ACS Medicinal Chemistry Letters

Letter

Varying Chirality Across Nicotinic Acetylcholine Receptor Subtypes – Selective Binding of Quinuclidine Triazole Compounds

Jiradanai Sarasamkan, Matthias Scheunemann, Nattayaporn Apaijai, Siripong Palee, Warisara Parichatikanond, Kuntarat Arunrungvichian, Steffen Fischer, Siriporn Chattipakorn, Winnie Deuther-Conrad, Gerrit Schüürmann, Peter Brust, and Opa Vajragupta

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.6b00146 • Publication Date (Web): 09 Aug 2016

ACS Publications

ACS Medicinal Chemistry Letters is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Varying Chirality Across Nicotinic Acetylcholine Receptor Subtypes–Selective Binding of Quinuclidine Triazole Compounds

Jiradanai Sarasamkan,[†,‡,§] Matthias Scheunemann,[‡] Nattayaporn Apaijai,[ᵖ] Siripong Palee,[ᵖ] Warisara Parichatikanond,[ᵠ] Kuntarat Arunrungvichian,[†] Steffen Fischer,[‡] Siriporn Chattipakorn,[ᵖ] Winnie Deuther-Conrad,[‡] Gerrit Schüürmann,[▽,#] Peter Brust,[‡] Opa Vajragupta[†,*]

[†]Center of Excellence for Innovation in Drug Design and Discovery, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayutthaya Road, Bangkok 10400, Thailand

[‡]Department of Neuroradiopharmaceuticals, Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Permoserstraße15, 04318 Leipzig, Germany

[§]National Cyclotron and PET Centre, Chulabhorn Hospital, 54 Kamphaengphet 6 Road, Bangkok 10210, Thailand

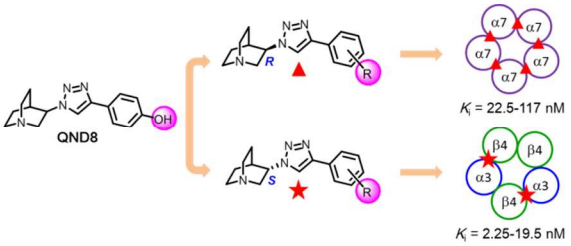
[ᵖ]Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

[ᵠ]Department of Pharmacology, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayutthaya Road, Bangkok 10400, Thailand

[▽]Department of Ecological Chemistry, Helmholtz Centre for Environmental Research – UFZ, Permoserstraße15, 04318 Leipzig, Germany

[#]Institute for Organic Chemistry, Technical University Bergakademie Freiberg, Leipziger Straße29, 09596 Freiberg, Germany

ABSTRACT: The novel quinuclidine *anti*-1,2,3-triazole derivatives **T1-T6** were designed based on the structure of **QND8**. The binding studies revealed that the stereochemistry at the C3 position of the quinuclidine scaffold plays an important role in the nAChR subtype selectivity. Whereas the (*R*)-enantiomers are selective to α7 over α4β2 (by factors of 44-225) and to a smaller degree over α3β4 (3-33), their (*S*)-counterparts prefer α3β4 over α4β2 (62-237) as well as over α7 (5-294). The (*R*)-derivatives were highly selective to α7 over α3β4 subtypes compared to (*RS*)- and (*R*)-**QND8**. The (*S*)-enantiomers are 5–10 times more selective to α4β2 than their (*R*) forms. The overall strongest affinity is observed for the (*S*)-enantiomer binding to α3β4 (K_i 2.25-19.5 nM) followed by their (*R*)-counterpart binding to α7 (K_i 22.5-117 nM), with a significantly weaker (*S*)-enantiomer binding to α4β2 (K_i 414-1980 nM) still above the very weak respective (*R*)-analog affinity (K_i 5059-10436 nM).



KEYWORDS: *Nicotinic acetylcholine receptor, positron emission tomography, quinuclidine anti-1,2,3-triazole, click chemistry*

Nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop receptor superfamily of ligand-gated ion channels that mediate fast synaptic transmission in the central (CNS) and peripheral (PNS) nervous systems.[1-4] They are pentameric structures of homomeric or heteromeric subunits symmetrically arranged around a central ion channel. There are 16 homologous mammalian nAChR subunits encoded by this multigene family. These subunits combine to form many different nAChR subtypes with various expression patterns, diverse functional properties, and different pharmacological characteristics.[1] Currently, there are at least 12 known nAChR subunits (α2-α10 and β2-β4) expressed in the brain.[4] The most predominant nAChRs found in the brain, associated with cognition, memory formation and behavior, are the α4β2 and α7 subtypes.[3] The α4β2 nAChR in mammalian brain accounts for the majority of the high affinity sites for nicotine[4] and is mainly found in the thalamus, cortical regions, striatum and colliculi whereas the distribution pattern of the α7 nAChR with high affinity for α-bungarotoxin is rather diffuse with focally high density in thalamus and rather low expression in the cerebellum.[3] A further important receptor subtype in the central nervous system (CNS) is the α3β4 nAChR because of its involvement in drug addiction and depression pathways.[5] In contrast to α4β2 and α7, this subtype is mainly expressed in the autonomic ganglia and only in selected brain regions (subsets of neurons in the medial habenula, nucleus interpeduncularis, dorsal medulla, pineal gland and retina).[4] The nAChRs are well recognized as key receptors involved not only in a variety of neuropsychiatric and neurodegenerative disorders, including Parkinson's disease (PD),

attention deficit hyperactivity disorder (ADHD), schizophrenia, Alzheimer's disease (AD), depression, epilepsy, nicotine and drug addiction,[6-12] but also in cancer.[13,14] The multiplicity of nAChR subtypes, their localization in the CNS and periphery along with their pharmacological, physiological and kinetic properties afford the opportunities to develop novel subtype-specific nAChR ligands for treatment of these diseases.[14,15]

Several compounds have been designed and synthesized based on known nAChR ligand recognition components covering the protonated amine that triggers the cation-π interaction, the hydrogen bond acceptor and the hydrophobic stabilization. The availability of aromatic moieties in this hydrophobic part may participate in favorable electron-π interactions which can be either electrostatic or dispersive. The tertiary quinuclidine amine is one of the most promising core structures used for the design and development of α7 nAChR ligands (Figure 1A). Besides the core structure, the effective pharmacophore linkers at position 3 of the quinuclidine scaffold are ether, carbamate, urea, amide, sulfonamide, oxazole, oxadiazole, and triazole.[16,17] Another approach in the design of α7 ligands was the synthesis of conformational restricted analogues such as oxazolidinone AR-R17779.[16] Among them, the compounds containing the quinuclidine *anti*-1,2,3-triazole skeleton bind with high affinity and selectivity to the α7 nAChR.[18] This hydrophobic motif in particular plays an important role in receptor subtype recognition through the interaction with amino acid residues of the complementary face which has been demonstrated as an essential part for ligand selectivity among various nAChR subtypes.[19-21] We recently reported three series of novel potent and selective α7 nAChR agonists featuring the quinuclidine *anti*-1,2,3-triazole containing molecules (**IND8** and **QND8**) that demonstrated cognitive enhancement in mice.[22] There is not only the pharmacological benefit of using the *anti*-1,2,3-triazole as the linker, but the triazole moiety itself is stable and largely resistant to metabolic degradation.[23,24]

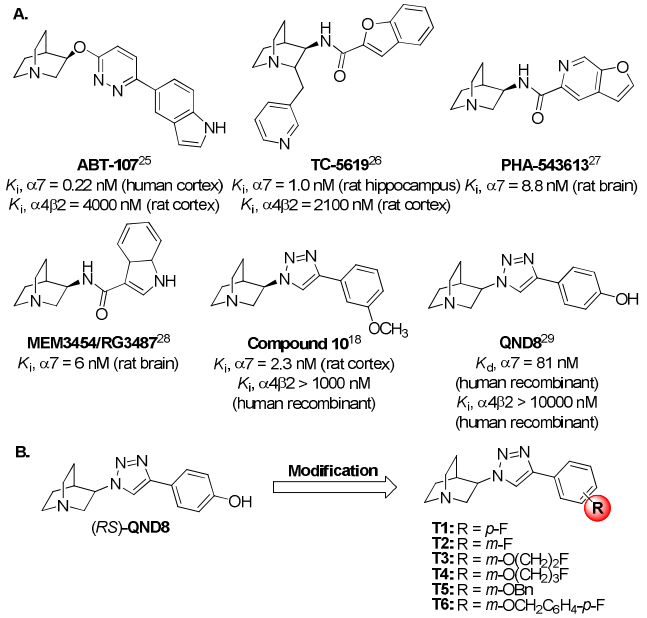
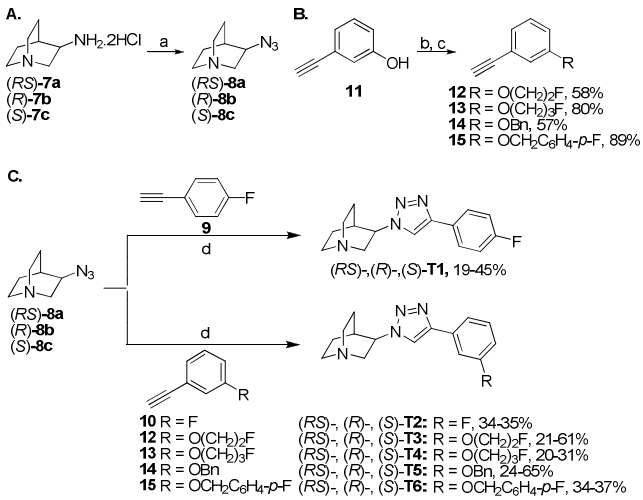


Figure 1. Structure of some nAChR ligands containing quinuclidine scaffold (A)[18,25-29] and design strategy (B).

In the present design, four different approaches were applied to the lead compound, (*RS*)-**QND8** (Figure 1B) to improve the potency and selectivity profiles at the most important neuronal nAChR subtypes (α7, α3β4, α4β2). First, the chirality at position 3 of the quinuclidine scaffold was investigated by individual analysis of the two enantiomers of the racemic compounds. Quinuclidine-containing compounds are well recognized for nAChR interaction and the selectivity of their (*R*)- enantiomers for α7 nAChR. However, detailed information with regard to their (*S*)-enantiomers was missing at the beginning of this study.[18,29] Thus, both enantiomers were prepared to investigate the influence of chirality on the selectivity as well as the affinity for nAChR. Second, the orientation of the hydrophobic aromatic ring was explored by altering the substitution from *para* to *meta*. With regard to α7 nAChR, the binding affinity of quinuclidine derivatives was found to prefer the *meta*-phenyl substitution, compared to the *para*-position.[18] The *m*-phenyl substitution was included to verify this notion. Third, the hydrophobic side chains were extended to enhance the binding affinity and selectivity, based on the increase in size as well as the lipophilicity for receptor binding. The expansion of the structure was conducted via an ether linkage (R in Figure 1B), where the phenol of **QND8** resides. Finally, the fluorine atoms were introduced into the structure for further development as potential positron emission tomography (PET) radioligands for imaging the nAChR in the brain.

Herein we report the synthesis and the binding affinity of quinuclidine derivatives **T1-T6** (*RS*, *R*, *S*) at neuronal α7, α3β4 and α4β2 nAChR subtypes. Promising compounds of high α3β4 binding affinity and good selectivity towards other nAChRs were further screened in functional assays using HEK cells expressing human nAChR subtypes.

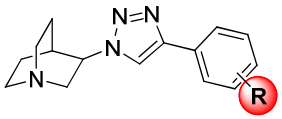
Scheme 1. Synthesis of Azide (A), Terminal Alkynes (B) and the Quinuclidine *anti*-1,2,3-Triazole Containing Molecules (C)[a]



[a]Reagents and conditions: (a) TfN_3, K_2CO_3, $CuSO_4.5H_2O$, CH_3OH, H_2O, rt, overnight, (b) 1-fluoro-2-iodoethane for **12** and 1-fluoro-3-iodopropane for **13**, K_2CO_3, CH_3CN, 70 °C, 10 h (c) benzyl chloride for **14** and 4-fluorobenzyl chloride for **15**, K_2CO_3, CH_3CN, 80 °C, 6 h (d) 5 mol% $CuSO_4.5H_2O$, 10 mol% sodium ascorbate, t-BuOH, H_2O, rt, overnight.

The general procedure for the preparation of the designed compounds started with the synthesis of the azide building block by a diazo transfer reaction and synthesis of terminal alkynes through the Sonogashira cross-coupling reaction, followed by the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction resulting in the

Table 1. ***In Vitro*** **Binding Affinities of 15 Quinuclidine** ***anti*****-1,2,3-Triazole Derivatives Towards nAChRs**[a]



R	Compd	Inhibition constant K_i in nM; mean ± SD			Selectivity[b] (inverse of respective K_i ratio)		
		α7[c]	α3β4[d]	α4β2[d]	α7 vs α3β4	α7 vs α4β2	α3β4 vs α4β2
p-F	(*RS*)-**T1**	72.8±13	8.50±0.50	449±127	1/8.6	6.2	53
	(*R*)-**T1**	73.0±15	1,010±162	10,436±1943	14	143	10
	(*S*)-**T1**	174.5±66	3.09±0.10	515±64	1/56	3.0	167
m-F	(*RS*)-**T2**	133±40	5.24±0.35	748±114	1/25	5.6	143
	(*R*)-**T2**	117±4	362±27	5,201±412	3.1	44	14
	(*S*)-**T2**	660.5±139	2.25±0.42	519±20	1/294	1/1.3	231
m-O$(CH_2)_2$F	(*RS*)-**T3**	98.7±39	20.9±0.7	1,962±228	1/4.7	20	94
	(*R*)-**T3**	38.8±8	558±34	7,050±200	14	182	13
	(*S*)-**T3**	74.9±20	11.8±0.3	1,262±187	1/6.3	17	107
m-O$(CH_2)_3$F	(*RS*)-**T4**	74.6±14	44.4±8.0	3,894±252	1/1.7	52	88
	(*R*)-**T4**	62.3±10	1,628±11	9,010±5,034	26	145	5.5
	(*S*)-**T4**	96.9±17	19.5±0.4	1,980±117	1/5.0	20	102
m-OBn	(*RS*)-**T5**	91.3±9	7.57±2.9	668±7	1/12	7.3	88
	(*R*)-**T5**	22.5±9	631±206	5,059±374	28	225	8.0
	(*S*)-**T5**	279±31	6.67±0.7	414±59	1/42	1.5	62
m-$OCH_2C_6H_4F$	(*RS*)-**T6**	127±5	13.9±2.8	1,013±107	1/9.1	8.0	73
	(*R*)-**T6**	33.2±7	1,090±163	6,392±230	33	193	5.9
	(*S*)-**T6**	149±42	7.17±1.2	537±11	1/21	3.6	75
p-OH	(*RS*)-**QND8**	9.61±1.47	3.44±0.04	627±52	1/2.8	65	182
	(*R*)- **QND8**	10.9±1.42	138±0	7389±42	12.7	678	54
	(*S*)- **QND8**	29.3±0.18	2.48±0.04	461±89	1/11.8	16	186

[a]Binding affinities are represented by the inhibition constant K_i determined for individual compounds in two separate experiments each performed as triplicate. [b]Selectivity is reported in terms of the reciprocal of the ratio of the respective K_i values. [c]Human α7 nAChR in stably transfected SH-SY5Y cells, with radiotracer [^{3}H]methyllycaconitine (0.5-1 nM), K_d = 2.0 nM. [d]Human α4β2 and α3β4 nAChR in stably transfected HEK-293 cells, with radiotracer [^{3}H]epibatidine (0.5-1 nM), K_d = 0.025 nM for hα4β2 nAChR, K_d = 0.117 nM for hα3β4 nAChR.

formation of the final *anti*-1,2,3-triazole-containing ligands (Scheme 1). The diazo transfer reaction for the preparation of azidoquinuclidine compounds (*RS*)-**8a**, (*R*)-**8b** and (*S*)-**8c** were achieved, respectively, by using 3-aminoquinuclidine dihydrochloride (*RS*)-**7a**, (*R*)-**7b** and (*S*)-**7c** interacted with freshly prepared trifluoromethanesulfonyl azide (TfN_3). The 3-ethynylphenol **11** was used as a starting material for the Williamson etherification with 1-fluoro-2-iodoethane, 1-fluoro-3-iodopropane, benzyl chloride and 4-fluorobenzyl chloride to afford the 3-ethynylphenylethers **12**, **13**, **14**, and **15**, respectively. The final compounds (*RS*)-**T1**, (*R*)-**T1** and (*S*)-**T1** were synthesized by the reaction of the respective azidoquinuclidines (*RS*)-**8a,** (*R*)-**8b** and (*S*)-**8c** with 4-fluoro-phenylacetylene **9** under CuAAC conditions. Cycloaddition of these azidoquinuclidines with 3-fluorophenylacetylene **10** yielded the final compounds (*RS*)-**T2**, (*R*)-**T2** and (*S*)-**T2**. CuAAC or click reactions between **8a-c** and terminal alkyne **12** resulted in the formation of its corresponding **T3** compounds. The remaining quinuclidine *anti*-1,2,3-triazoles **T4-T6** were prepared by the same synthetic route as described above.

The blood-brain barrier (BBB) penetration of CNS drugs depends on several physicochemical properties including molecular weight (MW), pK_a, hydrophobicity (logP & logD) and topological polar surface area (TPSA).These parameters were calculated for all synthesized compounds using the ACD/ADME Suite software, and the predicted values fall within the ranges of CNS drug-like

properties. Calculated logBB values of ≥ -1.0 indicate sufficient BBB permeation of all compounds[30] (Supplemental Table S1). Additional support for a good BBB penetration of the compounds is provided by calculated pK_a values in the range of 7.5-10.5 which furthermore suggest sufficient protonation of the compounds in the CNS for cation-π or other electrostatic interactions with conserved amino acid residues or residues comprising electron-rich aromatic moieties in the binding sites of nAChRs.

The *in vitro* binding affinities of all modified compounds for the nicotinic receptor subtypes were examined in competitive radioligand displacement studies using [^{3}H]methyllycaconitine and [^{3}H]epibatidine as selective radioligands for human α7 nAChR as well as human α4β2 and α3β4 nAChRs, respectively (supporting information). The binding affinities of the compounds tested are presented in Table 1. The racemic compounds showed K_i values at α7 nAChR in the range of 73-133 nM. In addition, all of them bound with higher affinity towards α3β4 nAChR. Replacement of the hydroxyl group of the lead compound **QND8** by a fluorine atom decreases the binding affinity on α7 as the K_i value of (*RS*)-**T1** at α7 nAChR is 7 times higher than that of (*RS*)-**QND8** (73 vs. 10 nM). However, this modified compounds showed better selectivity at α7 over α3β4.

The most important finding was the significant dependence of the nAChR subtype affinity on the handedness of the ligands. The chirality of the binding sites of the different nAChR subtypes offers a rationale for the enantioselective affinity profiles of chiral ligands for α7, α4β2 and α3β4 nAChRs. More specifically, the experimentally determined affinities (Table 1) reveal the following pattern: Firstly, all (*R*)-enantiomers showed a significant selectivity for α7 over α4β2 and to a lesser degree also over α3β4. They bind with low nanomolar affinity at α7, with high nanomolar affinity at α3β4 and with micromolar affinities at α4β2. The resulting selectivity values in the range of 3.1-33 and 44-225 for α7 vs. α3β4 and α4β2, respectively, indicate preference for the α7 nAChR binding site. Atypically, (*R*)-**T2** provides only weak preference for α7 over α3β4 (selectivity of 3.1). Secondly, all (*S*)-enantiomers are highly selective for α3β4 over α4β2 and mostly to a lesser degree also over α7. They bind with low nanomolar affinity at α3β4 and with high nanomolar to micromolar affinities at α7 and α4β2. The selectivity values of α3β4 vs. α7 and α4β2 were estimated in the range of 5-294 and 62-231 and indicate preference of the (*S*)-enantiomers of quinuclidine-*anti*-1,2,3-triazole containing nAChR ligands for the binding site of α3β4 nAChR. Compound **T2** plays again a special role, with its (*S*)-enantiomer showing the overall highest selectivity (294) for α3β4 over α7. Notably **AT-1001** previously reported as a high affinity and selective α3β4 nAChR antagonist,[31] also falls into this category, although with slightly lower affinity and selectivity than **T2**. However, two other recently reported ligands for the α3β4 nAChR, **CP-601932**[32] and an anabaseine analogue[33] showed modest selectivity of binding to α3β4 over α4β2 and α3β4 over α7, respectively (Figure 2). Thirdly, the preference of the (*S*)-enantiomers for α3β4 over α4β2 (selectivity 62-231) is more pronounced than the preference of the (*R*)-enantiomers for α7 over α3β4 (selectivity 3-33). Fourthly, the overall highest binding affinities for the herein assessed nAChR subtypes were observed for binding of the (*S*)-enantiomers to α3β4 (K_i = 2.25-19.5 nM) followed by the binding of the (*R*)-enantiomers to α7 (K_i= 22.5-117 nM). Concerning binding to α4β2, the affinity of the (*S*)-enantiomers is higher than of the (*R*)-enantiomers (414-1980 nM vs.5059-10436 nM).

By more detailed investigation of the interaction of the herein presented ligands with α7 nAChR, the fluorine-substituted enantiomers (*R*)-**T1** and(*R*)-**T2**, bearing F, at *p*- and *m*-position of the phenyl ring, respectively, showed binding profiles comparable to their racemic mixture with (*R*)-**T1** being 3-fold more selective for α7 over α4β2 than (*R*)-**T2** (factors 143 vs. 44). The binding affinity increases about two-fold when replacing the (*R*)-**T4** phenyl substituent *m*-O$(CH_2)_3$F (K_i= 62.3 nM) by the smaller and less hydrophobic *m*-O$(CH_2)_2$F group ((*R*)-**T3**, *K*i = 38.8 nM) as well as by the larger and more hydrophobic groups *m*-OBn ((*R*)-**T5**, K_i = 22.5 nM) and *m*-$OCH_2C_6H_4F$ ((*R*)-**T6**, K_i = 33.2 nM). A possible explanation would be that the respective variation in K_i might be triggered by the presence or absence of a spatially suitable electron donor functionality of the ligand (e.g. as H-bond acceptor), either through its F lone pair electrons (**T4**) or through the benzylic π-electrons (**T5**, **T6**).

For the corresponding (*S*)-enantiomers, (*S*)-**T2** shows a 5-fold higher selectivity for α3β4 over α7 in comparison to (*S*)-**T1**. Regarding further substituents, the following pattern is observed for the affinity of the (*S*)-enantiomers to α3β4 nAChR. Here, *m*-O$(CH_2)_2$F ((*S*)-**T3** with an K_i of 11.8 nM) is again ~2-fold more potent than *m*-O$(CH_2)_3$F ((*S*)-**T4**, *K*i = 19.5 nM), but now the two phenyl-containing (*S*)-**T5** (*K*i = 6.67 nM) and (*S*)-**T6** (*K*i = 7.17 nM) yield a further ~2-fold increase in binding affinity. These results support the suggestion that a ligand π-electron donor capability at a site distant from the quinuclidine triazole moiety may contribute to stabilizing the ligand-receptor complex. Interestingly, (*S*)-**T2** and (*S*)-**T1** as the two most promising α3β4 ligands (K_i= 2.25 vs. 3.09 nM) are the only derivatives with an F-substituted phenyl ring, where *m*-F (*S*)-**T2** yields the overall highest α3β4 selectivity over both α7 and α4β2 as discussed above. The significant difference in the selectivity profiles of the F-substituted isomers (*S*)-**T1** and (*S*)-**T2** suggests the following tentative explanation. In (*S*)-**T1**, the electronegative F is in *p*-position to the electron-rich triazole, the latter of which may partly compensate the electron shift from the benzene ring to F. By contrast, the respective conjugative substitution pattern is missing in (*S*)-**T2**, thus probably resulting in a stronger loss of π-electron density through inductive charge transfer to *m*-F. From this viewpoint it is likely that an electron-poor character of the benzylic moiety in compound (*S*)-**T2** triggers for a strong α3β4 selectivity, with K_i-based selectivity of 294 (over α7) and 231 (over α4β2), respectively. Interestingly, it was apparent that (*S*)-enantiomers were more selective to α3β4 (47-326 times) and α4β2 nAChR (4.5-20 times) than their corresponding (*R*)-counterparts. Up until now, the selective binding of (*S*)-quinuclidine based compounds on α3β4 and α4β2 subtypes have never been reported before.

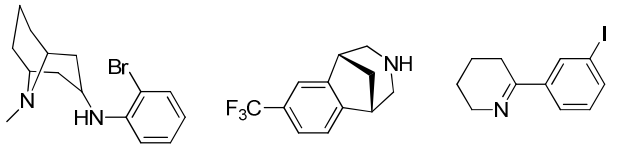
Four compounds with good affinity (K_i < 10 nM) which are (*S*)-enantiomers of **T1**, **T2**, **T5** and **T6** were evaluated for the binding at related receptors, these 4 compounds had no significant effect on N-muscle type nAChR (2-12% inhibition at 1 μM). For the 5-HT_3 receptor, (*S*)-enantiomers of **T1**, **T2**, **T5** and **T6** were tested at 100 nM, all except (*S*)-**T6** showed no significant binding at the 5-HT_3 receptor.

To see whether the designed compounds are agonist as **QND8** or antagonist as **AT-1001**, the functional assays of the potential compounds **T1** and **T2** in both (*R*) and (*S*) forms were performed using SH-SY5Y cells stably expressing human α7 nAChR and HEK 293 cells stably expressing human α3β4 nAChR. The screening results indicated that (*R*)**-T1**, (*R*)**-T2**, (*S*)-**T1** and (*S*)-**T2** are α7 nAChR agonists (Supplemental Figure S1). Compounds

(*R*)-**T2** and (*S*)-**T1** are α3β4 nAChR agonists whereas (*R*)-**T1** and (*S*)-**T2** are α3β4 nAChR antagonists (Supplemental Figure S2). However, the selectivity cannot be concluded due to the high testing concentration (10 μM) in the screening assay for functional effects.

Overall, according to the binding data of all novel analogues presented herein, the stereo center at C3 position of the quinuclidine motif has significant impact on the selectivity of modified compounds to nAChR subtypes. (*R*)-enantiomers bind preferably towards α7 while (*S*)-enantiomers were more selective for the α3β4 nAChR. Among all, (*R*)-**T5** was the most favorable ligand for the α7 nAChR, (*S*)-**T2** and (*S*)-**T1** for the α3β4 nAChR with almost equal affinities, and (although significantly less strong) (*S*)-**T5** was the most promising α4β2 nAChR ligand.

In conclusion, we have successfully designed two potent compounds (*S*)-**T1** and (*S*)-**T2** that bind selectively to α3β4 nAChR over α7 nAChR. Among the (*R*) and (*S*)-enantiomers of the quinuclidine base compounds, the binding affinities of (*S*)-enantiomers are higher compared to their (*R*) counterparts. This finding may have implications for the treatment of several brain diseases such as drug addiction and dependence. Compounds (*R*)-**QND8** and (*R*)-**T6** which displayed higher affinity for the α7 nAChR and higher selectivity towards the α3β4 nAChR than its parent (*R*S)-**QND8** might be useful candidate molecules in further drug development for the treatment of cognitive disorders. In addition, the introduction of a fluorine substituent in the structure makes it possible to develop the designed compounds as PET radioligands for brain imaging to localize the α3β4 and α7 nAChR subtypes in the brain and monitor drug efficacy.



AT-1001[31]	**CP-601932**[32]	**anabaseine analog**[33]
K_i, α3β4 = 2.64 nM	K_i, α3β4 = 21 nM	K_i, α3β4 = 4.7 nM
K_i, α4β2 = 476 nM	K_i, α4β2 = 21 nM	K_i, α4β2 = 3790 nM
K_i, α7 = 221 nM	K_i, α7 > 300 nM	K_i, α7 = 11.3 nM

Figure 2. Structures of selected α3β4 nAChR ligands.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +66-2-644-8695. E-mail: opa.vaj@mahidol.ac.th.

Funding Sources

This work was supported by Thailand Research Fund (TRF) through the Royal Golden Jubilee Ph.D. Program (Grant No.PHD/0272/2552) to J.S. and O.V., BRG 5780016 to S.C., the Office of the High Education Commission, Thailand; Thailand Research Fund (TRF) and Faculty of Pharmacy, Mahidol University (IRG578007) to O.V.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

nAChR, nicotinic acetylcholine receptor; PET, positron emission tomography; CuAAC, copper-catalyzed azide-alkyne cycloaddition; PD, Parkinson's disease; ADHD, attention deficit hyperactivity disorder; AD, Alzheimer's disease

REFERENCES

(1) Dani, J. A. Neuronal nicotinic acetylcholine receptor structure and function and response to nicotine. *Int. Rev. Neurobiol.* **2015**, *124*, 3-19.

(2) Dineley, K. T.; Pandya, A. A.; Yakel, J. L. Nicotinic ACh receptors as therapeutic targets in CNS disorders. *Trends Pharmacol. Sci.* **2015**, *36*, 96-108.

(3) Brust, P.; Deuther-Conrad, W.; Donat, C. K.; Barthel, H.; Riss, P.; Paterson, L.; Hoepping, A.; Sabri, O.; Cumming, P.: Preclinical aspects of nicotinic acetylcholine receptor imaging. In *PET and SPECT of Neurobiological Systems*; Dierckx, R. A. J. O., Ed.; Springer: Berlin Heidelberg, 2014; pp 465-512.

(4) Gotti, C.; Clementi, F.; Fornari, A.; Gaimarri, A.; Guiducci, S.; Manfredi, I.; Moretti, M.; Pedrazzi, P.; Pucci, L.; Zoli, M. Structural and functional diversity of native brain neuronal nicotinic receptors. *Biochem. Pharmacol.* **2009**, *78*, 703-711.

(5) Rahman, S.; Engleman, E. A.; Bell, R. L. Nicotinic receptor modulation to treat alcohol and drug dependence. *Front. Neurosci.* **2015**, *8*, 426.

(6) Posadas, I.; Lopez-Hernandez, B.; Cena, V. Nicotinic receptors in neurodegeneration. *Curr Neuropharmacol.* **2013**, *11*, 298-314.

(7) Toyohara, J.; Hashimoto, K. α7 Nicotinic receptor agonists: potential therapeutic drugs for treatment of cognitive impairments in Schizophrenia and Alzheimer's Disease. *Open Med. Chem. J.* **2010**, *4*, 37-56.

(8) Dani, J. A.; Bertrand, D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 699-729.

(9) Wilens, T. E.; Decker, M. W. Neuronal nicotinic receptor agonists for the treatment of attention-deficit/hyperactivity disorder: focus on cognition. *Biochem. Pharmacol.* **2007**, *74*, 1212-1223.

(10) Glick, S. D.; Maisonneuve, I. M.; Kitchen, B. A.; Fleck, M. W. Antagonism of a3b4 nicotinic receptors as a strategy to reduce opioid and stimulant self-administration. *Eur. J. Pharmacol.* **2002**, *438*, 99-105.

(11) Haydar, S. N.; Dunlop, J. Neuronal nicotinic acetylcholine receptors - targets for the development of drugs to treat cognitive impairment associated with schizophrenia and Alzheimer's disease. *Curr Top Med Chem* **2010**, *10*, 144-152.

(12) Taraschenko, O. D.; Panchal, V.; Maisonneuve, I. M.; Glick, S. D. Is antagonism of a3b4 nicotinic receptors a strategy to reduce morphine dependence? *Eur. J. Pharmacol.* **2005**, *513*, 207-218.

(13) Zhao, Y. The oncogenic functions of nicotinic acetylcholine receptors. *J.Oncol.* **2016**, DOI:10.1155/2016/9650481.

(14) Mucchietto, V.; Crespi, A.; Fasoli, F.; Clementi, F.; Gotti, C. Neuronal acetylcholine nicotinic receptors as new targets for lung cancer treatment. *Curr. Pharm. Des.* **2016**, *22*, 2160-2169.

(15) Gotti, C.; Zoli, M.; Clementi, F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol. Sci.* **2006**, *27*, 482-491.

(16) Pin, F.; Vercouillie, J.; Ouach, A.; Mavel, S.; Gulhan, Z.; Chicheri, G.; Jarry, C.; Massip, S.; Deloye, J. B.; Guilloteau, D.; Suzenet, F.; Chalon, S.; Routier, S. Design of a7 nicotinic acetylcholine receptor ligands in quinuclidine, tropane and quinazoline series. Chemistry, molecular modeling, radiochemistry, in vitro and in rats evaluations of a [^{18}F] quinuclidine derivative. *Eur. J. Med. Chem.* **2014**, *82*, 214-224.

(17) Mazurov, A. A.; Speake, J. D.; Yohannes, D. Discovery and development of a7 nicotinic acetylcholine receptor modulators. *J. Med. Chem.* **2011**, *54*, 7943-7961.

(18) Ouach, A.; Pin, F.; Bertrand, E.; Vercouillie, J.; Gulhan, Z.; Mothes, C.; Deloye, J. B.; Guilloteau, D.; Suzenet, F.; Chalon, S.; Routier, S. Design of a7 nicotinic acetylcholine receptor ligands using the (het)Aryl-1,2,3-triazole core: Synthesis, in vitro evaluation and SAR studies. *Eur. J. Med. Chem.* **2016**, *107*, 153-164.

(19) Cohen, B. N.; Figl, A.; Quick, M. W.; Labarca, C.; Davidson, N.; Lester, H. A. Regions of b2 and b4 responsible for differences between the steady state dose-response relationships of the a3b2 and a3b4 neuronal nicotinic receptors. *J. Gen. Physiol.* **1995**, *105*, 745-764.

(20) Parker, M. J.; Beck, A.; Luetje, C. W. Neuronal nicotinic receptor b2 and b4 subunits confer large differences in agonist binding affinity. *Mol. Pharmacol.* **1998**, *54*, 1132-1139.

(21) Luetje, C. W.; Patrick, J. Both a- and b-subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors. *J. Neurosci.* **1991**, *11*, 837-845.

(22) Arunrungvichian, K.; Boonyarat, C.; Fokin, V. V.; Taylor, P.; Vajragupta, O. Cognitive improvements in a mouse model with substituted 1,2,3-triazole agonists for nicotinic acetylcholine receptors. *ACS Chem. Neurosci.* **2015**, *6*, 1331-1340.

(23) Keck, T. M.; Banala, A. K.; Slack, R. D.; Burzynski, C.; Bonifazi, A.; Okunola-Bakare, O. M.; Moore, M.; Deschamps, J. R.; Rais, R.; Slusher, B. S. Using click chemistry toward novel 1, 2, 3-triazole-linked dopamine D3 receptor ligands. *Bioorg. Med. Chem.* **2015**, *23*, 4000-4012.

(24) Beltramo, M.; Robert, V.; Galibert, M.; Madinier, J.-B.; Marceau, P.; Dardente, H.; Decourt, C.; De Roux, N.; Lomet, D.; Delmas, A. F. Rational design of triazololipopeptides analogs of kisspeptin inducing a long-lasting increase of gonadotropins. *J. Med. Chem.* **2015**, *58*, 3459-3470.

(25) Malysz, J.; Anderson, D. J.; Gronlien, J. H.; Ji, J.; Bunnelle, W. H.; Haakerud, M.; Thorin-Hagene, K.; Ween, H.; Helfrich, R.; Hu, M.; Gubbins, E.; Gopalakrishnan, S.; Puttfarcken, P. S.; Briggs, C.; Li, J.; Meyer, M. D.; Dyhring, T.; Ahring, P. K.; Nielsen, E. O.; Peters, D.; Timmermann, D. B.; Gopalakrishnan, M. In vitro pharmacological characterization of a novel selective a7 neuronal nicotinic acetylcholine receptor agonist ABT-107. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 863-874.

(26) Hauser, T. A.; Kucinski, A.; Jordan, K. G.; Gatto, G. J.; Wersinger, S. R.; Hesse, R. A.; Stachowiak, E. K.; Stachowiak, M. K.; Papke, R. L.; Lippiello, P. M.; Bencherif, M. TC-5619: an alpha7 neuronal nicotinic receptor-selective agonist that demonstrates efficacy in animal models of the positive and negative symptoms and cognitive dysfunction of schizophrenia. *Biochem. Pharmacol.* **2009**, *78*, 803-812.

(27) Wishka, D. G.; Walker, D. P.; Yates, K. M.; Reitz, S. C.; Jia, S.; Myers, J. K.; Olson, K. L.; Jacobsen, E. J.; Wolfe, M. L.; Groppi, V. E.; Hanchar, A. J.; Thornburgh, B. A.; Cortes-Burgos, L. A.; Wong, E. H.; Staton, B. A.; Raub, T. J.; Higdon, N. R.; Wall, T. M.; Hurst, R. S.; Walters, R. R.; Hoffmann, W. E.; Hajos, M.; Franklin, S.; Carey, G.; Gold, L. H.; Cook, K. K.; Sands, S. B.; Zhao, S. X.; Soglia, J. R.; Kalgutkar, A. S.; Arneric, S. P.; Rogers, B. N. Discovery of *N*-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide, an agonist of the a7 nicotinic acetylcholine receptor, for the potential treatment of cognitive deficits in schizophrenia: synthesis and structure--activity relationship. *J. Med. Chem.* **2006**, *49*, 4425-4436.

(28) Wallace, T. L.; Callahan, P. M.; Tehim, A.; Bertrand, D.; Tombaugh, G.; Wang, S.; Xie, W.; Rowe, W. B.; Ong, V.; Graham, E.; Terry, A. V., Jr.; Rodefer, J. S.; Herbert, B.; Murray, M.; Porter, R.; Santarelli, L.; Lowe, D. A. RG3487, a novel nicotinic a7 receptor partial agonist, improves cognition and sensorimotor gating in rodents. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 242-253.

(29) Arunrungvichian, K.; Fokin, V. V.; Vajragupta, O.; Taylor, P. Selectivity optimization of substituted 1,2,3-triazoles as a7 nicotinic acetylcholine receptor agonists. *ACS Chem. Neurosci.* **2015**, *6*, 1317-1330.

(30) Ghose, A. K.; Herbertz, T.; Hudkins, R. L.; Dorsey, B. D.; Mallamo, J. P. Knowledge-based, central nervous system (CNS) lead selection and lead optimization for CNS drug discovery. *ACS Chem. Neurosci.* **2012**, *3*, 50-68.

(31) Toll, L.; Zaveri, N. T.; Polgar, W. E.; Jiang, F.; Khroyan, T. V.; Zhou, W.; Xie, X. S.; Stauber, G. B.; Costello, M. R.; Leslie, F. M. AT-1001: a high affinity and selective a3b4 nicotinic acetylcholine receptor antagonist blocks nicotine self-administration in rats. *Neuropsychopharmacology* **2012**, *37*, 1367-1376.

(32) Chatterjee, S.; Steensland, P.; Simms, J. A.; Holgate, J.; Coe, J. W.; Hurst, R. S.; Shaffer, C. L.; Lowe, J.; Rollema, H.; Bartlett, S. E. Partial agonists of the a3b4* neuronal nicotinic acetylcholine receptor reduce ethanol consumption and seeking in rats. *Neuropsychopharmacology* **2011**, *36*, 603-615.

(33) Matera, C.; Quadri, M.; Sciaccaluga, M.; Pome, D. Y.; Fasoli, F.; De Amici, M.; Fucile, S.; Gotti, C.; Dallanoce, C.; Grazioso, G. Modification of the anabaseine pyridine nucleus allows achieving binding and functional selectivity for the a3b4 nicotinic acetylcholine receptor subtype. *Eur. J. Med. Chem.* **2016**, *108*, 392-405.