Published in final edited form as:
Bioorg Med Chem Lett. 2014 January 15; 24(2): 674–678. doi:10.1016/j.bmcl.2013.11.049.

Synthesis and activity of substituted heteroaromatics as positive allosteric modulators for α4β2α5 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

Abstract

The design and synthesis of a series of substituted heteroaromatic α4β2α5 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; α4β2α5; 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 (α2–α10) and three β subunits (β2–β4).[3] The predominant nAChR subtypes in mammalian brain that have been heavily implicated in regulating the addiction properties of nicotine are those containing α4 and β2 subunits (denoted as α4β2 nAChRs).[4–8] The α4β2 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 α5/α3/β4 nAChR subunit gene cluster significantly increases the risk of tobacco addiction.[12,13] Specifically, polymorphism in the α5 subunit gene (*CHRNA5*) increases vulnerability to tobacco addiction.[14,15] These facts indicate that the α5 subunit plays an important role in the tobacco smoking habit. In

*Corresponding author. kameneck@scripps.edu (T.M. Kamenecka).

addition, in-house data has shown that habenular α5 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 α4β2 nAChR.[20–22] In-house, NS9283 was a PAM at α4β2 with an EC_{50} = 0.99 ± 0.6 μM. The compound had no agonist activity at the receptor. Perhaps not surprising, this compound NS9283 is also a PAM for α4β2α5 with an EC_{50} = 0.79 ± 0.2 μM (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 α4β2α5 as PAM's for smoking cessation therapy.

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.

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 it's quaternary salt (**1e**) led to complete loss of activity clearly showing that the 3-pyridinyl moiety is crucial for PAM activity in NS9283.

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

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]

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 α4β2α5. 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 heterocylcic 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 α4β2α5 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 α4β2α5. The pyridine ring isomers (**11c**–**d**) were equally inactive reconfirming the fact that the 3-pyridinyl moiety is critical for α4β2α5 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 α4β2α5. 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 α4β2α5.

To get a sense of the selectivity of NS9283 as well as **5k**, we counter-screened these α4β2α5 PAM's against a number of other nAChR's. These two compounds were screened against α3β4, α3β4α5, α3β2, and α3β2α5 and showed no activity. Further profiling against α2β4, α2β4α5, α6, and α7 is on-going. Both compounds were, however, equally potent PAM's on α4β2. It should also be mentioned that none of the analogs tested showed any agonist activity on α4β2α5, i.e., they only gave a response in the presence of nicotine. To target α4β2α5 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 11μM, and drug brain levels were 4.4 μM. Compound **5k** was also dosed in mice to look for standard pharmacokinetic parameters. The compound has moderate clearance, but very good oral exposure.

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 α4β2 and α4β2α5 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 α4β2 and α4β2α5 and will be reported in due course. Nonetheless, **5k** may be a useful tool compound to explore the PAM effects on α4β2 and α4β2α5 *in vivo* in animal models of nicotine addiction.

Cell Culture: The HEK tsA201 cell lines expressing human α4β2, α4β2α5, α3β2, α3β2α5, α3β4 or α3β4α5 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 μg/ml streptomycin, 2 mM L-glutamine (Invitrogen) and 10% fetal bovine serum (HyClone, Logan, UT) at 37 °C, 5% CO_2 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-α5-containing cell lines or 200,000/well for α5-containing cell lines on black-walled, clear-bottomed 96-well plates (Costar; Corning Life Sciences, Action, MA) coated with poly(D-lysine). For β2-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 μL/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): $I(x)=I_{max}[x^n/(x^n+EC_{50n})]$, 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.

Acknowledgments

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

References

1. Mikdad AH, Marks JS, Stroup DF, Gerberding JL. J. Am. Med. Assoc. 2004; 291:1238.
2. Stolerman IP, Jarvis MJ. Psychopharmacology. 1995; 117:2. [PubMed: 7724697]
3. Le NN, Corringer P-J, Changeux J-P. J. Neurobiol. 2002; 53:447. [PubMed: 12436412]

4. Corrigall WA, Franklin KBJ, Coen KM, Clarke PBS. Psychopharmacology. 1992; 107:285. [PubMed: 1615127]
5. Ikemoto S, Qin M, Liu Z-H. J. Neurosci. 2006; 26:723. [PubMed: 16421292]
6. Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, Dufour N, Cloez-Tayarani I, Bemelmans AP, Mallet J, Gardier AM, David V, Faure P, Granon S, Changeux JP. Nature. 2005; 436:103. [PubMed: 16001069]
7. Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux J-P. Nature. 1998; 391:173. [PubMed: 9428762]
8. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA. Science. 2004; 306:1029. [PubMed: 15528443]
9. Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, Shrikhande A, Heym JH, Schaeffer E, Rollema H, Lu Y, Mansbach RS, Chambers LK, Rovetti CC, Schulz DW, Tingley FD III, O'Neill BT. J. Med. Chem. 2005; 48:3474. [PubMed: 15887955]
10. Tønnesen P, Tonstad S, Hjalmarson A, Lebargy F, Van Spiegel PI, Hider A, Sweet R, Townsend J. J. Intern. Med. 2003; 254:184. [PubMed: 12859700]
11. West R, Zatonski W, Cedzynska M, Lewandowska D, Pazik J, Aveyard P, Stapleton J. N Engl J Med. 2011; 365:1193. [PubMed: 21991893]
12. Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H, Waterworth D, Muglia P, Mooser V. Mol. Psychiatry. 2008; 13:368. [PubMed: 18227835]
13. Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PAF, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, Swan GE, Goate AM, Rutter J, Bertelsen S, Fox L, Fugman D, Martin NG, Montgomery GW, Wang JC, Ballinger DG, Rice JP, Bierut LJ. Hum. Mol. Genet. 2007; 16:36. [PubMed: 17135278]
14. Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, Saccone NL, Saccone SF, Bertelsen S, Fox L, Horton WJ, Breslau N, Budde J, Cloninger CR, Dick DM, Foroud T, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Kuperman S, Madden PAF, Mayo K, Nurnberger J Jr, Pomerleau O, Porjesz B, Reyes O, Schuckit M, Swan G, Tischfield JA, Edenberg HJ, Rice JP, Goate AM. Am J Psychiatry. 2008; 165:1163. [PubMed: 18519524]
15. Kuryatov A, Berrettini W, Lindstrom J. Mol. Pharmacol. 2011; 79:119. [PubMed: 20881005]
16. Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ. Nature. 2011; 471:597. [PubMed: 21278726]
17. Lindsley CW, Niswender CM, Engers DW, Hopkins CR. Curr Top Med Chem. 2009; 9:949. [PubMed: 19754407]
18. Morrow JA, Maclean JKF, Jamieson C. Curr Opin Drug Discov Devel. 2006; 9:571.
19. Ward SE, Harries M. Curr Med Chem. 2010; 17:3503. [PubMed: 20738249]
20. Grupe M, Jensen AA, Ahring PK, Christensen JK, Grunnet M. Br. J. Pharmacol. 2013; 168:2000. [PubMed: 23278456]
21. Malysz J, Dyhring T, Ahring PK, Olsen GM, Peters D, Gronlien JH, Wetterstrand C, Ween H, Haakerud M, Thorin-Hagene K, Andersen E, Anderson DJ, Hu M, Kroeger PE, Lee CHL, Gopalakrishnan M, Timmermann DB. Biochem. Pharmacol. 2009; 78:919.
22. Rode F, Munro G, Holst D, Nielsen EO, Troelsen KB, Timmermann DB, Ronn LCB, Grunnet M. Brain Res. 2012; 1458:67. [PubMed: 22552114]
23. Gopalakrishnan, M.; Honore, MP.; Lee, C-H.; Malysz, J.; Ji, J.; Li, T.; Schrimpf, MR.; Sippy, KB.; Anderson, DJ. WO2008073942A2. 2008.
24. Chan DMT, Monaco KL, Wang R-P, Winters MP. Tetrahedron Lett. 1998; 39:2933.
25. Miyaura N, Suzuki A. J. Chem. Soc. Chem. Commun. 1979:866.
26. Bhagwat, SS.; Satoh, Y.; Sakata, ST.; Buhr, CA.; Albers, R.; Sapienza, J.; Plantevin, V.; Chao, Q.; Sahasrabudhe, K.; Ferri, R. US20040127536A1. 2004.
27. Himo F, Lovell T, Hilgraf R, Rostovtsev VV, Noodleman L, Sharpless KB, Fokin VV. J. Am. Chem. Soc. 2005; 127:210. [PubMed: 15631470]

28. Roppe J, Smith ND, Huang D, Tehrani L, Wang B, Anderson J, Brodkin J, Chung J, Jiang X, King C, Munoz B, Varney MA, Prasit P, Cosford NDP. J. Med. Chem. 2004; 47:4645. [PubMed: 15341479]

29. Dahl, BH.; Olsen, GM.; Peters, D.; Jensen, ML. WO2010106106A1. 2010. (Tetrazoles were recently reported as PAM's of a4b2).

30. Peters, D.; Grunnet, M.; Timmermann, DB.; Eriksen, BL.; Dyhring, T.; Ahring, PK. 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. [PubMed: 16183856]

32. Kuryatov A, Onksen J, Lindstrom J. Mol. Pharmacol. 2008; 74:132. [PubMed: 18381563]

33. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Mol. Pharmacol. 2003; 63:332. [PubMed: 12527804]

34. Wang F, Nelson ME, Kuryatov A, Olale F, Cooper J, Keyser K, Lindstrom J. J. Biol. Chem. 1998; 273:28721. [PubMed: 9786868]

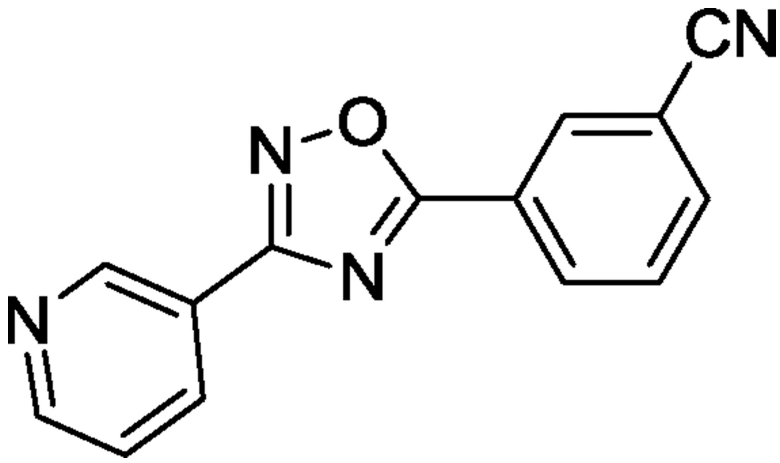
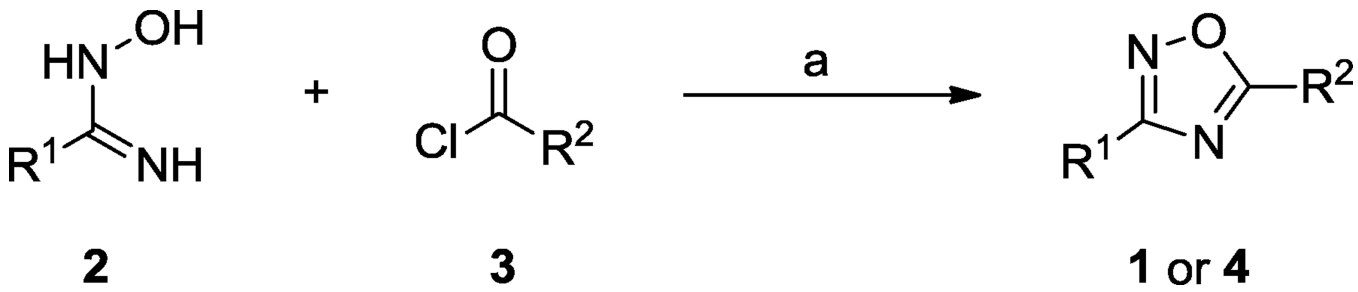
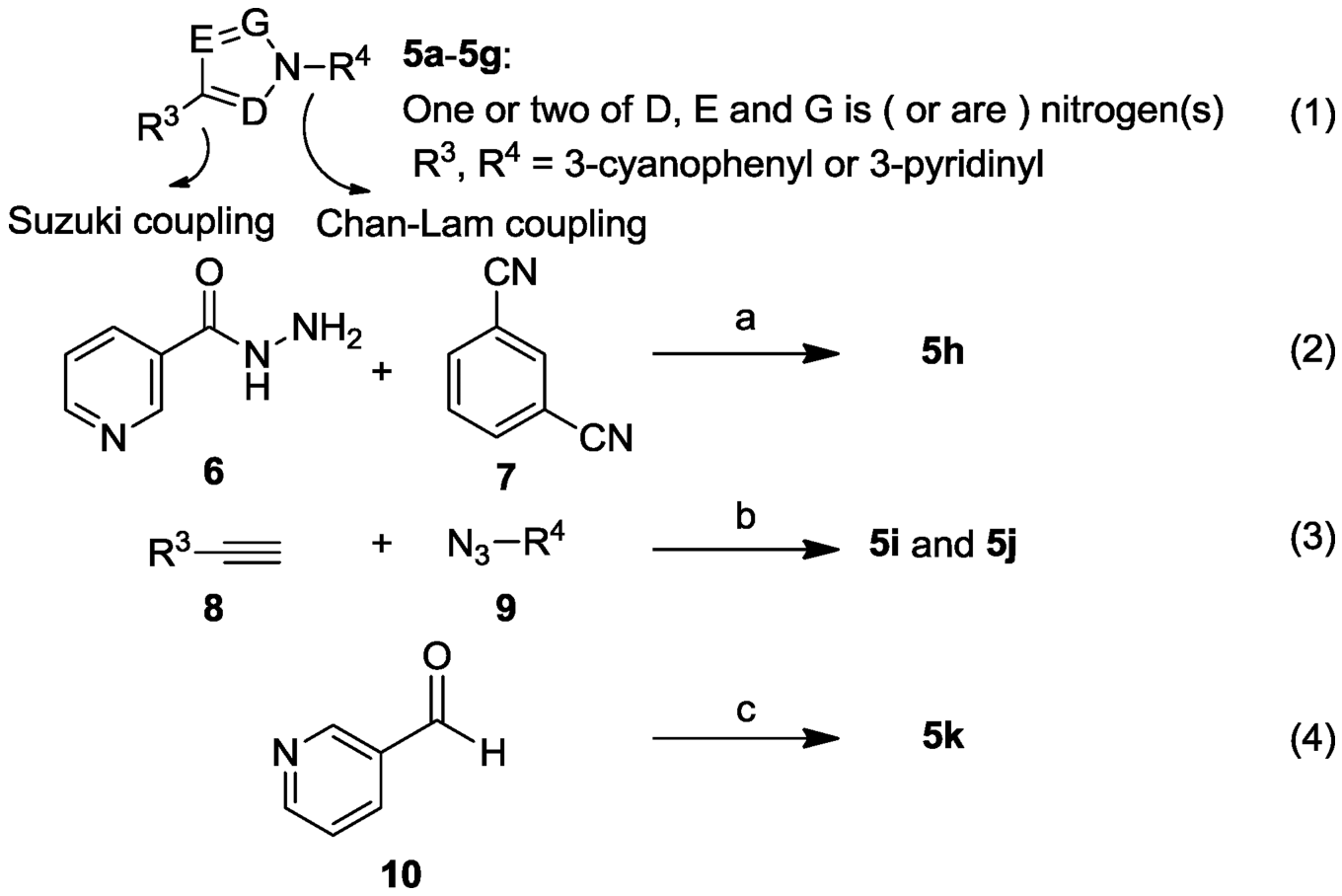


Figure 1.
Structure of NS9283



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.

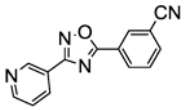
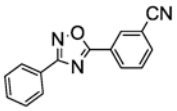
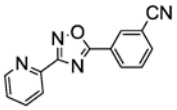
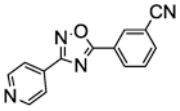
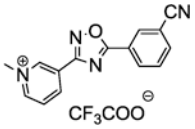


Scheme 2.

Syntheses of **5**. (a) K_2CO_3, *n*-butanol, 150 °C by microwave, 7 h. (b) $CuSO_4$, L-ascorbic acid sodium salt, Et_3N, Methanol-H_2O, rt, overnight. (c) (i) *p*-$CH_3C_6H_4SO_2NHNH_2$, EtOH, rt, 0.5 h; (ii) NaOH, H_2O, −5 °C – 0 °C; (iii) $NaNO_2$, H_2O, HCl, 3-aminobenzonitrile, −5 °C – 0 °C, 0.5 h.

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.

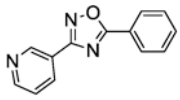
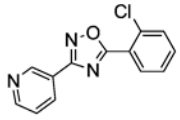
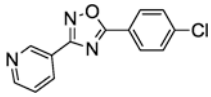
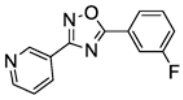
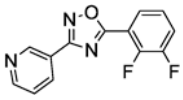
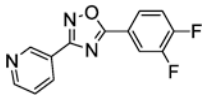
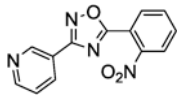
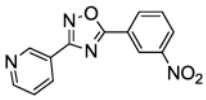
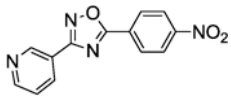
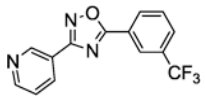
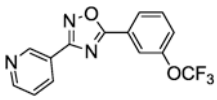
Y (for yes): 1 μM compound potentiates $EC_{20–30}$ nicotine >50%;

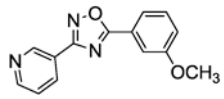
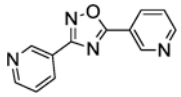
N (for no): 1 μM compound potentiates $EC_{20–30}$ nicotine <50%. Average of n = 2;

[b] Maximum efficacy relative to NS9283.

Table 2

SAR of the 3-cyanophenyl group of NS9283

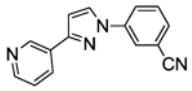
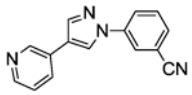
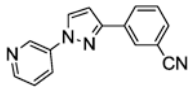
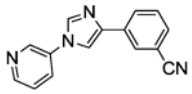
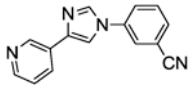
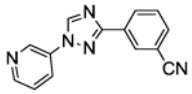
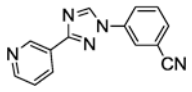
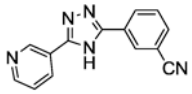
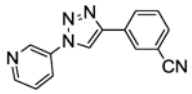
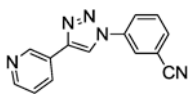
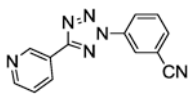
Compound	Structure	PAM(Y or N)[a]	EC_{50} (uM)	E_{max}[b]
4a		N	—	—
4b		N	—	—
4c		N	—	—
4d		N	—	—
4e		N	—	—
4f		Y	1.3±0.4	1.2
4g		N	—	—
4h		Y	0.69±0.35	1.2
4i		Y	1.7±0.5	0.81
4j		N	—	—
4k		N	—	—

Compound	Structure	PAM(Y or N)[a]	EC_{50} (uM)	E_{max} [b]
4l		N	—	—
4m		Y	1.0±0.3	0.98

[a,b] See Table 1 caption.

Table 3

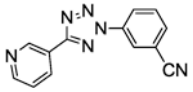
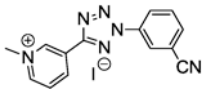
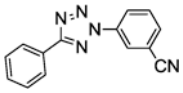
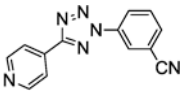
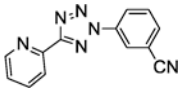
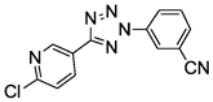
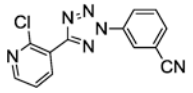
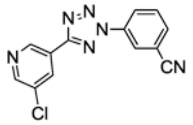
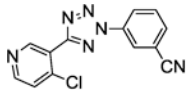
SAR of the 1,2,4-oxadiazole group of NS9283

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.

Table 4

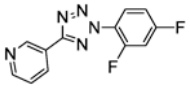
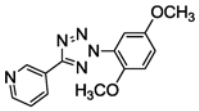
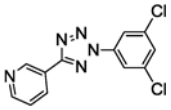
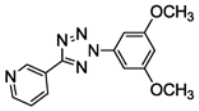
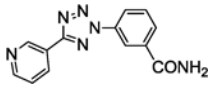
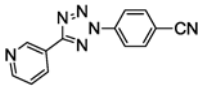
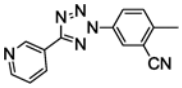
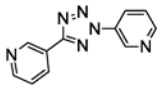
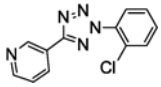
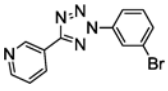
SAR of 2,5-disubstituted tetrazole analogs

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.

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.

Table 6

Pharmacokinetics of **5k** in mice

Cl (mL/min/kg)	$T_{1/2}$ (h)	AUCpo (μM•hr)	Cmax po (μM)	V_d (L/kg)	F%
46	0.9	21	4.1	2.7	100

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