

Accepted Manuscript

Synthesis and activity of substituted heteroaromatics as positive allosteric modulators for $\alpha 4\beta 2\alpha 5$ nicotinic acetylcholine receptors

Zhuang Jin, Pasha Khan, Youseung Shin, Jingyi Wang, Li Lin, Michael D. Cameron, Jon M. Lindstrom, Paul J. Kenny, Theodore M. Kamenecka

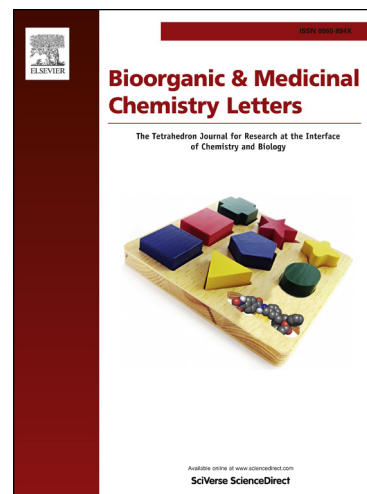
PII: S0960-894X(13)01340-1
DOI: <http://dx.doi.org/10.1016/j.bmcl.2013.11.049>
Reference: BMCL 21076

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 4 September 2013
Revised Date: 18 November 2013
Accepted Date: 20 November 2013

Please cite this article as: Jin, Z., Khan, P., Shin, Y., Wang, J., Lin, L., Cameron, M.D., Lindstrom, J.M., Kenny, P.J., Kamenecka, T.M., Synthesis and activity of substituted heteroaromatics as positive allosteric modulators for $\alpha 4\beta 2\alpha 5$ nicotinic acetylcholine receptors, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: <http://dx.doi.org/10.1016/j.bmcl.2013.11.049>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Synthesis and activity of substituted heteroaromatics as positive allosteric modulators for $\alpha 4\beta 2\alpha 5$ nicotinic acetylcholine receptors

Zhuang Jin^a, Pasha Khan^a, Youseung Shin^a, Jingyi Wang^b, Li Lin^a, Michael D. Cameron^a, Jon M. Lindstrom^b, Paul J. Kenny^a and Theodore M. Kamenecka^{a*}

^a Department of Molecular Therapeutics and Translational Research Institute, The Scripps Research Institute, Scripps Florida, 130 Scripps Way #A2A, Jupiter, FL 33458, USA

^b Department of Neuroscience, University of Pennsylvania, 217 Stemmler Hall, 36th & Hamilton Walk, Philadelphia, PA 19104, USA

* Corresponding author. E-mail address: kameneck@scripps.edu (T.M. Kamenecka).

Abstract: The design and synthesis of a series of substituted heteroaromatic $\alpha 4\beta 2\alpha 5$ positive allosteric modulators is reported. The optimization and development of the heteroaromatic series was carried out from NS9283, and several potent analogues, such as 3-(5-(pyridin-3-yl)-2H-tetrazol-2-yl)benzonitrile (**5k**) and 3,3'-(2H-tetrazole-2,5-diyl)dipyridine (**12h**) with good *in vitro* efficacy were discovered.

Keywords: Substituted heteroaromatics; $\alpha 4\beta 2\alpha 5$; Positive allosteric modulators

Tobacco smoking results in more than 5 million deaths each year and accounts for almost 90% of all deaths from lung cancer.¹ Nicotine is the principal reinforcing component in tobacco smoke responsible for addiction.² Nicotine acts in the brain through the neuronal nicotine acetylcholine receptors (nAChRs), which are ligand-gated ion channels consisting of five membrane-spanning subunits.³ Twelve neuronal nAChR subunits have been identified: nine α subunits ($\alpha 2$ - $\alpha 10$) and three β subunits ($\beta 2$ - $\beta 4$).³ The predominant nAChR subtypes in mammalian brain that have been heavily implicated in regulating the addiction properties of nicotine are those containing $\alpha 4$ and $\beta 2$ subunits (denoted as $\alpha 4\beta 2$ nAChRs).⁴⁻⁸ The $\alpha 4\beta 2$ nAChRs are also the targets for all current FDA-approved anti-smoking agents (ie: bupropion, Chantix).⁹⁻¹¹

Recent evidence has shown that allelic variation in the $\alpha 5/\alpha 3/\beta 4$ nAChR subunit gene cluster significantly increases the risk of tobacco addiction.^{12,13} Specifically, polymorphism in the $\alpha 5$ subunit gene (*CHRNA5*) increases vulnerability to tobacco addiction.^{14,15} These facts indicate that the $\alpha 5$ subunit plays an important role in the tobacco smoking habit. In addition, in-house data has shown that habenular $\alpha 5$ subunit signaling controls nicotine intake.¹⁶

The challenge in developing nicotine acetylcholine receptor modulators (agonists/antagonists) has been selectivity over closely related receptors. This is not unexpected given the highly conserved nature of the orthosteric site amongst nAChR's. One possible solution would be to target positive allosteric modulators (PAM's) which are ligands that bind to

allosteric sites and facilitate agonist induced stabilization of the "open" conformation or that reduce agonist-facilitated receptor desensitization. PAMs do not influence receptor function in the absence of orthosteric agonists. Instead, PAMs potentiate the stimulatory effects of low agonist concentrations on nAChR function. Hence, the receptor would only be activated in the presence of the orthosteric ligand, and may also avoid receptor desensitization issues often seen with full agonists.¹⁷⁻¹⁹

Without a high-throughput screen to look for novel structural leads, and given the high homology between nicotinic receptor subtypes, we considered other nAChR PAM's as potential starting points for medicinal chemistry. We initially focused on the bis-aryl isoxazole NS9283 (3-(3-(pyridin-3-yl)-1,2,4-oxadiazol-5-yl)benzonitrile) (Figure 1), which had previously been identified as a PAM for $\alpha 4\beta 2$ nAChR.²⁰⁻²² In-house, NS9283 was a PAM at $\alpha 4\beta 2$ with an $EC_{50} = 0.99 \pm 0.6 \mu M$. The compound had no agonist activity at the receptor. Perhaps not surprising, this compound NS9283 is also a PAM for $\alpha 4\beta 2\alpha 5$ with an $EC_{50} = 0.79 \pm 0.2 \mu M$ (compound **1a** in Table 1). Herein we describe our structure activity relationship studies (SAR) of this compound in an effort to optimize both the potency and selectivity towards $\alpha 4\beta 2\alpha 5$ as PAM's for smoking cessation therapy.

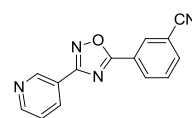
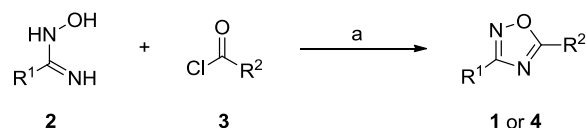


Figure 1. Structure of NS9283

NS9283 is easily envisioned to consist of 3 fragments that can be assembled in modular fashion- the pyridine ring, the central oxadiazole ring, and the substituted phenyl ring. Each portion of the molecule was selectively modified while keeping the other two fragments constant thereby allowing one to see the effects of single point changes to the molecule. Optimized portions would then be combined into the final compound.

The synthetic pathway for 3,5-disubstituted-1,2,4-oxadiazoles is straightforward (Scheme 1).²³ An appropriately substituted N-hydroxy-(carbox)imidamide (**2**) and an acid chloride (**3**) were mixed in dry pyridine, and warmed to reflux to afford 3,5-disubstituted-1,2,4-oxadiazoles in good yield.



Scheme 1. Syntheses of 3,5-disubstituted-1,2,4-oxadiazoles (**1** or **4**). Reagents and condition: (a) pyridine, r.t. 3 h; 120°C by microwave, 1 h.

First, we modified the 3-pyridinyl moiety, the left part of NS9283, and the SAR results are summarized in Table 1. Replacement of the 3-pyridinyl group with a phenyl ring abolished PAM activity (**1b**). Similarly, the corresponding pyridine ring isomers (**1c** and **1d**) as well as its quaternary salt (**1e**) led to complete loss of activity clearly showing that the 3-pyridinyl moiety is crucial for PAM activity in NS9283.

Table 1. SAR of the 3-pyridinyl group of NS9283

Analogue	Structure	PAM(Y or N) ^a	EC ₅₀ (uM)	E _{max} ^b
1a		Y	0.79±0.2	1
1b		N	—	—
1c		N	—	—
1d		N	—	—
1e		N	—	—

^a PAM: Positive Allosteric Modulator for α4β2α5.

Y (for yes): 1 μM compound potentiates EC₂₀₋₃₀ nicotine >50%;

N (for no): 1 μM compound potentiates EC₂₀₋₃₀ nicotine <50%. Average of n = 2;

^b Maximum efficacy relative to NS9283.

were conserved (Table 2). Different substituted phenyl groups (**4a-4l**) were incorporated into the molecule using the chemistry shown in Scheme 1. Phenyl (**4a**), 2-chlorophenyl (**4b**), 4-chlorophenyl (**4c**) and 3-fluorophenyl (**4d**) analogues all lost their PAM activities.

Interestingly, 2,3-difluorophenyl analogue (**4e**) has no PAM activity, while the 3,4-difluorophenyl analog (**4f**) is a PAM on α4β2α5. The potency of **4f** is lower than that of NS9283, but their maximum efficacies are similar. The 2-nitro phenyl analog (**4g**) did not show any PAM activity, however the 3-substituted analog (**4h**) exhibited similar activity to NS9283. The 4-substituted analog (**4i**) had some PAM activity on α4β2α5, but less so than NS9283. Other 3-substituted analogs (**4j-4l**) were inactive.

Table 2. SAR of the 3-cyanophenyl group of NS9283

Compound	Structure	PAM(Y or N) ^a	EC ₅₀ (uM)	E _{max} ^b
4a		N	—	—
4b		N	—	—
4c		N	—	—
4d		N	—	—
4e		N	—	—
4f		Y	1.3±0.4	1.2
4g		N	—	—
4h		Y	0.69±0.35	1.2
4i		Y	1.7±0.5	0.81
4j		N	—	—
4k		N	—	—
4l		N	—	—
4m		Y	1.0±0.3	0.98

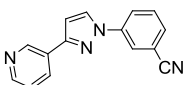
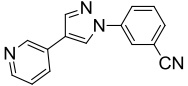
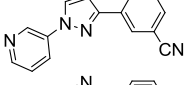
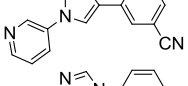
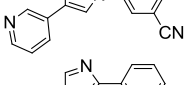
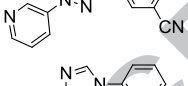
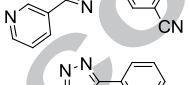
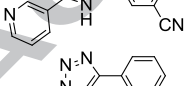
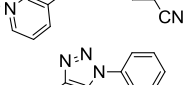
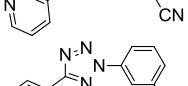
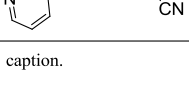
^{a,b} See Table 1 caption.

Second, a SAR study was conducted on the 3-cyanophenyl group while the 3-pyridinyl and 1,2,4-oxadiazole groups

Interestingly, the 3,5-di(pyridin-3-yl)-1,2,4-oxadiazole (**4m**) showed similar activity as NS9283. Overall, modifications to the 3-pyridinyl and 3-cyanophenyl rings of NS9283 were not well tolerated.

We then looked to modify the core 1,2,4-oxadiazole ring of NS9283, and these results are tabulated in Table 3. These analogs were synthesized as described in Scheme 2. Most of the compounds were made via Suzuki coupling or Chan-Lam type coupling using appropriate boronic acids.^{24,25} The 3-(5-(Pyridin-3-yl)-4H-1,2,4-triazol-3-yl)benzonitrile (**5h**) was prepared via the condensation reaction between nicotinohydrazide (**6**) and isophthalonitrile (**7**) in *n*-butanol (equation 2, scheme 2).²⁶ The 1,2,3-Triazole analogues **5i** and **5j** were made by Click chemistry (equation 3, scheme 2).²⁷ Analog **5k** was synthesized by the reaction of the diazonium salt generated from 3-aminobenzonitrile and 4-methyl-N'-(pyridin-3-ylmethylene)benzenesulfonohydrazide (equation 4, scheme 2).²⁸⁻³⁰

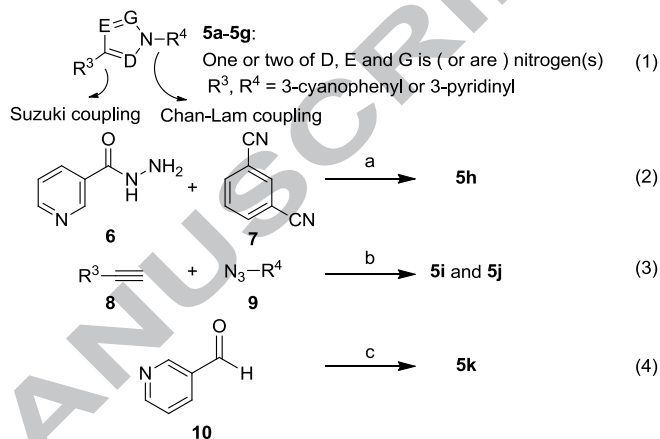
Table 3. SAR of the 1,2,4-oxadiazole group of NS9283

Compound	Structure	PAM(Y or N) ^a	EC ₅₀ (uM)	E _{max} ^b
5a		N	—	—
5b		N	—	—
5c		N	—	—
5d		N	—	—
5e		N	—	—
5f		N	—	—
5g		N	—	—
5h		N	—	—
5i		N	—	—
5j		N	—	—
5k		Y	2.0±0.9	2.5

^{a,b} See Table 1 caption.

Similarly, modifications to the central core were as intolerant as those to the rest of the molecule. We examined the three

pyrazole derivative isomers (**5a**, **5b** and **5c**), and none of them had PAM activity on $\alpha 4\beta 2\alpha 5$. On the other hand, two imidazole derivatives (**5d** and **5e**) did not show any activity either. Similarly, five differently disubstituted triazole derivatives (**5f-5j**) were devoid of activity. The only heterocyclic core tolerated was a tetrazole as in 3-(5-(pyridin-3-yl)-2H-tetrazol-2-yl)benzonitrile (**5k**). This compound showed similar potency and efficacy against $\alpha 4\beta 2\alpha 5$ as compared to NS9283. It is not clear the nature of the effect and why the only tolerable heterocyclic replacement for the oxadiazole is a tetrazole.



With **5k** in hand, we attempted to improve the potency and efficacy by similar modifications to the cyanophenyl and pyridine rings like what we performed on NS9283 (Tables 4 and 5). However, we experienced very similar SAR results, with not much toleration for substitutions. The quaternary salt (**11a**), and the phenyl ring substitution (**11b**) showed no PAM activity on $\alpha 4\beta 2\alpha 5$. The pyridine ring isomers (**11c-d**) were equally inactive reconfirming the fact that the 3-pyridinyl moiety is critical for $\alpha 4\beta 2\alpha 5$ modulation. The 3-pyridinyl ring did not tolerate substitution either (**11e-h**). Even a simple chlorine atom would ablate the PAM activity.

Attempts to alter the substitution of the N-linked tetrazole ring were equally frustrating (Table 5). Simple substitutions of the cyano group with alkoxy groups or halogens led to complete loss of activity (**12a-d**, **12i-j**). Considering that the 3-carboxamide analog (**12e**) showed no activity, it seems important to have a small polar substituent (like CN as in **5k**) for PAM activity on $\alpha 4\beta 2\alpha 5$. However, moving the cyano group to the 4-position of the ring as in **12f** led to a compound equipotent to **5k**. However, **5k** doesn't tolerate a 4-substituted methyl group (**12g**). Similarly to the oxadiazole series, a bis-3-pyridinyl substituted analog (**12h**) showed good PAM activity on $\alpha 4\beta 2\alpha 5$.

To get a sense of the selectivity of NS9283 as well as **5k**, we counter-screened these $\alpha 4\beta 2\alpha 5$ PAM's against a number of other nAChR's. These two compounds were screened against $\alpha 3\beta 4$, $\alpha 3\beta 4\alpha 5$, $\alpha 3\beta 2$, and $\alpha 3\beta 2\alpha 5$ and showed no activity. Further profiling against $\alpha 2\beta 4$, $\alpha 2\beta 4\alpha 5$, $\alpha 6$, and $\alpha 7$ is on-going. Both compounds were, however, equally potent

Table 4. SAR of 2,5-disubstituted tetrazole analogs

Compound	Structure	PAM(Y or N) ^a	EC ₅₀ (uM)	E _{max} ^b
5k		Y	2.0±0.9	2.5
11a		N	—	—
11b		N	—	—
11c		N	—	—
11d		N	—	—
11e		N	—	—
11f		N	—	—
11g		N	—	—
11h		N	—	—

^{a,b} See Table 1 caption.

PAM's on $\alpha 4\beta 2$. It should also be mentioned that none of the analogs tested showed any agonist activity on $\alpha 4\beta 2\alpha 5$, i.e., they only gave a response in the presence of nicotine. To target $\alpha 4\beta 2\alpha 5$ for smoking cessation, the compounds will have to penetrate the blood brain barrier. Hence, **5k** was dosed to mice (10 mg/kg, IP) to look for CNS exposure. Two hours after dosing, drug plasma levels were 11 μ M, and drug brain levels were 4.4 μ M. Compound **5k** was also dosed in mice to look for standard pharmacokinetic parameters. The compound has moderate clearance, but very good oral exposure.

Table 6. Pharmacokinetics of **5k** in mice

Cl (mL/min/kg)	T _{1/2} (h)	AUC _{po} (μ M•hr)	C _{max} po (μ M)	V _d (L/kg)	F%
46	0.9	21	4.1	2.7	100

Dosed 1 mg/kg IV, 2mg/kg PO.

In summary, the SAR about the lead oxadizaole NS9283 proved to be extremely narrow with most modifications to the structure leading to complete loss in activity. The 3-pyridine ring seemed essential for potency. The 3-cyanophenyl ring tolerated very few substitutions as well. With regards to the central core, of the eleven nitrogen heterocycles tested, only a tetrazole substitution was on-par with that of NS9283. Tetrazole **5k** is a moderately potent dual PAM of $\alpha 4\beta 2$ and $\alpha 4\beta 2\alpha 5$ with good CNS exposure following IP dosing and has good oral absorption. SAR is still on-going to see if selectivity can be achieved between $\alpha 4\beta 2$ and $\alpha 4\beta 2\alpha 5$ and will be reported in due course. Nonetheless, **5k** may be a useful tool compound to explore the PAM effects on $\alpha 4\beta 2$ and $\alpha 4\beta 2\alpha 5$ *in vivo* in animal models of nicotine addiction.

Table 5. SAR of 3-pyridinyl tetrazole analogs

Compound	Structure	PAM(Y or N) ^a	EC ₅₀ (uM)	E _{max} ^b
12a		N	—	—
12b		N	—	—
12c		N	—	—
12d		N	—	—
12e		N	—	—
12f		Y	2.2±0.4	0.96
12g		N	—	—
12h		Y	0.7±0.12	1.9
12i		N	—	—
12j		N	—	—

^{a,b} See Table 1 caption.

Cell Culture: The HEK tsA201 cell lines expressing human $\alpha 4\beta 2$, $\alpha 4\beta 2\alpha 5$, $\alpha 3\beta 2$, $\alpha 3\beta 2\alpha 5$, $\alpha 3\beta 4$ or $\alpha 3\beta 4\alpha 5$ AChRs were described previously.³¹⁻³⁴ All cell lines were maintained in Dulbecco's modified Eagle's medium (high glucose; Invitrogen) with 100 units/mL penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine (Invitrogen) and 10% fetal bovine serum (HyClone, Logan, UT) at 37 °C, 5% CO₂ at saturating humidity.

FLEXstation Experiments: For AChR functional tests, we used a FLEXstation II (Molecular Devices, Sunnyvale, CA) bench-top scanning fluorometer as described by Kuryatov.³¹ The cells were plated at 150,000/well for non- $\alpha 5$ -containing cell lines or 200,000/well for $\alpha 5$ -containing cell lines on black-walled, clear-bottomed 96-well plates (Costar; Corning Life Sciences, Action, MA) coated with poly(D-lysine). For $\beta 2$ -containing cells, to increase the AChR expression level, the plates were incubated at 29 °C for 20 hours before being tested. The membrane potential kit (Molecular Devices, Sunnyvale, CA) was used according to the manufacturer's protocols. Serial dilutions of the PAM candidates were manually added to the assay plate 15 min prior to the addition of nicotine. Agonists (i.e., acetylcholine or nicotine) were prepared in V-shaped 96-well plates (Fisher Scientific Co., Pittsburgh, PA) and were added in separate wells at a rate of 20 μ L/s during recording. Each data point was averaged from three to four responses from separate wells. The Hill equation was fitted to the concentration-response relationship using a non-linear least-squares error curve-fit method (KaleidaGraph, Synergy Software, Reading, PA): $I(x)=I_{\max}[x^n/(x^n+EC_{50}^n)]$, where $I(x)$ is the current measured at the agonist concentration x , I_{\max} is the maximal current response at the saturating agonist concentration, EC_{50} is the agonist concentration required for the half-maximal response, and n is the Hill coefficient. The potencies of the PAMs were obtained similarly except that the enhanced current relative to the current evoked by nicotine itself was used in the equation instead of the absolute current evoked by agonist. Efficacies of the PAMs were all compared to that of the control compound (NS9283) to minimize the differences between assays.

Acknowledgement

Supported by the National Institute on Drug Abuse (DA030929 to P.J.K. and J.M.L.).

References:

- (1) Mikdad, A. H.; Marks, J. S.; Stroup, D. F.; Gerberding, J. L. *J. Am. Med. Assoc.* **2004**, 291, 1238.
- (2) Stolerman, I. P.; Jarvis, M. J. *Psychopharmacology* **1995**, 117, 2.
- (3) Le, N. N.; Corringer, P.-J.; Changeux, J.-P. *J. Neurobiol.* **2002**, 53, 447.
- (4) Corrigan, W. A.; Franklin, K. B. J.; Coen, K. M.; Clarke, P. B. S. *Psychopharmacology* **1992**, 107, 285.
- (5) Ikemoto, S.; Qin, M.; Liu, Z.-H. *J. Neurosci.* **2006**, 26, 723.
- (6) Maskos, U.; Molles, B. E.; Pons, S.; Besson, M.; Guiard, B. P.; Guilloux, J. P.; Evrard, A.; Cazala, P.; Cormier, A.; Mameli-Engvall, M.; Dufour, N.; Cloez-Tayarani, I.; Bemelmans, A. P.; Mallet, J.; Gardier, A. M.; David, V.; Faure, P.; Granon, S.; Changeux, J. P. *Nature* **2005**, 436, 103.
- (7) Picciotto, M. R.; Zoli, M.; Rimondini, R.; Lena, C.; Marubio, L. M.; Pich, E. M.; Fuxe, K.; Changeux, J.-P. *Nature* **1998**, 391, 173.
- (8) Tapper, A. R.; McKinney, S. L.; Nashmi, R.; Schwarz, J.; Deshpande, P.; Labarca, C.; Whiteaker, P.; Marks, M. J.; Collins, A. C.; Lester, H. A. *Science* **2004**, 306, 1029.
- (9) Coe, J. W.; Brooks, P. R.; Vetelino, M. G.; Wirtz, M. C.; Arnold, E. P.; Huang, J.; Sands, S. B.; Davis, T. I.; Lebel, L. A.; Fox, C. B.; Shrikhande, A.; Heym, J. H.; Schaeffer, E.; Rollema, H.; Lu, Y.; Mansbach, R. S.; Chambers, L. K.; Rovetti, C. C.; Schulz, D. W.; Tingley, F. D., III; O'Neill, B. T. *J. Med. Chem.* **2005**, 48, 3474.
- (10) Tønnesen, P.; Tonstad, S.; Hjalmarson, A.; Leborg, F.; Van Spiegel, P. I.; Hider, A.; Sweet, R.; Townsend, J. J. *Intern. Med.* **2003**, 254, 184.
- (11) West, R.; Zatonski, W.; Cedzynska, M.; Lewandowska, D.; Pazik, J.; Aveyard, P.; Stapleton, J. *N Engl J Med* **2011**, 365, 1193.
- (12) Berrettini, W.; Yuan, X.; Tozzi, F.; Song, K.; Francks, C.; Chilcoat, H.; Waterworth, D.; Muglia, P.; Mooser, V. *Mol. Psychiatry* **2008**, 13, 368.
- (13) Saccone, S. F.; Hinrichs, A. L.; Saccone, N. L.; Chase, G. A.; Konvicka, K.; Madden, P. A. F.; Breslau, N.; Johnson, E. O.; Hatsukami, D.; Pomerleau, O.; Swan, G. E.; Goate, A. M.; Rutter, J.; Bertelsen, S.; Fox, L.; Fugman, D.; Martin, N. G.; Montgomery, G. W.; Wang, J. C.; Ballinger, D. G.; Rice, J. P.; Bierut, L. J. *Hum. Mol. Genet.* **2007**, 16, 36.
- (14) Bierut, L. J.; Stitzel, J. A.; Wang, J. C.; Hinrichs, A. L.; Grucza, R. A.; Xuei, X.; Saccone, N. L.; Saccone, S. F.; Bertelsen, S.; Fox, L.; Horton, W. J.; Breslau, N.; Budde, J.; Cloninger, C. R.; Dick, D. M.; Foroud, T.; Hatsukami, D.; Hesselbrock, V.; Johnson, E. O.; Kramer, J.; Kuperman, S.; Madden, P. A. F.; Mayo, K.; Nurnberger, J., Jr.; Pomerleau, O.; Porjesz, B.; Reyes, O.; Schuckit, M.; Swan, G.; Tischfield, J. A.; Edenberg, H. J.; Rice, J. P.; Goate, A. M. *Am J Psychiatry* **2008**, 165, 1163.
- (15) Kuryatov, A.; Berrettini, W.; Lindstrom, J. *Mol. Pharmacol.* **2011**, 79, 119.
- (16) Fowler, C. D.; Lu, Q.; Johnson, P. M.; Marks, M. J.; Kenny, P. J. *Nature* **2011**, 471, 597.
- (17) Lindsley, C. W.; Niswender, C. M.; Engers, D. W.; Hopkins, C. R. *Curr Top Med Chem* **2009**, 9, 949.
- (18) Morrow, J. A.; Maclean, J. K. F.; Jamieson, C. *Curr Opin Drug Discov Devel* **2006**, 9, 571.
- (19) Ward, S. E.; Harries, M. *Curr Med Chem* **2010**, 17, 3503.
- (20) Grupe, M.; Jensen, A. A.; Ahling, P. K.; Christensen, J. K.; Grunnet, M. *Br. J. Pharmacol.* **2013**, 168, 2000.
- (21) Malysz, J.; Dyhring, T.; Ahling, P. K.; Olsen, G. M.; Peters, D.; Gronlien, J. H.; Wetterstrand, C.; Ween, H.; Haakerud, M.; Thorin-Hagene, K.; Andersen, E.; Anderson, D. J.; Hu, M.; Kroeger, P. E.; Lee, C. H. L.; Gopalakrishnan, M.; Timmermann, D. B. *Biochem. Pharmacol.* **2009**, 78, 919.
- (22) Rode, F.; Munro, G.; Holst, D.; Nielsen, E. O.; Troelsen, K. B.; Timmermann, D. B.; Ronn, L. C. B.; Grunnet, M. *Brain Res.* **2012**, 1458, 67.
- (23) Gopalakrishnan, M.; Honore, M. P.; Lee, C.-H.; Malysz, J.; Ji, J.; Li, T.; Schrimpf, M. R.; Sippy, K. B.; Anderson, D. J.; WO2008073942A2: 2008.
- (24) Chan, D. M. T.; Monaco, K. L.; Wang, R.-P.; Winters, M. P. *Tetrahedron Lett.* **1998**, 39, 2933.
- (25) Miyaura, N.; Suzuki, A. *J. Chem. Soc., Chem. Commun.* **1979**, 866.

(26) Bhagwat, S. S.; Satoh, Y.; Sakata, S. T.; Buhr, C. A.; Albers, R.; Sapienza, J.; Plantevin, V.; Chao, Q.; Sahasrabudhe, K.; Ferri, R.; US20040127536A1: 2004.

(27) Himmo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210.

(28) Roppe, J.; Smith, N. D.; Huang, D.; Tehrani, L.; Wang, B.; Anderson, J.; Brodtkin, J.; Chung, J.; Jiang, X.; King, C.; Munoz, B.; Varney, M. A.; Prasit, P.; Cosford, N. D. *P. J. Med. Chem.* **2004**, *47*, 4645.

(29) Dahl, B. H.; Olsen, G. M.; Peters, D.; Jensen, M. L.; WO2010106106A1: 2010 (Tetrazoles were recently reported as PAM's of a4b2).

(30) Peters, D.; Grunnet, M.; Timmermann, D. B.; Eriksen, B. L.; Dyhring, T.; Ahring, P. K.; WO2010106106A1: 2011 (Tetrazoles were recently reported as PAM's of a4b2).

(31) Kuryatov, A.; Luo, J.; Cooper, J.; Lindstrom, J. *Mol. Pharmacol.* **2005**, *68*, 1839.

(32) Kuryatov, A.; Onksen, J.; Lindstrom, J. *Mol. Pharmacol.* **2008**, *74*, 132.

(33) Nelson, M. E.; Kuryatov, A.; Choi, C. H.; Zhou, Y.; Lindstrom, J. *Mol. Pharmacol.* **2003**, *63*, 332.

(34) Wang, F.; Nelson, M. E.; Kuryatov, A.; Olale, F.; Cooper, J.; Keyser, K.; Lindstrom, J. *J. Biol. Chem.* **1998**, *273*, 28721.

