Bioorganic & Medicinal Chemistry Letters 19 (2009) 1682–1685

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Preparation and characterization of *N*-(3-pyridinyl) spirocyclic diamines as ligands for nicotinic acetylcholine receptors

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ARTICLE INFO

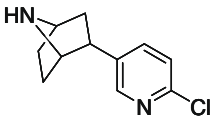
Article history:
Received 15 December 2008
Revised 28 January 2009
Accepted 29 January 2009
Available online 4 February 2009

Keywords:
Nicotinic
nAChR
Acetylcholine
Receptors
α4β2
ABT-594
Diamines

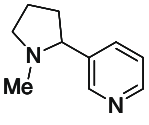
ABSTRACT

Several *N*-pyridin-3-yl spirobicyclic diamines, designed as conformationally restricted analogs of tebanicline (ABT-594), were synthesized as novel ligands for nicotinic acetylcholine receptors (nAChR). The spirocyclic compounds exhibited weaker binding affinity, than other constrained analogs in accord with a pharmacophore model. Nevertheless, some (**1a**, **1b**) possessed (partial) agonist potencies comparable to nicotine at the α4β2 subtype, but with greatly improved selectivity relative to the α3β4* nAChR.

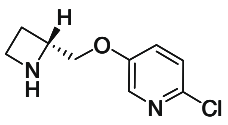
The potential of nicotinic acetylcholine receptor (nAChR) agonists as novel, broad-spectrum analgesics is now well recognized,[1–3] but development of effective drugs with suitable safety and tolerability profiles has proved challenging. Multiple subtypes of nAChRs are expressed throughout the central and peripheral nervous systems, and it is likely that receptor subtypes that mediate the analgesic response are different than those responsible for many of the side effects. For example, knock-out and antisense studies have implicated the α4β2 subtype as critical for analgesic efficacy,[4,5] while activation of α3β4* nAChRs, abundantly expressed in the autonomic ganglia, has been linked to side effect liabilities.[6] Therefore, our medicinal chemistry efforts are directed toward nAChR agonists that improved selectivity for the α4β2 subtype compared to the α3β4* nAChR.



epibatidine



nicotine



tebanicline

The frog skin alkaloid epibatidine,[7] has stimulated most of the recent interest in nAChRs as a target for treatment of pain, but has a poor subtype selectivity profile (four-fold more potent at the α3β4* nAChR compared to the α4β2 subtype) and accordingly exhibits a very narrow separation between analgesic efficacy and side effects in animal models.[6] Tebanicline (ABT-594) was developed at Abbott Laboratories as a potent analgesic with greater selectivity for the α4β2 subtype compared to epibatidine.[8,9] Although tebanicline exhibited an improved therapeutic index that enabled its advancement to human clinical trials, dose-limiting GI side effects precluded full development of this compound.[2] In an effort to identify analogs of tebanicline that discriminate between nAChR subtypes, we have sought to rigidify the core structure of tebanicline by replacing the ether linker with a cyclic secondary amine (Fig. 1). Cyclization via the aza linker can be accomplished in three distinct ways, to provide bridged (**A**) or fused (**B**) bicyclic diamines, which we have described elsewhere,[10,11] or spirobicyclic diamines (**C**), which are the subject of this report. These spirocyclic structures thus complete an interesting set of conformationally-rigidified bicyclic diamine nAChR ligands.

Construction of the diamines was accomplished as outlined in Scheme 1. Boc-protected methyl (*S*)-azetidine-2-carboxylate[12] was allylated via the enolate. Ozonolysis and reductive amination followed by thermal cyclization led to the lactam **8** which was reduced with $LiAlH_4$ to the differentially-protected diamine **9**. Hydrogenolysis, Pd-mediated coupling of the liberated secondary amine with 5-bromo-2-chloropyridine, and removal of the Boc-protecting group provided **1a**. Alternatively, coupling with 3-bromopyridine led to the des-chloro analog **1b**. In a similar fashion, *N*-benzoyl-(*S*)-proline methyl ester[12] was elaborated to benzyl-protected 1,7-diazaspiro[4.4]nonane **11** as described by Culbertson

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doi:10.1016/j.bmcl.2009.01.099

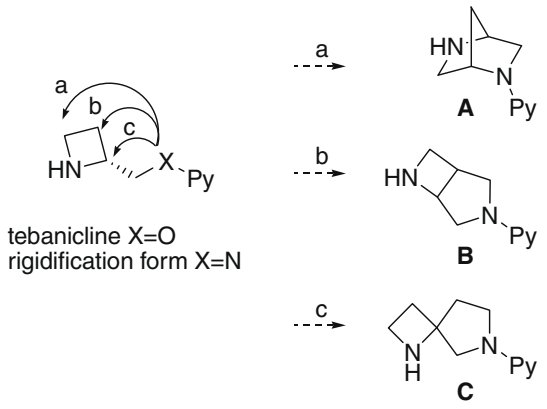
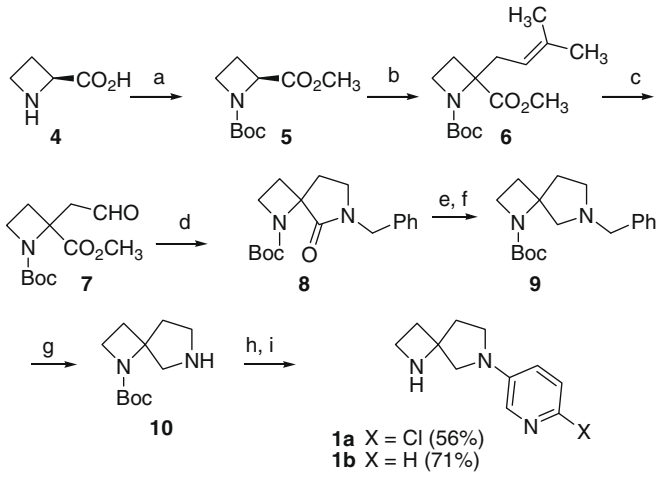
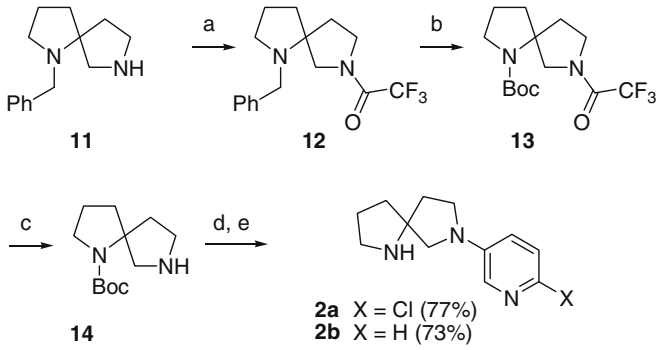


Figure 1. Cyclization of a secondary amine to provide bridged, fused or spirocyclic diamines.

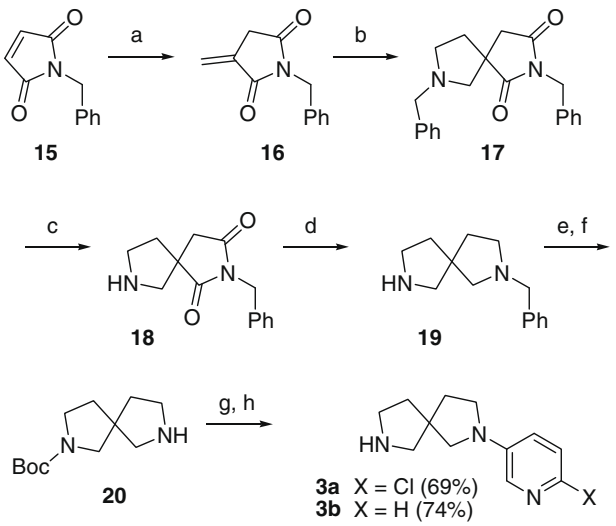


Scheme 1. Reagents and conditions: (a) (i) CH_3OH, $SOCl_2$, 0–90 °C; (ii) Boc_2O, Et_3N, CH_2Cl_2, (95%); (b) LiHMDS, THF, −65 °C, 1-bromo-3-methylbut-2-ene (92%) (c) O_3, Ph_3P, CH_3OH, CH_2Cl_2, −65 °C (75%); (d) (i) $PhCH_2NH_2$, $NaBH_3CN$, HOAc, CH_3OH, 0 °C, (ii) toluene, 110 °C (70%); (e) TFA, CH_2Cl_2 (f) (i) $LiAlH_4$, THF, 0 °C-reflux (ii) Boc_2O (64%); (g) H_2, Pd/C, EtOH (84%); (h) $Pd_2(dba)_3$, racemic-BINAP, sodium *tert*-butoxide, 5-bromo-2-X-pyridine, toluene (i) TFA, CH_2Cl_2.

et al.,[13] and after swapping the benzyl for Boc-protecting group as outlined in Scheme 2, palladium coupling and subsequent deprotection provided the 7-(6-chloro-pyridin-3-yl)-1,7-diaza-spiro[4.4]-nonane analog **2a** and the des-chloro analog **2b**.



Scheme 2. Reagents and conditions: (a) TFAA, Et_3N, THF, 0 °C (87%); (b) Boc_2O, EtOAc, 20% $Pd(OH)_2$, H_2 (75%); (c) K_2CO_3, CH_3OH (83%); (d) $Pd_2(dba)_3$, BINAP (rac.), sodium *tert*-butoxide, 5-bromo-2-X-pyridine, toluene (e) TFA, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) PPh_3, 37% CH_2O, AcOH (57%); (b) *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzyl amine, CH_2Cl_2, TFA, 0 °C (92%); (c) EtOH, H_2, 20% $Pd(OH)_2$, 50 °C (96%); (d) $LiAlH_4$, THF (98%); (e) CH_2Cl_2, Boc_2O (63%); (f) EtOH, 10% Pd/C, H_2 (100%); (g) $Pd_2(dba)_3$, BINAP (rac.), sodium *tert*-butoxide, 5-bromo-2-X-pyridine, toluene; (h) EtOAc, 4M HCl/dioxane (69%).

The 2,7-diazaspiro[4.4]nonanes were prepared via a 1,3-dipolar cycloaddition of 2-methylenesuccinimide **16** with an azomethine ylide[14] as shown in Scheme 3 to generate the spiroamine **17**. Imide reduction, Boc protection, and debenzylation provided the mono-protected diamine **20** which was carried through to the 3-pyridyl derivatives **3a** and **3b** as before.

Affinities of the compounds at the α4β2 nAChR were determined from displacement of [^{3}H]cytisine from rat brain membranes as described previously.[15] Agonist activities were evaluated from Ca^{2+} dynamics using FLIPR technology.[10,16] For α4β2, a cell line expressing the human recombinant receptor was employed, while a human neuroblastoma cell line (IMR-32) was used to assess activity at the α3β4∗ subtype.

Although pyridinyl ethers related to tebanicline are exceptionally potent ligands at nAChRs, simple replacement of oxygen by nitrogen causes a sharp decrement in binding potency (Fig. 2). Thus, **21** displaces [^{3}H]cytisine with sevenfold greater potency than nicotine,[15] while the aza-analog **22** is 11-fold weaker than nicotine.[17] N-Methylation of the linking nitrogen (**23**) is even more detrimental to binding affinity, resulting in this instance in a compound that is 700 times less potent than nicotine. Nevertheless, we have demonstrated that structures where the N-alkyl substituent is tethered to the azacyclic ring exhibit subnanomolar binding affinities for both bridged and fused bicyclic motifs.[10,11] From the data in Table 1, it is evident that the spirocyclic diamines exhibit binding potencies in the double-digit nanomolar range, ranking them as somewhat less potent than nicotine but substantially more potent than unconstrained analogs like **23**. As observed for the pyridinyl ethers,[9,18] the azetidines **1** are slightly more potent than the homologous pyrrolidines **2** and **3**. The 2,7-diazaspiro[4.4]nonanes **3**, where the distance between the secondary amine and the pyridine has been extended, show weaker binding affinity.

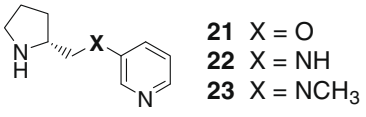


Figure 2. Pyridinyl ether and aza related analogs of tebanicline.

Table 1
In vitro pharmacological data for the spirocyclic diamines

Compound	Rat α4β2 K_i[a] (nM)	Human α4β2 EC_{50}[b] (μM)	Human α3β4 EC_{50}[b] (μM)
Nicotine	0.94	4.7 (100%)	9.4 (100%)
Epibatidine	0.047	0.12 (154%)	0.027 (132%)
Tebanicline	0.048	0.020 (115%)	0.19 (169%)
A[19]	0.15	nd	6.4 (108%)
B[20]	1.2	1.6 (122%)	6.1 (90%)
1a	21	2.5 (48%)	6.9 (23%)
1b	11	25 (44%)	>100 (4%)
2a	48	>100 (6%)	>100 (8%)
2b	33	>100 (20%)	>100 (15%)
3a	240	>100 (9%)	>100 (2%)
3b	74	>100 (4%)	>100 (10%)

[a] Values are means of at least three experiments.
[b] Average of six replicates; maximal response normalized to 100 μM nicotine is given in parenthesis. nd, not determined.

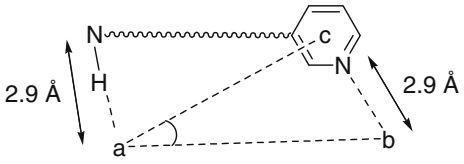


Figure 3. The definition of a novel nicotinic pharmacophore model[22], where **a** is the site point corresponding to the protonated nitrogen atom, **b** is the site point corresponding to the electronegative atom capable of forming a hydrogen bond, **c** is the center of a heteroaromatic ring or a C=O bond. ∠bac is the angle measured between the interatomic distance vectors **a**–**b** and **a**–**c**.

Of these spirocyclic diamines, only the azetidines **1a,b** display any agonist activity. Both exhibit potencies comparable to nicotine at the α4β2 nAChR that is considered to be crucial to the analgesic response, but they are only partial agonists (40–50% of the full nicotine response) at this receptor. Interestingly, although **1b** has somewhat higher affinity than **1a** for the α4β2 nAChR, it is about 10-fold weaker as an agonist. This might reflect a species difference (rat receptor for binding, human for functional activity), or may indicate that the structural requirements for the binding conformation of the receptor (considered to be the desensitized state) are changed in the activated (channel-opening) conformation. On the other hand, neither has much activity at the α3β4∗ receptor, with **1a** producing a maximal response of only 23% efficacy (relative to nicotine), and **1b** a barely detectable response up to the maximum concentration of 100 μM.

Both **1a** and **1b** were tested in the mouse hot plate model for analgesia. While **1a** does exhibit an analgesic response at the highest dose tested (62 μmol/kg, ip), hypolocomotion and decreased body temperature were observed at the same dose. The des-chloro analog **1b** showed no behavioral effects at 62 μmol/kg, consistent with the lower agonist activity relative to **1a**.

Pharmacophore models for the α4β2 nAChR place importance on the spacing and orientation between a basic (or quaternized) amine and a hydrogen bond acceptor (heterocycle or carbonyl group). For example, Figure 3 depicts the model developed by Tønder and Olessen[21,22] that employs two receptor interaction points (a and b) located 2.9 Å along the trajectory of the NH bond of the basic amine and the lone pair of the pyridine ring, respectively, as well as the centroid (c) of the heterocycle. According to this model, the optimum geometry for high affinity at the α4β2 nAChR is achieved when distances a–b and a–c fall in the ranges 7.3–8.0 Å and 6.5–7.4 Å, respectively, with ∠bac within 30.4–35.8°. To a first approximation, structures **A**, **B**, and **C** (see Fig. 1) represent different rotamers about the exocyclic C—N bond external to the pyridine substituted ring. This has the effect of changing orientation of the N—H bonds relative to the pyridine ring, and consequently, the ability of the respective compounds to fit the parameters of the pharmacophore model. Structures **A, B**, and **C** were generated and the geometry optimized using CFF force field.[23] Rotation about the N-pyridine bond was then allowed to produce the best fit within the parameters of the model.[22]

Table 2
Calculated pharmacophore modeling values for bridged, fused and spiro diamines

Structure	**a**–**b** (Å)	**a**–**c** (Å)	∠bac (°)
Optimal range[22]	7.3–8.0	6.5–7.4	30.4–35.8
A (bridged)	7.4	6.5	34.97
B (fused)	7.6	6.5	34.09
C (spiro)	8.1	5.4	28.2

As shown in Table 2, the bridged and fused ring diamine structures **A** and **B**, respectively, both achieve a good fit to the pharmacophore model, with each of the three calculated parameters within the optimal range of the model. This is consistent with the potent (nanomolar) binding affinity for these series (Table 1). In contrast, spirocyclic structure **C** cannot achieve a conformation that satisfies the criteria of the model. The best-fit conformation for **C** approaches the optimal ranges for a–b and ∠bac, but the a–c distance is too short by 1.1 Å. Moreover, this conformer involves substantial twisting of the pyridinyl-N bond away from the minimal energy conformation, an energy cost that will further disfavor fit to the pharmacophore model. Thus, the reduced binding affinity of **C** relative to **A** and **B** is in accord with the pharmacophore model.

In summary, the spirocyclic diamine represents a new core for construction of nAChR ligands. The lower affinity of the spirocyclic structures relative to fused- and bridged-bicyclic counterparts is consistent with recently developed pharmacophore models. On the other hand, the spirocyclic amines may exhibit enhanced selectivity for the α4β2 subtype over other nAChRs.

References and notes

1. Jain, K. K. *Curr. Opin. Inv. Drugs* **2004**, *5*, 76.
2. Meyer, M. D. *Drug Dev. Res.* **2006**, *67*, 355.
3. Bunnelle, W. H.; Decker, M. W. *Exp. Opin. Ther. Pat.* **2003**, *13*, 1003.
4. Bitner, R. S.; Nikkel, A. L.; Curzon, P.; Donnelly-Roberts, D. L.; Puttfarcken, P. S.; Namovic, M.; Jacobs, I. C.; Meyer, M. D.; Decker, M. W. *Brain Res.* **2000**, *871*, 66.
5. Marubio, L. M.; del Mar Arroyo-Jimenez, M.; Cordero-Erausquin, M.; Lena, C.; Le Novere, N.; de Kerchove d'Exaerde, A.; Huchet, M.; Damaj, M. I.; Changeux, J.-P. *Nature* **1999**, *398*, 8085.
6. Sullivan, J. P.; Bannon, A. W. *CNS Drug Rev.* **1996**, *2*, 21.
7. Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3475.
8. Bannon, A. W.; Decker, M. W.; Holladay, M. W.; Curzon, P.; Donnelly-Roberts, D. L.; Puttfarcken, P. S.; Bitner, R. S.; Diaz, A.; Dickenson, A. H.; Porsolt, R. D.; Williams, M.; Arneric, S. P. *Science* **1998**, *279*, 77.
9. Holladay, M. W.; Wasicak, J. T.; Lin, N.-H.; He, Y.; Ryther, K. B.; Bannon, A. W.; Buckley, M. J.; Kim, D. J. B.; Decker, M. W.; Anderson, D. J.; Campbell, J. E.; Kuntzweiler, T.; Donnelly-Roberts, D. L.; Piattoni-Kaplan, M.; Briggs, C. A.; Williams, M.; Arneric, S. P. *J. Med. Chem.* **1998**, *41*, 407.
10. Bunnelle, W. H.; Daanen, J. F.; Ryther, K. B.; Schrimpf, M. R.; Dart, M. J.; Gelain, A.; Meyer, M. D.; Frost, J. M.; Anderson, D. J.; Buckley, M. J.; Curzon, P.; Cao, Y.-J.; Puttfarcken, P. S.; Searle, X.; Ji, J.; Putman, C. B.; Surowy, C. S.; Toma, L.; Barlocco, D. *J. Med. Chem.* **2007**, *50*, 3627.
11. Ji, J.; Schrimpf, M. R.; Sippy, K. B.; Bunnelle, W. H.; Li, T.; Anderson, D. J.; Faltynek, C.; Surowy, C. S.; Dyhring, T.; Ahring, P. K.; Meyer, M. D. *J. Med. Chem.* **2007**, *50*, 5493.
12. The chiral amino acids were commercially available, but the stereogenic center is racemized during the synthesis.
13. Culbertson, T. P.; Sanchez, J. P.; Gambino, L.; Sesnie, J. S. *J. Med. Chem.* **1990**, *32*, 2270.
14. Padwa, A.; Dent, W. *Org. Synth.* **1989**, *67*, 133.
15. Anderson, D. J.; Arneric, S. P. *Eur. J. Pharmacol.* **1994**, *253*, 261.
16. Kuntzweiler, T. A.; Arneric, S. P.; Donnelly-Roberts, D. L. *Drug Dev. Res.* **1998**, *44*, 14.
17. Balboni, G.; Marastoni, M.; Merighi, S.; Borea, P. A.; Tomatis, R. *Eur. J. Med. Chem.* **2000**, *35*, 979.
18. Abreo, M. A.; Lin, N.-H.; Garvey, D. S.; Gunn, D. E.; Hettinger, A.-M.; Wasicak, J. T.; Pavlik, P. A.; Martin, Y. C.; Donnelly-Roberts, D. L.; Anderson, D. J.;

Sullivan, J. P.; Williams, M.; Arneric, S. P.; Holladay, M. W. *J. Med. Chem.* **1996**, *39*, 817.
19. Data for bridged compound **A** is that of (1*S*,4*S*)-2-(3-pyridyl)-2,5-diazabicyclo[2.2.1]heptane taken from Ref. 10.
20. Data for fused compound **B** is that of (1*S*,5*S*)-3-(3-pyridyl)-3,6-diazabicyclo[3.2.0]heptane taken from Ref. 11.
21. Tønder, J. E.; Olesen, P. H. *Curr. Med. Chem.* **2001**, *8*, 651.
22. Tønder, J. E.; Olesen, P. H.; Hansen, J. B.; Begtrup, M.; Pettersson, I. *J. Comput. Aided Mol. Des.* **2001**, *15*, 247.
23. Ewig, C. S.; Berry, R.; Dinur, U.; Hill, J. R.; Hwang, M. J.; Li, H.; Liang, C.; Maple, J.; Peng, Z.; Stockfisch, T. P.; Thacher, T. S.; Yan, L.; Ni, X.; Hagler, A. T. *J. Comput. Chem.* **2001**, *22*, 1782.