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Tying up Nicotine: New Selective Competitive Antagonist of the Neuronal Nicotinic Acetylcholine Receptors

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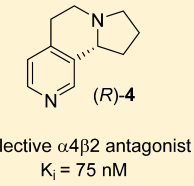
Supporting Information

ABSTRACT: Conformational restriction of the pyrrolidine nitrogen in nicotine by the introduction of an ethylene bridge provided a potent and selective antagonist of the $\alpha4\beta2$-subtype of the nicotinic acetylcholine receptors. Resolution by chiral SFC, pharmacological characterization of the two enantiomers, and determination of absolute configuration via enantioselective synthesis showed that the pharmacological activity resided almost exclusively in the (*R*)-enantiomer.



KEYWORDS: *Bridged-nicotine analogue, nAChRs, selective antagonist, Negishi cross-coupling reaction, oocyte*

The neuronal nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop receptor family of ligand-gated ion channels. They are assembled by either α-subunits or a combination of α- and β-subunits and play an important role in the peripheral and central nervous systems where they mediate neurotransmission in response to acetylcholine (ACh).[1,2] The involvement of nAChRs in a wide range of disease states as well as psychiatric and neurodegenerative disorders (such as depression, schizophrenia, attention deficit hyperactivity disorder, Alzheimer's and Parkinson's diseases, substance abuse, and pain) has made this class of receptors a highly pursued target for drug discovery.[3−6]

Nicotine is the principal psychoactive ingredient in tobacco and acts as a potent agonist of the nAChRs. Structural modifications of nicotine have been the starting point for many drug discovery programs, and the introduction of conformational restraint as in compounds **1**,[7,8] **2**,[9] and **3**[10,11] has previously been investigated to probe the different possible conformations of nicotine (see Figure 1). Ligands **1** and **2** lock the pyrrolidine nitrogen in nicotine in the "down" position, whereas compound **3** locks it in the "up" position.

In continuation of that line of thinking we recently reported the synthesis of new bridged-nicotine analogues locked in the "up" position, i.e., compounds **rac-4** and **rac-5**.[12]

Rigid nicotine analogue **1** was previously shown to be a selective partial agonist of rat $\alpha7$ receptor, while **3** had no agonist activity at any nAChR of the investigated subtypes, whereas conformational restriction in **2** was reported to attenuate nicotinic activity.

Herein we report the pharmacological characterization of the racemic compounds **rac-4** and **rac-5** at several nAChR subtypes. Furthermore, we describe the resolution of **rac-4** by chiral supercritical fluid chromatography (SFC on chiral phase)

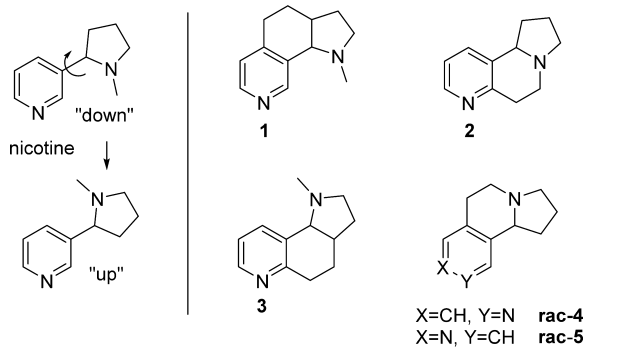


Figure 1. Structure of nicotine and selected conformational restricted analogues.

and determination of absolute configuration of the most potent enantiomer via enantioselective synthesis.

As depicted in Table 1, compound **rac-5** exhibited low binding affinity at $\alpha4\beta2$ nAChRs (K_i = 13 μM), while its affinity for other nAChR subtypes was negligible. In contrast, the direct analogue of nicotine, **rac-4**, was a potent and highly subtype selective ligand for $\alpha4\beta2$ receptors in terms of affinity. Compound **rac-4** displayed nanomolar affinity (K_i = 71 nM) toward the $\alpha4\beta2$ nAChR with more than 100-fold selectivity over $\alpha4\beta4$, $\alpha3\beta4$, and $\alpha7$ (K_i values of 7.2, ∼100, and ∼500 μM, respectively).

In the fluorescence-based FLIPR membrane potential assay **rac-4** was found to be an antagonist at the $\alpha4\beta2$ receptor (IC_{50} = 0.12 μM), whereas it acted as a weak agonist at the $\alpha3\beta4$

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Table 1. Binding Affinities of Constrained Analogues, rac-4, (*R*)-4, (*S*)-4, and rac-5 at the nAChRs[a]

	K_i (μM)			
compds	$\alpha4\beta2$	$\alpha4\beta4$	$\alpha3\beta4$	$\alpha7/5\text{-HT}_{3A}$
(*S*)-nicotine	0.011	0.051	0.23	15
rac-4	0.071	7.2	~100	~500
(*R*)-**4**	0.075	3.7	~50	~300
(*S*)-**4**	3.4	~100	~100	>300
rac-5	13	~100	~100	

[a]The K_i values for the compounds at the rat $\alpha4\beta2$, $\alpha4\beta4$, and $\alpha3\beta4$ nAChR subtypes were obtained in a [^{3}H]epibatidine binding assay, and the K_i values for the compounds at the $\alpha7/5\text{-HT}_{3A}$ chimera were determined in a [^{3}H]MLA binding assay. The K_i values are given in μM. The data are the means of 3−5 individual experiments performed in duplicate. The complete pharmacological data (including the SEM values) are given in the Supporting Information.

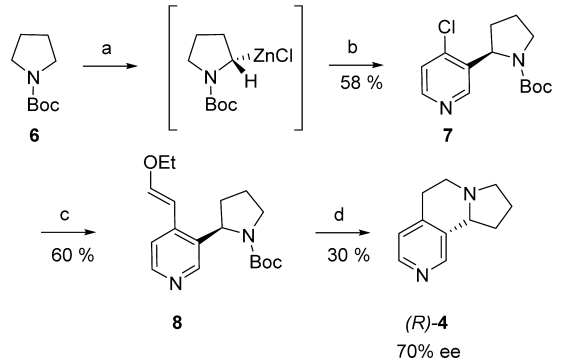
nAChR. It is noteworthy that this simple manipulation of the structure of nicotine converts it from a full agonist to a competitive antagonist at the $\alpha4\beta2$ receptor.

Naturally occurring (*S*)-nicotine is a considerably more potent nAChR agonist than (*R*)-nicotine,[13,14] and we wanted to investigate if the two enantiomers of **4** would exhibit a similar tendency. Thus, the racemic mixture (**rac-4**) was resolved by SFC (see Supporting Information for details). The two enantiomers[15] (*R*)-**4** (corresponding to the first eluted compound, 97.8% ee) and (*S*)-**4** (corresponding to the second eluted compound, 98.8% ee) were tested for binding affinity showing that the activity mainly resided in the first eluting enantiomer with K_i values of 0.075, 3.7, ~50, and ~300 μM at the $\alpha4\beta2$, $\alpha4\beta4$, $\alpha3\beta4$, and $\alpha7$ receptors, respectively. Thus, the pharmacological activity resides almost exclusively in the (*R*)-enantiomer, which in terms of absolute stereochemistry is opposite to nicotine for which the eutomer has (*S*)-configuration.

The absolute configuration of the two enantiomers of **4** was determined in the following way: several attempts to crystallize the compound as different salts for X-ray crystallographic analysis were made, albeit without success. Therefore, a synthetic pathway that would give enantio-enriched material with a known configuration was pursued. Several different approaches toward the enantioselective synthesis of **4** were initially explored. Eventually a strategy based on the enantioselective metalation of *N*-Boc-pyrrolidines[16−22] (which has been demonstrated to proceed with a predictable stereo chemical outcome) proved to be fruitful. This required an enantioselective Negishi cross-coupling reaction with a 3-bromo-4-chloropyridine giving an enantioenriched version of known intermediate in our previous synthesis of rac-**4**.[12]

The synthesis of (*R*)-**4** using this approach is depicted in Scheme 1. Stoichiometric lithiation of *N*-Boc-protected pyrrolidine **6** using (−)-sparteine/*s*-BuLi, subsequent transmetalation with $ZnCl_2$, and palladium-catalyzed cross-coupling reaction with 3-bromo-4-chloropyridine at 65 °C gave α-arylpyrrolidine **7** in 58% yield. Suzuki−Miyaura cross-coupling between (*E*)-2-(2-ethoxyvinyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane and **7** in a CH_3CN−water (3:2) mixture under microwave (MW) conditions at 85 °C for 3 h delivered **8** in 60% yield. Subsequent treatment in a TFA−CH_2Cl_2 (1:1) mixture led to a presumed iminium salt intermediate, which was reduced with an excess of $NaBH_4$ in MeOH providing bridged-nicotine analogue (*R*)-**4** in a 3-step synthesis in 10% overall

Scheme 1. Enantioselective Synthesis of (*R*)-4[a]



[a]Reaction conditions: (a) *s*-BuLi (1 equiv), (−)-sparteine (1 equiv), MTBE, −78 °C, 3 h then $ZnCl_2$ (1 equiv), −78 °C, 30 min then to rt, 30 min; (b) 3-bromo-4-chloropyridine (0.73 equiv), $Pd(OAc)_2$ (5 mol %), TTBP−HBF_4 (5 mol %), 65 °C, 16 h, 58% (2 steps); (c) (*E*)-2-(2-ethoxyvinyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.9 equiv), SPhos (6 mol %), $Pd(OAc)_2$ (3 mol %), K_3PO_4 (2 equiv), CH_3CN−water (3:2), MW, 85 °C, 3 h, 60%; (d) TFA−CH_2Cl_2 (1:1), 0 °C to rt, 3 h then $NaBH_4$ (5 equiv), MeOH, 0 °C to rt, 15 h, 30%.

yield from **6** with 70% ee. The HPLC chromatogram showed that (*R*)-**4** had a retention time identical to that of the eutomer isolated by chromatographic separation of the racemic mixture (see Supporting Information for details).

The reason for the moderate ee of **4** compared to those reported in the literature[16] could be due to a possible racemization during the last reaction step, i.e., the imine intermediate could potentially isomerize to an achiral iminium intermediate affording **rac-4** after reduction, as shown in Figure 2.

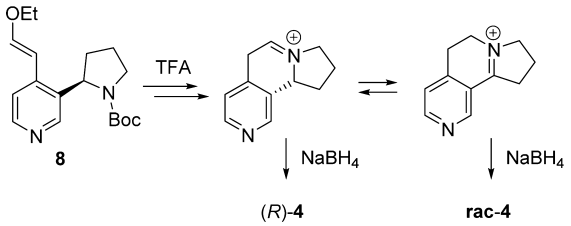


Figure 2. Possible isomerization of the iminium intermediate causing racemization.

To investigate the pharmacological properties of (*R*)-**4** at $\alpha4\beta2$ nAChRs in more detail, we determined whole cell currents in *Xenopus laevis* oocytes[23] expressing the two $\alpha4\beta2$ nAChR stoichiometries upon application of (*R*)-**4** alone and upon coapplication of (*R*)-**4** with acetylcholine (see Figure 3). While there was no change in baseline current when 100 μM (*R*)-**4** was applied, ACh (30 μM) induced current was inhibited when the same concentration of (*R*)-**4** was coapplied (data not shown). This verified the findings from the FLIPR membrane potential assay: (*R*)-**4** acts as an antagonist of $\alpha4\beta2$ nAChR.

The antagonist potency of (*R*)-**4** at the $\alpha4\beta2$ nAChRs was subsequently determined in *Xenopus* oocytes measuring the peak responses to a fixed ACh concentration in the presence of increasing concentrations of (*R*)-**4**. These recordings were performed on both receptor stoichiometries $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$ that are controlled by maintaining subunit mRNA injection ratios of $\alpha4$:$\beta2$ at 10:1 and 1:10, respectively. (*R*)-**4**

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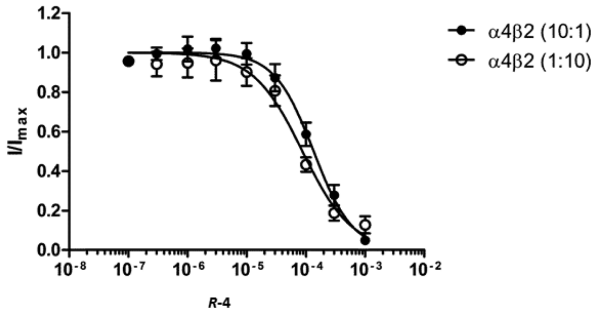

Figure 3. Concentration−inhibition curves of (*R*)-**4**. Concentration−inhibition curve for (*R*)-**4** in oocytes injected with $\alpha4\beta2$ mRNA in 10:1 (●) and 1:10 (○) injection ratios to express $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$, respectively. One millimolar ACh was used for 10:1 and 100 μM ACh was used for 1:10 injection ratios.

inhibited maximal ACh (1 mM) current in $(\alpha4)_3(\beta2)_2$ nAChR ($\alpha4/\beta2$, 10:1) with an IC_{50} value of 135 μM (K_i = 15 μM). On $(\alpha4)_2(\beta2)_3$ receptors ($\alpha4/\beta2$, 1:10), (*R*)-**4** inhibited the max current elicited by ACh (100 μM), with an IC_{50} of 87 μM (K_i = 0.34 μM) (see Figure 3). Although, there is marginal difference in the inhibition curve (1.5-fold difference in IC_{50}) between two stoichiometries, the inhibition constants (K_i) of (*R*)-**4** are significantly different (45-fold) between $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$. As the $(\alpha4)_2(\beta2)_3$ receptor contains only $\alpha4(+)$-$\beta2(-)$ binding sites whereas $(\alpha4)_3(\beta2)_2$ contains both $\alpha4(+)\beta2(-)$ and $\alpha4(+)\alpha4(-)$ sites,[24] the results suggest that (*R*)-**4** exhibits higher affinity to the $\alpha4(+)\beta2(-)$ binding site.

Given the high in vitro potency of (*R*)-**4** we wanted to conduct a preliminary evaluation of the compounds' *in vivo* potential. Antidepressant effects of both nAChR agonists and antagonists have been reported,[25] and we previously evaluated another $\alpha4\beta2$-selective antagonist in the mouse forced swim test and found it to have antidepressant-like activity.[26] Thus, (*R*)-**4** was tested in the mouse forced swim test (1, 3, and 10 mg/kg, subcutaneous administration), but no effects on swimming was found at the tested doses (see Supporting Information for details).

In conclusion, we have shown that **4** is a new potent antagonist of the $\alpha4\beta2$ nAChR. The pharmacological activity resides almost exclusively in the (*R*)-enantiomer, which in terms of absolute stereochemistry is opposite to nicotine for which the eutomer has the (*S*)-configuration. (*R*)-**4** holds significant selectivity for $\alpha4\beta2$ nAChRs over the other nAChR subtypes tested in this study and is an interesting scaffold for development of new nAChR antagonists. In comparison, **3** (another conformationally restrained ligand with the pyrrolidine nitrogen fixed in the "up" position, see Figure 1) showed no affinity toward the nAChRs, highlighting the profound effect on the pharmacological profile, depending on the placement of the conformational restraint in the ligand. Furthermore, we have shown that it is possible to convert nicotine from a full agonist to a competitive antagonist with a very minor structural modification, and further studies with (*R*)-**4** may pave the way for a better understanding of the structural requirement for agonism versus competitive antagonism at the nAChRs.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full experimental details and pharmacological characterization of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

I.N.P., F.C., and J.L.K. conceived the experiments. I.N.P. and F.C. synthesized the compounds. H.P. performed the chiral separation. A.A.J., D.C.I., and T.B. tested the compounds *in vitro*. J.T.A. performed the *in vivo* experiments. I.N.P., F.C., A.A.J., T.B., and J.L.K. wrote the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; SFC, supercritical fluid chromatography; mRNA, messenger ribonucleic acid; CNS, central nervous system; MTBE, methyl *tert*-butyl ether; MW, microwave; TFA, trifluoroacetic acid; TTBP−HBF_4, tri-*tert*-butylphosphonium tetrafluoroborate; ee, enantiomeric excess

■ REFERENCES

(1) Dougherty, D. A. Cys-Loop Neuroreceptors: Structure to the Rescue? *Chem. Rev.* **2008**, *108*, 1642−1653.

(2) Albuquerque, E. X.; Perpeira, E. F. R.; Alkondon, M.; Rogers, W. R. Mammalian Nicotinic Acetylcholine Receptors: From Structure to Function. *Physiol. Rev.* **2009**, *89*, 73−120.

(3) Jensen, A. A.; Frolund, B.; Liljefors, T.; Krogsgaard-Larsen, P. Neuronal Nicotinic Acetylcholine Receptors: Structural Revelations, Target Identifications, and Therapeutic Inspirations. *J. Med. Chem.* **2005**, *48*, 4705−4745.

(4) Arneric, S. P.; Holladay, M.; Williams, M. Neuronal Nicotinic Receptors: A Perspective on Two Decades of Drug Discovery Research. *Bio. Pharmacol.* **2007**, *74*, 1092−1101.

(5) Steinlein, O. K. Ion Channel Mutations in Neuronal Diseases: A Genetics Perspective. *Chem. Rev.* **2012**, *112*, 6334−6352.

(6) Lemoine, D.; Jiang, R.; Taly, A.; Chataigneau, T.; Specht, A.; Grutter, T. Ligand-Gated Ion Channels: New Insights into Neurological Disorders and Ligand Recognition. *Chem. Rev.* **2012**, *112*, 6285−6318.

(7) Glassco, W.; Suchocki, J.; George, C.; Martin, B. R.; May, E. L. Synthesis, Optical Resolution, Absolute Configuration, and Preliminary Pharmacology of (+)- and (−)-cis-2,3,3a,4,5,9b-Hexahydro-1-methyl-1*H*-pyrrolo[3,2-*h*]isoquinoline, a Structural Analog of Nicotine. *J. Med. Chem.* **1993**, *36*, 3381−3385.

(8) Damaj, M. I.; Glassco, W.; Marks, M. J.; Slobe, B.; James, J. R.; May, E. L.; Rosecrans, J. A.; Collins, A. C.; Martin, B. R. Pharmacological Investigation of (+)- and (−)-cis-2,3,3a,4,5,9b-Hexahydro-1-methyl-1H-pyrrolo[3,2-*h*]isoquinoline, a Bridged-Nicotine Analog. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 1425−1434.

(9) Kachur, J. F.; May, E. L.; Awaya, H.; Egle, J. L., Jr.; Aceto, M. D.; Martin, B. R. Pharmacological Effects of 1,2,3,5,6,10b-Hexahydropyrido[2,3-*g*]indolizine, a Bridged-Nicotine Analog. *Life Sci.* **1986**, *38*, 323−330.

(10) Xu, R.; Dwoskin, L. P.; Grinevich, V.; Sumithran, S. P.; Crooks, P. A. Synthesis and Evaluation of Conformationally Restricted Pyridino *N*-Alkylated Nicotine Analogs as Nicotinic Acetylcholine Receptor Antagonists. *Drug Dev. Res.* **2002**, *55*, 173−186.

DOI: 10.1021/acsmedchemlett.5b00028
ACS Med. Chem. Lett. 2015, 6, 472−475

(11) Papke, R. L.; Zheng, G.; Horenstein, N. A.; Dwoskin, L. P.; Crooks, P. A. The Characterization of a Novel Rigid Nicotine Analog with α7-selective nAChR Agonist Activity and Modulation of Agonist Properties by Boron Inclusion. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3874−3880.
(12) Crestey, F.; Hooyberghs, G.; Kristensen, J. L. Concise Synthesis of New Bridged-Nicotine Analogues. *Tetrahedron* **2012**, *68*, 1417−1421.
(13) Copeland, J. R.; Adem, A.; Jacob, P., III; Nordberg, A. A Comparison of the Binding of Nicotine and Nornicotine Stereoisomers to Nicotinic Binding Sites in Rat Brain Cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, *343*, 123−127.
(14) Zhang, X.; Nordberg, A. The Competition of (−)-[3H]Nicotine Binding by the Enantiomers of Nicotine, Nornicotine and Anatoxin-a in Membranes and Solubilized Preparations of Different Brain Regions of Rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1993**, *348*, 28−34.
(15) The absolute configuration of two enantiomers (*R*)-**4** and (*S*)-**4** has been assigned accordingly throughout the Letter even though at the time of the study the absolute configuration was not yet established. The ee of (*R*)-**4** (the first compound to elute) after chiral SFC separation was 97.8% while the ee of (*S*)-**4** (the second compound to elute) was 98.8%. See Supporting Information for more details.
(16) Kopach, M. E.; Meyers, A. I. Metalation−Alkylation of N-Activated Pyrrolidines. Rigorous Proof of Retention for Both Steps. *J. Org. Chem.* **1996**, *61*, 6764−6765.
(17) Gross, K. M. B.; Beak, P. Complex-Induced Proximity Effects: The Effect of Varying Directing-Group Orientation on Carbamate-Directed Lithiation Reactions. *J. Am. Chem. Soc.* **2001**, *123*, 315−321 and references cited therein.
(18) Campos, K. R.; Klapars, A.; Waldman, J. H.; Dormer, P. G.; Chen, C.-Y. Enantioselective, Palladium-Catalyzed α-Arylation of *N*-Boc-pyrrolidine. *J. Am. Chem. Soc.* **2006**, *128*, 3538−3539.
(19) Bilke, J. L.; O'Brien, P. On the Two-Ligand Catalytic Asymmetric Deprotonation of *N*-Boc Pyrrolidine: Probing the Effect of the Stoichiometric Ligand. *J. Org. Chem.* **2008**, *73*, 6452−6454.
(20) Stead, D.; O'Brien, P.; Sanderson, A. A New Sparteine Surrogate for Asymmetric Deprotonation of *N*-Boc Pyrrolidine. *Org. Lett.* **2008**, *10*, 1409−14012.
(21) Barker, G.; McGrath, J. L.; Klapars, A.; Stead, D.; Zhou, G.; Campos, K. R.; O-Brien, P. Enantioselective, Palladium-Catalyzed α-Arylation of *N*-Boc Pyrrolidine: *In Situ* React IR Spectroscopic Monitoring, Scope, and Synthetic Applications. *J. Org. Chem.* **2011**, *76*, 5936−5953.
(22) Cordier, C. J.; Lundgren, R. J.; Fu, G. C. Enantioconvergent Cross-Couplings of Racemic Alkylmetal Reagents with Unactivated Secondary Alkyl Electrophiles: Catalytic Asymmetric Negishi α-Alkylations of *N*-Boc-pyrrolidine. *J. Am. Chem. Soc.* **2013**, *135*, 10946−10949.
(23) Absalom, N. L.; Quek, G.; Lewis, T. M.; Qudah, T.; von Arenstorff, I.; Ambrus, J. I.; Harpsøe, K.; Karim, N.; Balle, T.; McLeod, M. D.; Chebib, M. Covalent Trapping of Methyllycaconitine at the α4-α4 Interface of the α4β2 Nicotinic Acetylcholine Receptor. *J. Biol. Chem.* **2013**, *288*, 26521−26532.
(24) Harpsøe, K.; Ahring, P. K.; Christensen, J. K.; Jensen, M. L.; Peters, D.; Balle, T. Unraveling the high and low sensitivity agonist responses of nicotinic acetylcholine receptors. *J. Neurosci.* **2011**, *31*, 10759−10766.
(25) Philip, N. S.; Carpenter, L. L.; Tyrka, A. R.; Price, L. H. Nicotinic Acetylcholine Receptors and Depression: A Review of the Preclinical and Clinical Literature. *Psychopharmacology* **2010**, *212*, 1−12.
(26) Crestey, F.; Jensen, A. A.; Borch, M.; Andreasen, J. T.; Andersen, J.; Balle, T.; Kristensen, J. L. Design, Synthesis, and Biological Evaluation of Erythrina Alkaloid Analogues as Neuronal Nicotinic Acetylcholine Receptor Antagonists. *J. Med. Chem.* **2013**, *56*, 9673−9682.

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ACS Med. Chem. Lett. 2015, 6, 472−475