(bi)cyclic systems to avoid bis-coupling product formation. The diazabicyclic system bispidine was prepared using our published method.[50,51] Subsequent removal of the N-*t*boc-protecting group by HCl in dioxane led to the desired products. The highest purity (95-99%) was obtained when purified on preparative HPLC using a gradient of MeOH/H_2O.

3.2. In vitro characterization and structure-activity relationship (SAR) studies

The binding affinities of our synthesized compounds were determined for α4β2*, α3β4*, α7*, and $(\alpha1)_2\beta1\gamma\delta$ ("muscle type") nAChRs in radioligand binding assays previously described.[51,52] [^{3}H]Epibatidine and [^{3}H]MLA were used as radioligands. Crude membrane fractions were prepared from Sprague-Dawley rat forebrains, pig adrenals or Torpedo californica electroplax to serve as receptor sources. The K_i values are summarized in Table 2.

*Compound **10***: The "twin" compound **10** based on two molecules of *N,N*-dimethyl-2-(pyridin-3-yloxy)ethan-1-amine **9** possesses a K_i value of 290 nM for the α4β2* subtype, therefore an approximately nine-fold decreased affinity in comparison with the single entity **9**. The second molecule of **9** was attached using the no-linker mode at position 5 of the pyridyl moiety where larger substituents are normally tolerated best.[23] There was no interaction with the α3β4* or α7* nAChR subtypes, whereas a K_i of 905 nM was observed for the muscle type which could be related to a more favorable distance between both basic nitrogen atoms similar to suxamethonium **5**. An ideal distance of 10-12 carbon atoms between the two basic nitrogen atoms has been discussed for muscle relaxants in the past.[13] Since we aimed for this project to develop CNS based drugs, we focused on our non-identical twin compounds using the overlap mode which shortens the distance between the two basic nitrogen atoms to avoid the interaction with the muscle subtype.

Non-identical "twin" compounds: The SAR for the non-identical "twin" compounds **17a**-**21c** can be discussed in the context of the type of diaza(bi)cyclic scaffolds attached and the influence of the HBA system used here. As mentioned earlier the dimethylaminoethoxy group of **9** was kept constant. The dimethylaminoethoxy group shows a choline like motif and an acyclic "cation" part. Compounds (**17a**, **18a**, **19a**, **20a**, **21a**) with a pyridyl moiety serving as the HBA

system displayed higher affinity for α4β2* nAChR along with high subtype selectivity (Tab. 2). Larger diaza(bi)cyclic scaffolds increased the affinity for the α4β2* subtype in the pyridyl series which has been also found in non-twin drug based nAChR ligands.[7] The "twin" compound **21a** shows higher affinity than **13b** for α4β2*, whereas the non-methylated analogs (**20a** versus **13a**) have the opposite trend (Tab. 1 and 2). The homopiperazine based "twin" compounds (**18a**, **19a**) have slightly reduced affinities for α4β2* nAChR, but improved subtype selectivity comparing with compounds **12a** and **12b** which interact with the α7* subtype. The "twin" compound **17a** (BPC) with the highest affinity in this series is bearing the bispidine (3,7-diazabicyclo[3.3.1]nonane) scaffold. The K_i value obtained is 0.188 nM for α4β2* nAChR with high subtype selectivity. The affinity for the single entity (**11**) developed by Abbott Laboratories is higher (K_i = 0.02 nM) and its minimally effective dose in the mouse hot plate paradigm for antinociceptive effect is 1.9 μmol/kg.[32] There are no further data on subtype selectivity available for **11.** Our "twin" compound **17a** (BPC) shows superior subtype selectivity and functionality in comparison with the smoking cessation drugs cytisine and varenicline, and the efficacy in the mouse tail suspension test underlines its potential use in mood disorders.[22] Previous studies have shown that larger diaza(bi)cyclic systems providing the protonated nitrogen have higher affinity for α4β2* nAChR due to lipophilic interactions.[7,28] The piperazine derivative **20a** displays the lowest affinity among the three compounds, which is about 770-fold lower in comparison with compound **17a**. Its piperazine scaffold is less bulky than the homopiperazine one in **18a** and especially the diazabicylic system bispidine in **17a**.

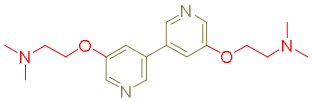
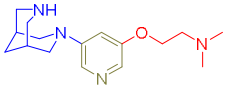
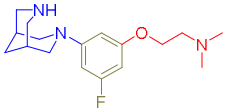
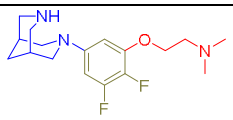
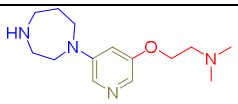
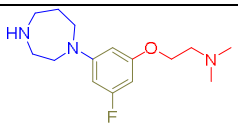
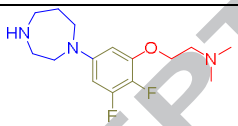
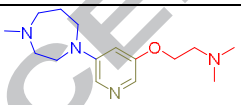
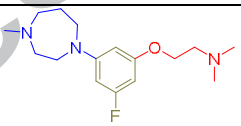
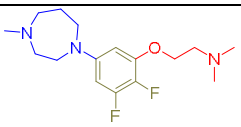
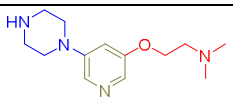
N-methylated analogs of compounds **18a** and **20a**: In case of the piperazines, introduction of a methyl group (**21a**) results in a higher affinity for α4β2* (K_i = 66.8 nM vs K_i = 145.2 nM for **20a**). In contrast, N-methylation of **18a** decreases the affinity (K_i = 212 nM vs K_i = 6.9 nM for **19a**). Taken together, the bulkiness of diaza(bi)cyclic cation part along with its orientation in space are important for high affinity for the α4β2* subtype.

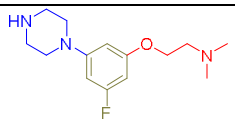
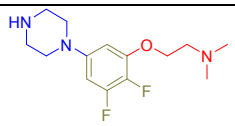
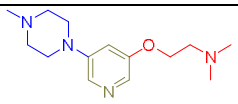
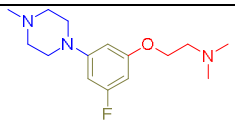
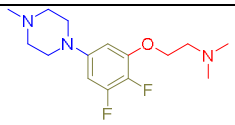
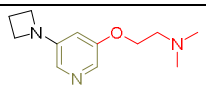
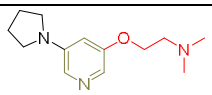
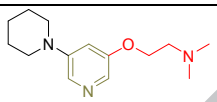
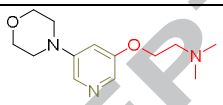
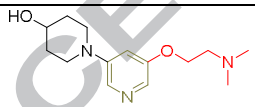
HBA system: Among the "twin" drugs, compound **17a** (BPC) has the highest affinity for α4β2* nAChR (K_i = 0.188 nM). To improve lipophilicity and blood brain barrier penetration, the pyridine ring (HBA system) was replaced by a monofluoro-phenyl (**17b**) or a difluoro-phenyl moiety (**17c**).[54,55] The difluoro-phenyl derivative **17c** possesses a K_i value of 65 nM, and the determined K_i value for the monofluoro-phenyl analog **17b** is 186 nM. The HBA strength of the fluorophenyl moieties is lower in comparison with the pyridyl moiety explaining the reduced

affinities obtained. Since the electron density has an impact of the HBA strength, partial charges of the aromatic system were calculated using the Gasteiger-Marsilli method (Discovery Studio 3.1). Table S2 summarizes the partial charges for each atom within the aromatic system. The total charge distribution is 0.666 for the pyridine ring. This value is lower for the fluoro- and difluoro-phenyl moieties resulting in a decrease in HBA strength and subsequently in reduced affinity (**17b**, **17c**). There is a complete loss of affinity for non-bispidine based compounds in this series.

*Compound **9** analogs*: Since bulkier basic nitrogen containing scaffolds fit better into the nAChR binding pocket, and we assume that the diaza(bi)cyclic moieties in our "twin" drugs are responsible for the cation-π interaction instead of the dimethylamine group in the parent compound **9**, we studied the influence of some small monoazacyclic systems in position 5 for compound **9** (Fig. 5B). Few derivatives of **9** have been synthesized by Abbott Laboratories presenting different substituent size in position 5, but non-aromatic cyclic systems were not among them.[23] Introduction of different e.g. halogen atoms, trifluoromethane or a nitro group decreased the affinity for α4β2* in comparison with the parent compound **9**.[23] When we introduced small monoazacyclic systems, only the azetidine (**22**, K_i = 784 nM) and the piperidin-4-ol (**26**, K_i = 613 nM) moieties were tolerated, but with reduced affinities for α4β2*, and no activity on other nAChR subtypes tested. A pyrrolidine (**23**), piperidine (**24**) or morpholine (**25**) substituent resulted in a complete loss of affinity for all nAChR subtypes. Steric aspects/bulkiness can be considered here, when there is not an additional nitrogen atom present which has higher basicity or hydrogen bond donor effects. This means that an azetidine substituent is just tolerated, whereas bigger azacyclic systems are only allowed, when a second nitrogen atom is present (e.g. **20a**) capable for strong cation-π interaction. An HBD system or a motif which provides also hydrogen bond donor interaction (OH group in **26**) seems to recover affinity for the α4β2* subtype. Other monoazacylic substituents with shifted positions of the nitrogen atom need to be studied in the future along with implemented HBD systems to explore this chemical space further.

Table 2. Affinities (K_i values ± SEM) and calculated physicochemical parameters (ClogP, TPSA, logBB) of "twin compounds", their mono- or difluoro analogs (**10**, **17a-21c**), and compound **9** analogs (**22-26**).

Cpd #	Structure	α4β2* K_i [nM]	α3β4* K_i [nM]	α7* K_i [nM]	$(\alpha1)_2\beta1\gamma\delta$ K_i [nM]	M_r	ClogP[a]	TPSA[a]	logBB[a]
"Twin" compounds and their fluoro analogs									
10		290 ± 20	> 1,000	> 1,000	905 ± 13	330.42	1.46	50.72	0.04
17a (BPC)		0.188 ± 0.02	> 1,000	> 1,000	> 1,000	290.40	1.16	40.63	0.11
17b		186 ± 11	> 1,000	> 1,000	> 1,000	307.40	2.05	27.74	0.22
17c		65 ± 4.5	> 1,000	> 1,000	> 1,000	325.39	1.99	27.74	0.19
18a		6.9 ± 1.2	> 1,000	> 1,000	> 1,000	264.36	0.84	40.63	0.08
18b		> 1,000	> 1,000	n.d.	n.d.	281.36	1.73	27.74	0.29
18c		> 1,000	> 1,000	n.d.	n.d.	299.35	1.68	27.74	0.30
19a		212 ± 17	> 1,000	n.d.	n.d.	278.39	1.38	31.84	0.19
19b		> 1,000	> 1,000	n.d.	n.d.	295.39	2.27	18.95	0.52
19c		> 1,000	> 1,000	n.d.	n.d.	313.38	2.22	18.95	0.55
20a		145.2 ± 15.6	> 1,000	> 1,000	> 1,000	250.33	0.38	40.63	0.01

20b		> 1,000	> 1,000	n.d.	n.d.	267.34	1.27	27.74	0.16
20c		> 1,000	> 1,000	n.d.	n.d.	285.33	1.22	27.74	0.17
21a		66.8 ± 3.5	> 1,000	> 1,000	> 1,000	264.36	1.07	31.84	0.14
21b		> 1,000	> 1,000	n.d.	n.d.	281.36	1.96	18.95	0.39
21c		> 1,000	n.d.	n.d.	n.d.	299.35	1.91	18.95	0.40
Compound **9** analogs									
22		784 ± 45	> 1,000	> 1,000	n.d.	221.29	1.12	28.60	0.12
23		> 1,000	> 1,000	> 1,000	n.d.	235.32	1.68	28.60	0.25
24		> 1,000	> 1,000	> 1,000	n.d.	249.35	2.25	28.60	0.31
25		> 1,000	> 1,000	n.d.	n.d.	251.32	0.63	37.83	-0.03
26		613 ± 13	> 1,000	> 1,000	n.d.	265.35	0.72	48.83	0.02

Values are generated from 2-10 independent experiments; n. d. = not determined; [a]ClogP, TPSA, and logBB values were calculated using ACD/ADME Suite 5.0.

Pharmacophore: A detailed crystal structure of an entire nAChR is still not available, but 3D QSAR pharmacophore models have been created.[27,56-61] Those models display two essential pharmacophoric elements: a cationic center and a hydrogen bond acceptor (HBA). The distance between these two elements can vary from 4-7Å. To illustrate and identify pharmacophoric

features (HBA, HBD, positive ionizable, aromatic ring) present in the "twin" compounds and its derivatives, a 3D QSAR pharmacophore model was developed based on compounds **17a, 18a, 19a, 20a, 21a, 22-26** using Discovery Studio 3.1. The full ligand set was sorted by a random index fixed at 60. All the ligands are considered to be active on the same binding site. A set of 10 pharmacophores was generated from six training set compounds (**17a, 18a, 20a, 23, 24, 26,**) and validated by the test set of four compounds (**21a, 19a, 22, 25**). Furthermore, a cross-validation was used based on the Fisher randomization test method with a significance of 90% to evaluate the statistical relevance of the pharmacophore model. Five features were considered in all models (HBA, HBD, ring aromatic, hydrophobic and positive ionizable). One pharmacophore model was eliminated because the significance value was only 80%. The selection for the best pharmacophore model was made by the best fit and lowest error values on the predicted activity for the most active compounds (Tab. 3, e.g. **17a** and **18a**). The pharmacophore model #9 was considered as the best one, and it displayed a q^2 of 0.733, a RMS error of 0.965 and a mean absolute error of 0.862. The model obtained displays two positive ionizable features (Red), one HBA (Green) and an aromatic feature (Orange) (Fig. 6). The two different positive ionizable features will be given an arbitrary name for a better understanding when discussing the mapping of the "twin" compounds with pharmacophore #9. This denomination will be based on their position related to the mapping of compound **17a** with pharmacophore #9 (Fig. 6). Positive ionizable feature #1 (PI-1) is the positive ionizable feature that matches the N-dimethyl group of the choline-like motif, and positive ionizable feature #2 (PI-2) is the positive ionizable feature fitting the azacyclic system. The distances between the pharmacophoric features are displayed in Figure **S1**.

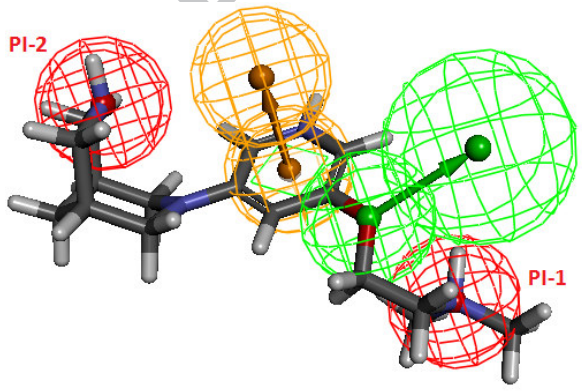


Figure 6: Mapping of compound **17a** onto pharmacophore #9. Positive charges, hydrogen bond acceptor (HBA) and aromatic ring features are shown in Red, Green and Orange, respectively.

PI-1 is the positive ionizable feature #1 that fits the N-dimethyl group of the choline-like motif, PI-2 (the positive ionizable feature #2) fits the azacyclic system. The attribution is based on the fitting of **17a** with the best pharmacophore generated.

Compound **17a** has the best fit value for the generated model (Tab. 3). The secondary amine of the bispidine moiety fits one of the positive ionizable features (PI-2, Fig. 6). The second positive ionizable feature (PI-1, Fig. 6) matches the N-dimethyl group of the choline-like motif. The centroid of the pyridine ring maps onto the aromatic ring feature (shown in Orange in Fig. 6). The ether oxygen of the dimethylaminoethoxy moiety fits with an HBA feature (shown in Green in Fig. 6).

The mapping of the "twin" compounds (**18a**, **19a**, **20a**, **21a**) and compounds **22-26** with pharmacophore #9 are shown in Figures S2, S3 and S4 (supporting material). Table 3 displays the fit values of the compounds with pharmacophore #9. Compounds with the highest affinity (e.g. **17a** and **18a**) have the highest fit value to pharmacophore #9 (Table 3). However, one inversion is observed: Compound **20a** displays a higher fit value (9.97) to the pharmacophore than **21a** (9.60) whereas **21a** has higher affinity than **20a** (Tab. 2 and Tab. 3). Figure S2 shows the mapping of compounds **17a**, **18a** and **20a**. These three compounds map all features of pharmacophore #9 with the highest fit value (11.55, 10.20 and 9.97, respectively) and display high affinity (0.19, 6.9 and 145.2 nM, respectively). The N-dimethyl nitrogen atom fits the positive ionizable feature (PI-1) for compounds **19a, 21a**, **22** and **26** (Fig. S3). The centroid of the pyridine ring does not perfectly fit the aromatic ring feature of compounds **22** and **26**, which could explain the decrease in affinity when comparing with **19a** and **21a**. A greater difference is observed for the second positive ionizable feature (PI-2, Fig. S3). The piperidin-4-ol part of compound **26** only partially fits the positive ionizable feature (PI-2). There is no match of the azetidine ring with the ionizable feature (PI-2) for compound **22**, which has greatly reduced affinity.

Mapping of the non-active compounds **23**, **24**, and **25** is shown in Figure S4. These three compounds fit only one positive ionizable feature (PI-1), the HBA feature and the aromatic ring feature. A second positive charge (PI-2) seems to be necessary to keep the affinity related to this model. Compound **9** analogs (**22**-**26**) which are not fulfilling the criteria of a "twin" compound are non-active (**23**-**25**) or have greatly reduced affinity (**22**, **26**).

The estimated affinities, error values and fit values are listed in Table 3. A "ligand pharmacophore mapping" protocol was used to obtain predicted affinity values for the test set (see Experimental Part). The error value was defined as the ratio of predicted affinity to measured affinity when the error value is > 1 (or as the ratio of measured affinity to predicted affinity, when the error value is negative). Error values between actual and predicted affinity were < 5, only one compound (**17a**) had an error value larger than 5, but lower than 10, which is still within an acceptable error range. The most active compound (**17a**) has a predicted affinity of 1.3 nM whereas its measured value is 0.188 nM. The error values demonstrate a model with good prediction. All compounds are classified by their affinities as highly active (K_i < 150 nM), moderately active (150 < K_i < 1,000 nM) and inactive (K_i > 1,000 nM). Most of the active compounds were correctly predicted. Only one moderately active compound (**19a**) was predicted to be highly active and one moderately active compound (**22**) was predicted to be inactive.

Herein, we have generated a good theoretical model for our "twin" compounds that correlates the experimental data measured: a) The predicted affinity values obtained from pharmacophore #9 are in correlation with the K_i values obtained (e.g. **17a**, **18a**, **19a**, **20a**, **21a**); b) The mapping of the "twin" compounds with the pharmacophore #9 can explain the differences observed for the activities (e.g. **22-26**).

Table 3. Experimental and estimated K_i values of training set compounds based on the best model generated.

Cpds	Measured K_i for α4β2*	Predicted K_i for α4β2[a]	"Error" Value[b]	Fit Value[c]	Measured activity scale[d]	Predicted activity scale[d]
17a	0.19	1.3	6.73	11.55	+++	+++
18a	6.9	27.7	4.01	10.20	+++	+++
19a	212	147.9	-1.43	9.48	++	+++
20a	145.2	47.2	-3.08	9.97	+++	+++
21a	66.8	110.3	1.65	9.60	+++	+++
22	864.9	3,159	3.65	8.15	++	+
23	> 5,000[#]	2,748	-3.63	8.21	+	+
24	> 5,000[#]	2,748	-3.63	8.21	+	+
25	> 1,000[#]	2,748	-3.63	8.21	+	+
26	632.2	972.8	1.54	8.66	++	++

[#]A K_i value of 10,000 was used for generating the pharmacophore model. [a]The value was predicted by the best pharmacophore generated. [b]The error value is a ratio between the predicted and measured value or the inverse. [c]The fit value is a measure of how well a ligand fits the

pharmacophore; the higher the value, the better the fitting. [d] Activity scale: highly active +++, K_i < 150 nM; moderately active ++, 150 nM < K_i < 1,000 nM; inactive +, K_i > 1,000 nM.

3.3. Physicochemical Properties and ADME Calculation

The design of the new ligands was guided by *in silico* ADME calculations.[62,63] ADME related properties like BBB penetration were calculated using Discovery Studio 3.1. The blood-brain penetration model was derived from over 800 compounds classified as CNS therapeutics. The plot (Fig. 7) displays the drug-like properties with logP and polar surface area (PSA) values (absorption and BBB penetration). Compounds **17a-26** have promising properties for absorption and blood brain barrier penetration, since their values appear within the "Absorption" and "BBB" ellipses (Fig. 7).

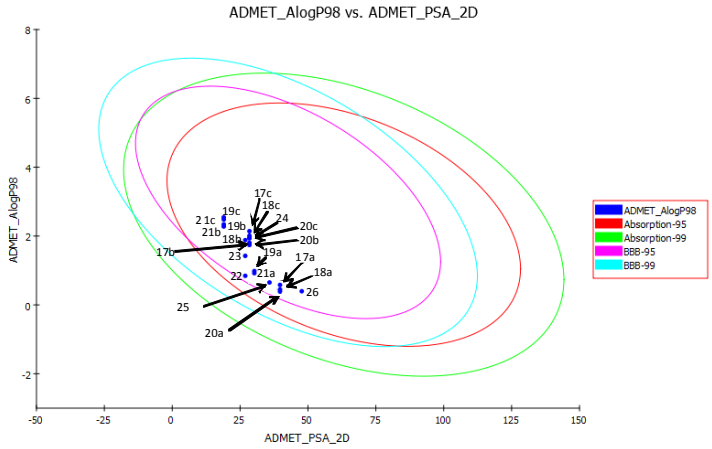


Figure 7: Plot of PSA (Polar Surface Area) vs. LogP for "twin" compounds and compound **9** analogs showing confidence limit ellipses (95 % and 99 %) corresponding to the BBB (Blood Brain Barrier) and Intestinal Absorption models. The plot was generated using Discovery Studio 3.1 (Accelrys, San Diego, CA).

In addition, other physicochemical properties can be considered to be important for oral absorption and BBB.[64-66] E.g. the CNS multiparameter optimization (MPO) is based on six fundamental physicochemical properties (a) lipophilicity (ClogP); (b) distribution coefficient (ClogD); (c) molecular weight (MW); (d) topological polar surface area (TPSA); (e) number of hydrogen bond donors (NHD); (f) most basic center (pK_a). Based on the drug set, median values

were found to be ClogP = 2.8, ClogD = 1.7, MW = 305.3 Da, TPSA = 44.8 Å^2, NHD = 1, and pKa = 8.4. Ghose *et al.* compared physicochemical property of CNS and non-CNS drugs.[25] This study provides the following guideline for CNS drug design, TPSA < 76 Å^2 (25-60 Å^2), more than one nitrogen (one or two, including one aliphatic amine), fewer than seven (two to four) linear chains outside of rings, fewer than three (zero or one) polar hydrogen atoms, volume of 740-970 Å^3, solvent accessible surface area of 460-580 Å^2.

We calculated important parameters (pKa, MolRefrac, NHA, NHD, Rings, Aromatic rings, logBB) for all synthesized compounds using ACD/ADME Suite 5.0 (ACD/Labs) software. Data are listed in Table S1 (supporting information). Values in Green are within the range of 50 % of marketed CNS drugs. Values in Black are in "acceptable" ranges, but would need further profiling. Most of our compounds are in the range for CNS drug-likeness parameter values. In general, the twin drug compounds and analogs of compound **9** (**10**, **17a-26**) present a better CNS drug-likeness profile than the single entities (**9**, **11**-**13b**). Most active compounds showed "good" or "acceptable" values (e.g. **17a**, **18a** and **21a**). No correlations were identified between affinity and physicochemical properties. However a trend can be highlighted: one hydrogen bond donor (NHD) and five hydrogen bond acceptors (NHA) seems a favorable combination to maintain good affinity (e.g. **17a** and **18a**). The lack of one of them (NHD = 0 and/or NHA = 4) results in a decrease or loss of activity. A few exceptions can be pointed out: compound **26** possesses NHD = 1 and NHA = 5, but only displays a moderate activity (K_i = 613 nM). Also, compound **10** shows a moderate activity (K_i = 290 nM) with NHD = 0 and NHA = 6.

The concept of binding efficiency is used for any drug development process. It correlates potency, size and lipophilicity. Ligand Efficiency (LE) represents the efficiency of binding with respect to size as measured by the number of heavy atoms. It has a minimum of 0.3 for hit compounds. Lipophilic Ligand Efficiency (LLE) focuses on maximizing lipophilicity efficiency. LLE mean values for lead compounds and marketed drugs are respectively 3.8 and 5-6.2.[67-69]

In order to identify lead compounds, ligand efficiency index (LE) and lipophilic ligand efficiency (LLE) were calculated for our compound set and the single entities (Tab. S3). LE and LLE values were obtained by using the following equations: LE = (1.37 x pK_i)/HA where HA is the number of heavy atoms, and LLE = pK_i-clogP.[70,71] The LE minimum value for hit compounds is 0.3.[68] The LLE mean value for lead compounds is 3.8, and it is in the range of 5-6.2 for marketed drugs.[68] LE and LLE values for all compounds (**9**, **10**, **17a-c**, **18a**, **19a**, **20a**,

21a, **22**, **26**) are above the mean values. Single entity compounds **9**, **11-13b** show good LE (0.67-0.98) and LLE (5.63-9.73) properties to be considered as lead compounds. Compounds **11** and **12a** have the highest LE and LLE values. Among the "twin" compounds (**10**, **17a**, **18a**, **19a**, **20a**, **21a**), two compounds have shown high LE and LLE, compounds **17a** (LE = 0.63; LLE = 8.6) and **18a** (LE = 0.59; LLE = 9.7). **17a** is the combination of **9** and **11**. **18a** is the combination of **9** and **12a**. Among compounds **9**, **11-13b**, compounds **9**, **11** and **12a** are the most active compounds with the best LE and LLE confirming their role of lead compounds. Taken together, the "twin" compounds **17a** and **18a** can be considered as lead compounds as well.

4. Conclusion

In summary, the "twin drug" approach proves to be a successful strategy for the development of α4β2* nAChR ligands with improved subtype selectivity and desired functionality profile recently described especially for our lead compound BPC (**17a**). Using the "overlap mode" in the design strategy provides compounds with a "good" or "acceptable" drug-likeness profile. The compounds were evaluated for their affinities at four different nAChRs. Active compounds displayed α4β2* subtype selectivity. The bispidine scaffold containing compound **17a** (BPC) showed the highest affinity among the "twin" compounds and even kept high affinities for α4β2* nAChRs when the strength of its HBA system was reduced (**17b**, **17c**) in contrast to the piperazine and homopiperazine analogs. Not following the "twin" design and introducing small monoazacyclic substituents at position 5 of compound **9** caused a dramatically drop or complete loss in affinity for nAChRs.

5. Experimental Section

All reagents and solvents were obtained from various suppliers (Acros, Aldrich, Alfa Aesar, Fisher Scientific, Frontier Scientific Merck, Oakwood or Sigma) and used without further purification unless otherwise noted. Tetrahydrofuran (THF), 1,4-dioxane and toluene were dried over sodium. Water was taken from a water purification system Direct-Q^{TM} 5 (Millipore). Reactions were monitored by thin layer chromatography (TLC) using aluminium sheets coated with silica gel 60 F254 (Merck). Compounds were visualized using UV light (254 nm) and/or using a $KMnO_4$ solution (1%). Column chromatography was carried out on silica gel 60 (0.063-0.200 mm) using gradient mixtures of CH_2Cl_2 with MeOH (20:1, 9:1 or 8:2) as mobile phases.

^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on an Avance 400 spectrometer (Bruker). All NMR spectra were recorded at rt. Chemical shifts (δ) are given in parts per million (ppm) relative to the remaining protons of the deuterated solvent used as internal standard. Coupling constants *J* are given in Hertz (Hz) and spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), t (triplet), q (quartet), m (multiplet) and br (broad). Mass spectra were recorded on a Varian 500-MS mass spectrometer with an electron spray ionization source (ESI-MS). HRMS runs were performed on Agilent Technologies 6530 Accurate Mass Q-TOF/LCMS. The purity of the compounds was determined by a Shimadzu Prominence HPLC system at an appropriate wavelength. All compounds proved to possess ≥ 95% purity.

5.1. General procedure A: Mitsunobu reaction

In a round bottom flask, DIAD (1-1.2 eq) and N-*t*boc-ethanolamine (1.3 eq) were dissolved in THF (15 mL). Then the 3-bromo-5-hydroxypyridine (1 eq) and the triphenylphosphine (1.2-1.5 eq) were added. The reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure and the residue was used without any further purification for deprotection.

5.2. General procedure B: Mitsunobu reaction

In a round bottom flask, DIAD (1 eq), triphenylphosphine (1.1 eq) and the appropriate nucleophile (1 eq) were dissolved in THF (15 mL). The reaction mixture was stirred at room temperature for 1 h. Then N-*t*boc-ethanolamine was added (1.2 eq) The reaction mixture was stirred at room temperature for an additional 5 h. The solvent was removed under reduced pressure and the residue was used without any further purification for deprotection.

5.3. General procedure C: Cleavage of the N-tboc protecting group from N-tboc protected ethanolamine derivatives

HCl in 1,4-dioxane (4 N, 5-6 mL) was added to a stirred solution of the N-*t*boc protected compound (2.5-4 mmol), dissolved in 5-6 mL of 1,4-dioxane, and the mixture was allowed to stir at rt for 2-15 h. The white solid was filtered and washed with cyclohexane and diethylether.

*5.4. 2-[(5-bromopyridin-3-yl)oxy]ethanamine (**15a**)*

The Mitsunobu reaction was performed according to general procedure A with 3-bromo-5-hydroxypyridine **14a** (500 mg, 2.87 mmol), N-*t*boc-ethanolamine (620 mg, 3.85 mmol), DIAD (576 mg, 2.85 mmol), triphenylphosphine (1.13 g, 4.31 mmol) and THF. The reaction time was 5 h. Then the general procedure C was used for the N-*t*boc deprotection. Final product **15a** was obtained as a white solid (336 mg, 54%). ^{1}H NMR (MeOD, d_4, 400 MHz) δ 8.75 (s, 1H), 8.70 (d, J = 2.4 Hz, 1H), 8.49 (t, J = 1.6 Hz, 1H), 4.53 (t, J = 4.4 Hz, 2H), 3.47 (t, J = 4.4 Hz, 2H) ; ^{13}C NMR (MeOD, d_4, 100 MHz) δ 158.11, 138.61, 134.41, 132.04, 123.65, 67.84, 39.82.

*5.5. 2-(3-bromo-5-fluorophenoxy)ethanamine (**15b**)*

The Mitsunobu reaction was performed according to general procedure A with 3-bromo-5-fluorophenol **14b** (565 mg, 2.96 mmol), N-*t*boc-ethanolamine (607 mg, 3.77 mmol), DIAD (698 mg, 3.45 mmol), triphenylphosphine (920 mg, 3.51 mmol) and THF. The reaction time was 5 h. Then the general procedure C was used for the N-*t*boc deprotection. Final product **15b** was obtained as a white solid (162 mg, 23%). ^{1}H NMR (MeOD, d_4, 400 MHz) δ 7.00 (s, br, 1H), 6.98 (dt, J = 1.2-3.6 Hz, 1H), 6.83 (dt, J = 2-10.4 Hz, 1H), 4.25 (t, J = 4 Hz, 2H), 3.37 (t, J = 4.8 Hz, 2H) ; ^{13}C NMR (MeOD, d_4, 100 MHz) δ 161.26, 132.07, 123.84, 115.43, 113.07, 102.65, 66.08, 40.05.

*5.6. 2-(5-bromo-2,3-difluorophenoxy)ethanamine (**15c**)*

The Mitsunobu reaction was performed according to general procedure B with 5-bromo-2,3-difluorofluorophenol **14c** (814 mg, 3.90 mmol), N-*t*boc-ethanolamine (635 mg, 3.94 mmol), DIAD (714 mg, 3.53 mmol), triphenylphosphine (1.32 g, 5.03 mmol) and THF. The total reaction time was 6 h. Then the general procedure C was used for the N-*t*boc deprotection. Final product **15c** was obtained as a white solid (403 mg, 36%). ^{1}H NMR (MeOD, d_4, 400 MHz) δ 7.22 (d, J = 3.2 Hz, 1H), 7.19 (dt, J = 2.4-3.2 Hz, 1H), 4.35 (t, J = 4.8 Hz, 2H), 3.40 (t, J = 4.8 Hz, 2H); ^{13}C NMR (MeOD, d_4, 100 MHz) δ 154.26, 149.24, 143.50, 115.53, 114.83, 67.76, 40.07.

5.7. General procedure D: Eschweiler-Clarke methylation

The appropriate ethanamine (**15a**, **15b** or **15c**) (1eq) was taken into aqueous formaldehyde solution (3-6 mL) and formic acid (5-6 mL) was added. The resulting mixture was refluxed for 6 h. After cooling down, the solution was basified to pH = 9-10 with NaOH 10 N. The aqueous solution was extracted with 4 x 20 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give a yellowish/brownish oil. The crude material was purified using silicagel chromatography with a gradient of CH_2Cl_2/MeOH 10% to give the desired product.

*5.8. 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine (**16a**)*

The methylation reaction was achieved according to general procedure D with 2-[(5-bromopyridin-3-yl)oxy]ethanamine **15a** (750 mg, 3.46 mmol), aqueous formaldehyde (4 mL) solution and formic acid (4 mL) The resulting mixture was refluxed for 6h The crude compound was purified on flash chromatography using a gradient of CH_2Cl_2/MeOH. Final product **16a** was obtained as a yellow oil (522 mg, 62%). ^{1}H NMR ($CDCl_3$, 400 MHz): 8.25 (d, J=1.2 Hz, 1H), 8.23 (d, J=2.4 Hz, 1H), 7.36 (t, J= 2.4 Hz, 1H), 4.07 (t, J=5.6 Hz, 2H), 2.71 (t, J=5.2 Hz, 2H), 2.31 (s, 6H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : 155.3, 142.9, 136.4, 123.9, 120.3, 66.7, 57.9, 45.8.

*5.9. 2-(3-bromo-5-fluorophenoxy)-N,N-dimethylethanamine (**16b**)*

The methylation reaction was achieved according to general procedure D with 2-(3-bromo-5-fluorophenoxy)ethanamine **15b** (656 mg, 2.80 mmol), aqueous formaldehyde (3 mL) solution and formic acid (3 mL) The resulting mixture was refluxed for 6h The crude compound was purified on flash chromatography using a gradient of CH_2Cl_2/MeOH. Final product **16b** was obtained as a white oil (197 mg, 27%). ^{1}H NMR ($CDCl_3$, 400 MHz): 6.97 (s, br, 1H), 6.90 (dt, J=2.2-8.2 Hz, 1H,), 6.74 (dt, J= 2.2-10.8 Hz, 1H), 4.09 (t, J=5.6 Hz, 2H), 2.76 (t, J=5.2 Hz, 2H), 2.33 (s, 6H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : 166.09, 162.21, 123.8, 115.29, 112.33, 102.49, 67.35, 58.78, 47.77.

*5.10. 2-(5-bromo-2,3-difluorophenoxy)-N,N-dimethylethanamine (**16c**)*

The methylation reaction was achieved according to general procedure D with 2-(5-bromo-2,3-difluorophenoxy)ethanamine **15c** (430 mg, 1.71 mmol), aqueous formaldehyde (3 mL) solution and formic acid (3 mL) The resulting mixture was refluxed for 6h The crude compound

was purified on flash chromatography using a gradient of CH_2Cl_2/MeOH. Final product **16c** was obtained as a white oil (111 mg, 23%). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.13 (d, J=10.9 Hz), 7.08 (dt, J=2.2-8.5 Hz, 1H), 4.19 (t, J=5.3 Hz, 2H), 2.82 (t, J=5.4 Hz, 2H), 2.36 (s, 6H) ; ^{13}C NMR ($CDCl_3$, 100 MHz): 151.27, 150.34, 143.19, 116.53, 114.97, 113.81, 69.11, 58.70, 45.92.

5.11. General Procedure E: Buchwald-Hartwig cross coupling reaction / microwave procedure

In a microwave vessel, palladium acetate (5-10 mol%), (R,S)- BINAP or XPhos (10-30 mol%), sodium *tert*-butoxide (1.5-3 eq), the appropriate amine (1-1.3 eq), 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (1 eq) were added and dissolved in a mixture of toluene with *tert*-butanol (5:1) (1 mL). The reaction vessel was sealed with a septum and placed into the microwave cavity. Microwave irradiation of 150 W was used and the temperature ramped from rt to 140°C. Once 140°C was reached the reaction mixture was held for 80 min. After cooling down, the reaction vessel was opened and the reaction mixture was filtered on a C-18 SPE column and eluted with methanol. The solvent was removed under reduced pressure. The crude compound was purified by preparative HPLC system using RP C-18 silicagel and appropriate MeOH/H_2O gradients. The chromatograms were scanned at 254 and 220 nm. The appropriate fractions were collected. The fractions containing the desired product were concentrated under reduced pressure.

5.12. General Procedure F: Buchwald-Hartwig cross coupling / Q-tube procedure

A Q-tube was loaded with palladium acetate (5-10 mol%), (R,S)-BINAP or XPhos (10-30 mol%), sodium *tert*-butoxide (1.5-3 eq), the appropriate amine (1.2-1.3 eq), the appropriate bromo-containing compounds **16a**, **16b**, or **16c** (1 eq) and then dissolved in a mixture of toluene with *tert*-butanol (5:1) (2 mL). The Q-tube was flushed with nitrogen and the reaction mixture was heated at 160°C for 2 h. The pressure was released and the reaction vessel was opened. The reaction mixture was filtered on a C-18 SPE column and eluted with cyclohexane. The solvent was removed under reduced pressure. The crude compound was purified by preparative HPLC system using RP C-18 silicagel and appropriate MeOH/H_2O gradients. The chromatograms were scanned at 254 and 220 nm. The appropriate fractions were collected. The fractions containing the desired product were concentrated under reduced pressure.

5.13. General procedure G: Cleavage of the N-tboc protecting group

HCl in 1,4-dioxane (4 N, 1-2 mL) was added to a stirred solution of the N-*t*boc protected compound (0.15-0.55 mmol), dissolved in 1-2 mL of 1,4-dioxane, and the mixture was allowed to stir at rt for 15 h. The solvent was removed. The residue was taken into KOH 0.2 N (5 mL) and extracted with 5x10 mL of CH_2Cl_2. The organic layers were collected and dried over $MgSO_4$. The solution was filtered and the solvent removed to give the desired compound.

*5.14. 2,2'-([3,3'-bipyridine]-5,5'-diylbis(oxy))bis(N,N-dimethylethanamine) (**10**)*

A Q-tube was loaded with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (93 mg, 038 mmol), $Pd(OAc)_2$ (6 mg, 0.027 mmol), tetrabutylammonium bromide (71 mg, 0.22 mmol), K_2CO_3 (98 mg, 0.71 mmol) dissolved in 2 mL DMF/H_2O (1/1). The reaction mixture was heated at 115°C for 4 h. After cooling down, water (20 mL) and Et_2O (30 mL) were added to the reaction mixture. The organic layer was extracted and washed with 3x15mL of water, then it was dried over MgSO4, filtererd and the solvent removed. The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (33 mg, 20%). HRMS (ESI) m/z calcd for $C_{18}H_{26}N_4O_2$ $(M+H)^+$: 331.2129, found: 331.2126.; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 8.32 (d, J = 2 Hz, 2H), 8.20 (t, J = 2.4 Hz, 2H), 7.21 (d, J = 1.2 Hz, 2H), 4.10 (t, J = 5.6 Hz, 4H), 2.74 (t, J = 5.2 Hz, 4H), 2.33 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.3, 140.65, 137.40, 134.04, 120.11, 66.63, 58.25, 45.99.

*5.15. 2-((5-(3,7-diazabicyclo[3.3.1]nonan-3-yl)pyridin-3-yl)oxy)-N,N-dimethylethanamine (**17a**)*

Compound **17a** was prepared according to the general procedure E for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (91 mg, 0.37 mmol), N*t*boc-bispidine (145 mg, 0.64mmol), palladium acetate (6 mg, 0.027 mmol), BINAP (16 mg, 0.026 mmol), sodium *tert*-butoxide (58 mg, 0.60 mmol). The crude product was purified by preparative HPLC: 60-100-60/10 mL x min^{-1}/30 min (H_2O/MeOH). Then the N-*t*Boc protective group was removed using the general procedure G with 1,4-dioxane (1.5 mL) and HCl in 1,4-dioxane (1.5 mL) for 0.15 mmol of material to obtain (18 mg, 42%). HRMS (ESI) m/z calcd for $C_{16}H_{26}N_4O$ $(M+H)^+$: 291.2177, found: 291.2176.; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 7.95 (d, J = 1.6 Hz, 1H), 7.78 (d, J = 2.4 Hz, 1H), 6.74 (t, J = 2.4 Hz, 1H), 4.09 (t, J = 6 Hz, 2H), 3.73 (d, J = 11.6 Hz, 2H), 3.14-3.00 (m, 6H), 2.71 (t, J = 6 Hz, 2H), 2.66 (s, broad, 3H), 2.31 (s, 6H),

1.96-1.81 (m, 4H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.44, 147.74, 130.59, 126.97, 108.27, 66.28, 58.26, 53.53, 52.17, 45.83, 31.01, 28.93.

*5.16. 2-(3-(3,7-diazabicyclo[3.3.1]nonan-3-yl)-5-fluorophenoxy)-N,N-dimethylethanamine (**17b**)*

Compound **17b** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(3-bromo-5-fluorophenoxy)-N,N-dimethylethanamine **16b** (136 mg, 0.52 mmol), N-*t*boc-bispidine (143 mg, 0.63mmol), palladium acetate (5 mg, 0.022 mmol), BINAP (25 mg, 0.040 mmol), sodium *tert*-butoxide (99 mg, 1.03 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH). Then the N-*t*boc protective group was removed using the general procedure G with 1,4-dioxane (1 mL) and HCl in 1,4-dioxane (1 mL) for 0.055 mmol of material to yield (10 mg, 6%). HRMS (ESI) m/z calcd for $C_{17}H_{26}FN_3O$ $(M+H)^+$: 308.2133, found: 308.2134.; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 6.27 (d, J = 2 Hz, 21H), 6.12 (d, J = 10.4 Hz, 1H), 4.03 (t, J = 5.6 Hz, 2 H), 3.71 (d, J = 8 Hz, 3H), 3.15-3.10 (m, 6H), 2.71 (t, J = 5.6 Hz, 2H), 2.33 (s, 6H), 1.96-1.81 (m, 4H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 165.77, 163.38, 160.78, 92.97, 95.14, 92.34, 66.22, 58.35, 53.93, 52.32, 46.01, 31.24, 29.15.

*5.17. 2-(5-(3,7-diazabicyclo[3.3.1]nonan-3-yl)-2,3-difluorophenoxy)-N,N-dimethylethanamine (**17c**)*

Compound **17c** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(5-bromo-2,3-difluorophenoxy)-N,N-dimethylethanamine **16c** (186 mg, 0.66 mmol), N-*t*boc-bispidine (145 mg, 0.64 mmol), palladium acetate (9 mg, 0.040 mmol), BINAP (47 mg, 0.075 mmol), sodium *tert*-butoxide (138 mg, 1.43 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH). Then the N-*t*boc protective group was removed using the general procedure G with 1,4-dioxane (1 mL) and HCl in 1,4-dioxane (1 mL) for 0.015 mmol of material to yield 14 mg, (6%). HRMS (ESI) m/z calcd for $C_{17}H_{25}F_2N_3O$ $(M+H)^+$: 326.2038, found : 326.2047.; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 6.50 (s, 1H), 6.35 (d, J = 11.2 Hz, 1H), 4.17 (d, J = 12.8 Hz, 2H), 3.62 (d, J = 11.6 Hz, 2H), 3.32 (d, J = 12.8 Hz, 2H), 3.12 (d, J = 13.2 Hz, 2H), 3.01 (d, J = 11.6 Hz, 2H), 2.76 (t, J = 5.6 Hz, 2H), 2.35 (s, 6H) 1.98-1.81 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 152.74, 150.31, 148.55, 147.28, 100.15, 97.46, 68.51, 58.25, 55.26, 51.08, 46.10, 30.84, 28.58.

*5.18. 2-((5-(1,4-diazepan-1-yl)pyridin-3-yl)oxy)-N,N-dimethylethanamine (**18a**)*

Compound **18a** was prepared according to general procedure F for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (134 mg, 0.55 mmol), homopiperazine (200 mg, 1.00 mmol), palladium acetate (11 mg, 0.049 mmol), BINAP (52 mg, 0.083 mmol), sodium *tert*-butoxide (180 mg, 1.87 mmol). The product was purified by preparative HPLC: 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH). Then the N-*t*boc protective group was removed using the general procedure G with 1,4-dioxane (1 mL) and HCl in 1,4-dioxane (1 mL) for 0.081 mmol of crude material to yield (9 mg, 6%). HRMS (ESI) m/z calcd for $C_{14}H_{24}N_4O$ $(M+H)^+$ 265.2023, found: 265.2028; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 7.78 (d, J = 2.4 Hz, 1H), 7.65 (d, J = 2Hz, 1H), 6.52 (t, J = 2 Hz, 1H), 4.09 (t, J = 5.6 Hz, 2H), 3.57-3.51 (m, 4H,),3.47 (s, 1H), 3.02 (t, J = 5.2 Hz, 2H), 2.83 (t, J = 5.6 Hz, 2H), 2.72 (t, J = 5.6 Hz, 2H), 2.33 (s, 6H), 1.91 (q, J = 6 Hz, 2H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ155.99, 145.55, 128.06, 124.17, 105.04, 66.28, 58.49, 51.86, 48.22, 48.16, 47.97, 46.00, 29.35.

*5.19. 2-(3-(1,4-diazepan-1-yl)-5-fluorophenoxy)-N,N-dimethylethan-1-amine (**18b**)*

Compound **18b** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(3-bromo-5-fluorophenoxy)-N,N-dimethylethanamine **16b** (113 mg, 0.43 mmol), N-*t*boc-homopiperazine (144 mg, 0.72 mmol), palladium acetate (9 mg, 0.038 mmol), BINAP (60 mg, 0.096 mmol), sodium *tert*-butoxide (104 mg, 1.08 mmol). The product was purified by preparative HPLC method 80-100-80/10mL x min^{-1}/30min (H_2O/MeOH). Then the N-tboc protective group was removed using the general procedure G with 1,4-dioxane (1 mL) and HCl in 1,4-dioxane (1 mL) for 0.080 mmol of material to yield 18 mg (10%). HRMS (ESI) m/z calcd for $C_{15}H_{24}FN_3O$ $(M+H)^+$: 282.1976, found: 282.1995.; ^{1}H NMR (MeOD d_4, 400 MHz) : δ 6.09, (t, J = 8-4 Hz, 2H), 5.99 (dt, J = 8-4 Hz, 1H), 4.05 (t, J = 4 Hz, 2H), 3.54 (q, J = 4 Hz, 4H), 2.99 (t, J = 4 Hz, 2H), 2.81 (t, J = 4 Hz, 2H), 2.74 (t, J = 4 Hz, 2H), 2.33 (s, 6H), 1.94 (J = 4 Hz, 2H); ^{13}C NMR (MeOD d_4, 100 MHz) : δ 167.61, 165.24, 162.54, 151.84, 95.30, 92.24, 90.53, 66.63, 59.10, 51.95, 45.82, 29.71.

*5.20. 2-(5-(1,4-diazepan-1-yl)-2,3-difluorophenoxy)-N,N-dimethylethan-1-amine (**18c**)*

Compound **18c** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(5-bromo-2,3-fluorophenoxy)-N,N-dimethylethanamine **16c** (100 mg, 0.36 mmol), N-*t*boc-homopiperazine (184 mg, 0.92mmol), palladium acetate (8 mg, 0.036 mmol), BINAP (33 mg, 0.052 mmol), sodium *tert*-butoxide (43 mg, 0.44 mmol). The product was purified by preparative HPLC method 80-100-80/10mL x min^{-1}/30min (H_2O/MeOH). Then the N-*t*boc protective group was removed using the general procedure G with 1,4-dioxane (0.5 mL) and HCl in 1,4-dioxane (0.4 mL) for 0.032 mmol of material to yield to yield 8 mg (8%). HRMS (ESI) m/z calcd for $C_{15}H_{23}F_2N_3O$ $(M+H)^+$: 300.1882, found: 300.1888.; ^{1}H NMR (MeOD d_4, 400 MHz) : δ 6.20-6.13 (m, 2H), 4.16 (t, J = 4 Hz, 2H), 3.67 (dq, J = 4 Hz, 1H), 3.58 (q, J = 4 Hz, 1H), 3.53 (q J = 4 Hz, 4H), 2.95 (t, J = 4 Hz, 1H), 2.78 (q, J = 4 Hz, 3H), 2.35 (s, 6H), 1.96-1.89 (m, 2H); ^{13}C NMR (MeOD d_4, 100 MHz) : δ 162.30, 161.41, 151.76, 149.89, 95.22, 93.40, 93.17, 68.86, 59.04, 52.56, 52.24, 45.96, 30.13.

*5.21. N,N-dimethyl-2-((5-(4-methyl-1,4-diazepan-1-yl)pyridin-3-yl)oxy)ethanamine (**19a**)*

Compound **19a** was prepared according to general procedure F for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (95 mg, 0.38 mmol), N-methyl homopiperazine (73 mg, 0.64 mmol), palladium acetate (9 mg, 0.040 mmol), BINAP (25 mg, 0.040 mmol), sodium *tert*-butoxide (64 mg, 0.66 mmol). The product was purified by preparative HPLC: 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (55 mg, 51%). HRMS (ESI) m/z calcd for $C_{15}H_{26}N_4O$ $(M+H)^+$: 279.2179, found: 279.2181; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 7.76 (d, J = 2.4 Hz, 1H), 7.64 (d, J = 2 Hz, 1H), 6.50 (t, J = 2.4 Hz, 1H), 4.08 (t, J = 5.6 Hz, 2H), 3.54 (t, J = 4.4 Hz, 2H,),3.46 (q, J = 3.6 Hz, 2H), 2.72-2.67 (m, 4H), 2.54 (t, J = 5.2 Hz, 2H), 2.36 (s, 3H), 2.32 (s, 6H), 2.03 (q, J = 6.4 Hz, 2H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.82, 146.15, 127.98, 124.06, 104.96, 66.20, 58.43, 57.83, 57.17, 48.47, 48.15, 46.75, 45.95, 27.57.

*5.22. 2-(3-fluoro-5-(4-methyl-1,4-diazepan-1-yl)phenoxy)-N,N-dimethylethan-1-amine (**19b**)*

Compound **19b** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(3-bromo-5-fluorophenoxy)-N,N-dimethylethanamine **16b** (144 mg, 0.55 mmol), N-methylhomopiperazine (88 mg, 0.77mmol), palladium acetate (11 mg, 0.051 mmol), BINAP (37 mg, 0.059 mmol), sodium *tert*-butoxide (140 mg, 1.45 mmol). The product was

purified by preparative HPLC method 80-100-80/10mL x min^{-1}/35min (H_2O/MeOH) yield (22.4 mg, 14%). HRMS (ESI) m/z calcd for $C_{16}H_{26}FN_3O$ $(M+H)^+$: 296.2133, found: 296.2129; ^1H NMR ($CDCl_3$, 400 MHz) : δ 5.99 (d, J = 14 Hz, 2H), 5.96 (d, J = 8.8 Hz, 1H), 3.99 (t, J = 5.6 Hz, 2H), 3.49 (t, J = 4.8 Hz, 2H), 3.41 (t, J = 6.4 Hz, 2H), 2.70-2.64 (m, 4H), 2.53 (t, J = 5.2 Hz, 2H), 2.34 (s, 3H), 1.99 (m, 2H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 163.73, 161.05, 151.16, 94.48, 92.08, 89.48, 65.98, 58.32, 57.96, 57.09, 48.68, 48.40, 46.66, 45.92, 27.63.

*5.23. 2-(2,3-difluoro-5-(4-methyl-1,4-diazepan-1-yl)phenoxy)-N,N-dimethylethan-1-amine (**19c**)*

Compound **19c** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(5-bromo-2,3-difluorophenoxy)-N,N-dimethylethanamine **16c** (130 mg, 0.47 mmol), N-methylhomopiperazine (97 mg, 0.85mmol), palladium acetate (16 mg, 0.070 mmol), BINAP (44 mg, 0.071 mmol), sodium *tert*-butoxide (123 mg, 1.28 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (12 mg, 8%). HRMS (ESI) m/z calcd for $C_{16}H_{25}F_2N_3O$ $(M+H)^+$: 314.2038, found: 314.2031; ^1H NMR ($CDCl_3$, 400 MHz) : δ 6.06 (t, J = 3 Hz, 1H), 6.02 (t, J = 3Hz, 1H), 4.12 (t, J = 6 Hz, 2H), 3.48 (t, J = 3 Hz, 2H), 3.39 (t, J = 3 Hz, 2H), 2.75 (t, J = 6 Hz, 2H), 2.66 (t, J = 3 Hz, 2H), 2.55 (t, J = 3 Hz, 2H), 2.38 (s, 3H), 2.35 (s, 6H), 1.98 (q, J = 3 Hz, 2H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 153.17, 150.67, 148.60, 145.43, 128.80, 94.55, 92.58, 68.44, 58.24, 57.88, 57.13, 48.94, 48.49, 46.71, 46.03, 27.67.

*5.24. N,N-dimethyl-2-{[5-(piperazin-1-yl)pyridin-3-yl]oxy}ethanamine (**20a**)*

Compound **20a** was prepared according to the general procedure E for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (92 mg, 0.37 mmol), N*t*boc-piperazine (114 mg, 0.61 mmol), palladium acetate (6 mg, 0.027 mmol), XPhos (23 mg, 0.048 mmol), sodium *tert*-butoxide (52 mg, 0.54 mmol). Then the N-*t*boc protective group was removed using the general procedure G with 1,4-dioxane (6 mL) and HCl in 1,4-dioxane (6 mL) for 0.5 mmol of crude material. The product was purified by preparative HPLC: 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (53 mg, 43%). HRMS (ESI) m/z calcd for $C_{13}H_{22}N_4O$ $(M+H)^+$: 251.1865, found: 251.1866. ^1H NMR ($CDCl_3$, 400 MHz) : δ 7.65 (d, J = 2 Hz, 1H), 7.53 (d, J = 2 Hz, 1H), 6.64 (t, J = 2 Hz, 1H), 3.93 (t, J = 5.6 Hz, 2H), 3.04 (t, J = 5.2 Hz, 4H), 2.87 (t, J = 5.2 Hz, 4H), 2.61 (t, J = 2 Hz, 2H), 2.17 (s, 6H).

*5.25. 2-(3-fluoro-5-(piperazin-1-yl)phenoxy)-N,N-dimethylethan-1-amine (**20b**)*

Compound **20b** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(3-bromo-5-fluorophenoxy)-N,N-dimethylethanamine **16b** (123 mg, 0.47 mmol), N-*t*boc-piperazine (123 mg, 0.66mmol), palladium acetate (6 mg, 0.027 mmol), BINAP (48 mg, 0.078 mmol), sodium *tert*-butoxide (136 mg, 1.41 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH). Then the N-*t*boc protective group was removed using the general procedure G with 1,4-dioxane (1 mL) and HCl in 1,4-dioxane (1 mL) for 0.067 mmol of material to yield 18 mg, (14%). HRMS (ESI) m/z calcd for $C_{14}H_{22}FN_3O$ $(M+H)^+$: 268.182, found: 268.1846.; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 6.21 (d, J = 14.3 Hz, 2H), 6.12 (dd, J = 12.5-2.0 Hz, 1H), 4.00 (t, J = 5.6 Hz, 2H), 3.12 (t, J = 4.6 Hz, 4H), 2.99 (t, J = 5.5 Hz, 4H), 2.70 (t, J = 5.7 Hz, 2H), 2.32 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 165.78, 163.38, 160.79, 153.67, 98.38, 95.78, 92.84, 66.15, 58.30, 49.79, 45.96.

*5.26. 2-(2,3-difluoro-5-(piperazin-1-yl)phenoxy)-N,N-dimethylethan-1-amine (**20c**)*

Compound **20c** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(5-bromo-2,3-fluorophenoxy)-N,N-dimethylethanamine **16c** (98 mg, 0.35 mmol), N-*t*boc-piperazine (91 mg, 0.49 mmol), palladium acetate (7 mg, 0.032 mmol), BINAP (24 mg, 0.039 mmol), sodium *tert*-butoxide (106 mg, 1.10 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH). Then the N-*t*boc protective group was removed using the general procedure G with 1,4-dioxane (1 mL) and HCl in 1,4-dioxane (1 mL) for 0.065 mmol of material to yield 13 mg (13%). HRMS (ESI) m/z calcd for $C_{14}H_{21}F_2N_3O$ $(M+H)^+$: 286.1725, found: 286.1746.; ^{1}H NMR (MeOD-d_4, 400 MHz) : δ 6.49 (dt, J = 8-4 Hz, 1H), 6.43 (dt, J = 16-4 Hz, 1H), 4.18 (t, J = 4 Hz, 2H), 3.12 (t, J = 4 Hz, 4H), 2.99 (t, J = 4 Hz, 4H), 2.81 (t, J = 4 Hz, 2H), 2.37 (s, 6H); ^{13}C NMR (MeOD-d_4, 100 MHz) : δ 153.89, 151.55, 149.68, 149.42, 137.94, 100.17, 98.03, 68.77, 58.95, 50.71, 46.28, 45.94.

*5.27. N,N-dimethyl-2-{[5-(4-methylpiperazin-1-yl)pyridin-3-yl]oxy}ethanamine (**21a**)*

Compound **21a** was prepared according to general procedure E for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (92 mg, 0.37 mmol), N-methyl piperazine (55 mg, 0.55 mmol), palladium acetate (6 mg, 0.027 mmol), XPhos (19 mg,

0.040 mmol), sodium *tert*-butoxide (53 mg, 0.55 mmol). The product was purified by preparative HPLC: 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (56 mg, 57%). HRMS (ESI) m/z calcd for $C_{14}H_{24}N_4O$ $(M+H)^+$: 265.2018, found: 265.2023; ^1H NMR ($CDCl_3$, 400 MHz) : δ 7.68 (d, J = 2Hz, 1H), 7.55 (d, J = 1.6 Hz, 1H), 6.64 (t, J = 2.4 Hz, 1H), 3.94 (t, J = 5.6 Hz, 2H), 3.08 (t, J = 4.8 Hz, 4H,), 2.61 (t, J = 5.6 Hz, 2H), 2.44 (t, J = 4.8 Hz, 4H), 2.19 (s, 9H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.40, 147.78, 130.26, 126.74, 108.99, 65.57, 57.54, 54.19, 47.54, 45.34, 45.02.

*5.28. 2-(3-fluoro-5-(4-methylpiperazin-1-yl)phenoxy)-N,N-dimethylethan-1-amine (**21b**)*

Compound **21b** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(3-bromo-5-fluorophenoxy)-N,N-dimethylethanamine **16b** (117 mg, 0.44 mmol), N-methylpiperazine (77 mg, 0.77mmol), palladium acetate (6 mg, 0.026 mmol), BINAP (30 mg, 0.048 mmol), sodium *tert*-butoxide (130 mg, 1.35 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) to yield 50 mg (40%). HRMS (ESI) m/z calcd for $C_{15}H_{24}FN_3O$ $(M+H)^+$: 282.1976, found: 282.1983.; ^1H NMR (MeOD d_4, 400 MHz) : δ 6.28 (d, J = 1.8 Hz, 2H), 6.18 (dt, J = 7.5, 1.5 Hz, 1H), 4.05 (t, J = 4.2 Hz, 2H), 3.20 (t, J = 3.6 Hz, 4H), 2.74 (t, J = 4.2 Hz, 2H), 2.57 (t, J = 3.6 Hz, 4H), 2.33 (s, 9H); ^{13}C NMR (MeOD d_4, 100 MHz) : δ 167.07, 164.68, 162.17, 154.66, 99.18, 96.59, 93.93, 66.80, 59.03, 55.83, 46.09, 45.82.

*5.29. 2-(2,3-difluoro-5-(4-methylpiperazin-1-yl)phenoxy)-N,N-dimethylethan-1-amine (**21c**)*

Compound **21c** was prepared according to the general procedure F for Buchwald-Hartwig cross coupling with 2-(5- bromo-2,3-fluorophenoxy)-N,N-dimethylethanamine **16c** (151 mg, 0.54 mmol), N-methylpiperazine (100 mg, 1.00 mmol), palladium acetate (9 mg, 0.040 mmol), BINAP (62 mg, 0.099 mmol), sodium *tert*-butoxide (124 mg, 1.29 mmol). The product was purified by preparative HPLC method 80-100-80/10mL x min^{-1}/30min (H_2O/MeOH) to yield 32 mg (40%). HRMS (ESI) m/z calcd for $C_{15}H_{23}F_2N_3O$ $(M+H)^+$: 300.1882, found: 300.1888.; ^1H NMR ($CDCl_3$, 400 MHz) : δ 6.29 (d. J = 8 Hz, 1H), 6.26 (t, J = 4 Hz, 1H), 4.10 (t, J = 8 Hz, 2H), 3.10 (t, J = 4 Hz, 4H), 2.73 (t, J = 8 Hz, 2H), 2.53 (t, J = 8 Hz, 4H), 2.32 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 152.78, 150.32, 148.39, 147.32, 136.92, 134.53, 99.14, 97.26, 68.25, 58.11, 54.87, 49.33, 46.02, 45.91.

*5.30. 2-{[5-(azetidin-1-yl)pyridin-3-yl]oxy}-N,N-dimethylethanamine (**22**)*

Compound **22** was prepared according to general procedure F for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (97 mg, 0.40 mmol), azetidine hydrochloride (45 mg, 0.48 mmol), palladium acetate (7 mg, 0.031 mmol), BINAP (25 mg, 0.040 mmol), sodium *tert*-butoxide (143 mg, 1.48 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (1.6 mg, 2%). HRMS (ESI) m/z calcd for $C_{12}H_{19}N_3O$ $(M+H)^+$: 222.1601, found: 222.1604. ^1H NMR (MeOD d_4, 400 MHz) : δ 7.60 (d, J = 2.4 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 6.44 (t, J = 2.2 Hz, 1H), 4.15 (t, J = 5.2 Hz, 2H), 3.94 (t, J = 7.2 Hz, 4H), 2.83 (t, J = 5.6 Hz, 2H), 2.44-2.39 (m, 8H). ^{13}C NMR (MeOD d_4, 100 MHz) : δ 157.15, 150.67, 127.17, 126.87, 105.25, 58.92, 53.51, 45.69, 18.22.

*5.31. N,N-dimethyl-2-{[5-(pyrrolidin-1-yl)pyridin-3-yl]oxy}ethanamine (**23**)*

Compound **23** was prepared according to general procedure E for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (88 mg, 0.36 mmol), pyrrolidine (35 mg, 0.49 mmol), palladium acetate (4 mg, 0.018 mmol), BINAP (47 mg, 0.075 mmol), sodium *tert*-butoxide (47 mg, 0.48 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (73 mg, 86%). HRMS (ESI) m/z calcd for $C_{13}H_{21}N_3O$ $(M+H)^+$: 236.1749, found: 236.1757. ^1H NMR ($CDCl_3$, 400 MHz) : δ 7.67 (d, J = 2.0 Hz, 1H), 7.64 (d, J = 2.0 Hz, 1H,), 6.38 (t, J = 2.4 Hz, 1H), 4.11 (t, J = 6 Hz, 2H), 3.28 (t, J = 6.4 Hz, 4H), 2.73 (t, J = 5.6 Hz, 2H), 2.34 (s, 6H), 2.02 (t, J = 3.2 Hz, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.66, 144.68, 127.88, 124.10, 104.41, 66.03, 58.32, 47.42, 45.88, 25.37.

*5.32. N,N-dimethyl-2-{[5-(piperidin-1-yl)pyridin-3-yl]oxy}ethanamine (**24**)*

Compound **24** was prepared according to general procedure E for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (101 mg, 0.41 mmol), piperidine (113 mg, 0.13 mmol), palladium acetate (15 mg, 0.067 mmol), XPhos (30 mg, 0.063 mmol), sodium *tert*-butoxide (70 mg, 0.73 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (6 mg, 6%). HRMS (ESI) m/z calcd for $C_{14}H_{23}N_3O$ $(M+H)^+$: 250.1924, found: 250.1914; ^1H NMR ($CDCl_3$, 400 MHz) : δ 7.95 (d, J = 2.4 Hz, 1H), 7.77 (d, J = 2.4 Hz, 1H), 6.73 (t, J = 2.4 Hz, 1H), 4.09 (t, J = 5.6 Hz,

2H), 3.17 (t, 5.6 Hz, 4H), 2.72 (t, J = 5.6 Hz, 2H), 2.33 (s, 6H), 1.68 (q, J = 5.6 Hz, 4H), 1.59 (q, J = 4.8Hz, 2H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.47, 148.60, 132.04, 126.88, 109.18, 66.21, 58.28, 49.83, 45.84, 25.47, 24.14.

*5.33. N,N-dimethyl-2-{[5-(morpholin-4-yl)pyridin-3-yl]oxy}ethanamine (**25**)*

Compound **25** was prepared according to general procedure E for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (92 mg, 0.37 mmol), morpholine (65 mg, 0.75 mmol), palladium acetate (8 mg, 0.036 mmol), XPhos (25 mg, 0.053 mmol), sodium *tert*-butoxide (51 mg, 0.53 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (49 mg, 53%). HRMS (ESI) m/z calcd for $C_{13}H_{21}N_3O_2$ $(M+H)^+$: 252.1669, found: 252.1707; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 7.79 (d, J = 2 Hz, 1H), 7.69 (d, J = 2 Hz, 1H), 6.70 (t, J = 2.4 Hz, 1H), 4.03 (t, J = 5.6 Hz, 2H), 3.78 (t, J = 4.8 Hz, 4H), 3.11 (t, J = 4.8 Hz, 4H), 2.68 (t, J = 6 Hz, 2H), 2.26 (s, 6H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.50, 147.99, 130.31, 127.19, 108.92, 66.39, 65.83, 57.75, 48.25, 45.34.

*5.34. 1-(5-(2-(dimethylamino)ethoxy)pyridin-3-yl)piperidin-4-ol (**26**)*

Compound **26** was prepared according to general procedure F for Buchwald-Hartwig cross coupling with 2-[(5-bromopyridin-3-yl)oxy]-N,N-dimethylethanamine **16a** (93 mg, 0.37 mmol), piperidin-4-ol (82 mg, 0.81 mmol), palladium acetate (9 mg, 0.040 mmol), BINAP (45 mg, 0.072 mmol), sodium *tert*-butoxide (111 mg, 1.16 mmol). The product was purified by preparative HPLC method 60-100-60/10mL x min^{-1}/30min (H_2O/MeOH) yield (14 mg, 14%). HRMS (ESI) m/z calcd for $C_{14}H_{23}N_3O_2$ $(M+H)^+$:266.1863, found: 266.1862; ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 7.94 (d, J = 2 Hz, 1H), 7.77 (d, J = 2.4 Hz, 1H), 6.75 (t, J = 2.4 Hz, 1H), 4.10 (t, J = 5.6 Hz, 2H),3.95 (m, 1H), 3.55 (m, 2H), 2.96 (t, J = 12.4 Hz, 2H), 2.33 (s, 6H), 1.98 (m, 4H), 1.68 (m, 2H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 155.63, 147.95, 131.96, 127.16, 109.51, 67.43, 66.34, 58.38, 46.58, 45.96, 33.88.

5.35. Computational Studies

The partial charge calculation and the 3D pharmacophore model were generated using Discovery Studio 3.1. Physicochemical properties were calculated using the ACD/ADME suite 5.0 software (ACD/Labs). Details on the protocols and experiments are described below.

Partial charges were calculated for each atom of the HBA system (**17a**, **17b**, **17c**) using the Gasteiger-Marsili method. The geometry of the compounds was cleaned using a "fast Dreiding-like" forcefield.

3D QSAR pharmacophore model was developed based on compounds **17a, 18a, 19a, 20a, 21a, 22-26** using Discovery Studio 3.1. The full ligand set was sorted by a random index fixed at 60. All the ligands are considered to be active on the same binding site. A set of 10 pharmacophores was generated from six training set compounds (**17a, 18a, 20a, 23, 24, 26,**) and validated with test set of four compounds (**21a, 19a, 22, 25**). The parameters for the generation of the model were set up as followed: ligand conformational models were generated using CAESAR, with an energy cutoff of 20 kcal/mol and the maximum number of conformations set to 255. Minimum inter-feature distance was set to 1.0. Five features were considered: HBA, HBD, hydrophobic, positive ionizable, ring aromatic. A Fischer validation test with 90% confidence level was performed. All other parameters were kept as default. The "ligand pharmacophore mapping" protocol was used to determine the predicted activity for the test set. The parameters were set up as followed: ligand conformational models were generated using CAESAR, with an energy cutoff of 20 kcal/mol and the maximum number of conformations set to 255. "Maximum omitted features" parameter was set at -1. All other parameters were kept as the default ones.

The 2D ADMET plot of designed and synthesized compounds (**17**-**26**) was generated using Discovery Studio 3.1. Compounds (**17**-**26**) were drawn and "cleaned up" using a **"**fast Dreiding-like**"** force field. All structures were launched into the ADMET Descriptors protocol considering the following parameters: aqueous solubility, blood brain barrier penetration, cytochrome P450 2D6 inhibition, hepatotoxicity, human intestinal absorption and plasma protein binding. When the job was completed the computed properties were displayed as a 2D ADMET Plot using AlogP98 and PSA_2D.

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Supplementary data

Supplementary data associated with this article can be found in the online version at

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Graphical Abstract

The twin drug approach for novel nicotinic acetylcholine receptor ligands

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