Bioorganic & Medicinal Chemistry Letters 22 (2012) 2380–2384

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Consequences of linker length alteration of the α7 nicotinic acetylcholine receptor (nAChR) agonist, SEN12333

Corinne Beinat [a], Samuel D. Banister [a,b], Saundra van Prehn [b], Munikumar Reddy Doddareddy [c], David Hibbs [c], Michael Sako [c], Mary Chebib [c], Thao Tran [d], Nour Al-Muhtasib [d], Yingxian Xiao [d], Michael Kassiou [a,b,e,*]

[a] School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia
[b] Brain and Mind Research Institute, Sydney, NSW 2050, Australia
[c] School of Pharmacy, The University of Sydney, Sydney, NSW 2006, Australia
[d] Department of Pharmacology and Physiology, Georgetown University, Washington, DC 20057, USA
[e] Discipline of Medical Radiation Sciences, The University of Sydney, Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 23 January 2012
Revised 13 February 2012
Accepted 16 February 2012
Available online 24 February 2012

Keywords:
α7 Nicotinic receptors
Molecular modeling
CNS
Structure–activity relationships

ABSTRACT

A series of ligands based on SEN12333, containing either contracted or elongated alkyl chains, were synthesized and evaluated in molecular docking studies against a homology model of the α7 nicotinic acetylcholine receptor (nAChR) subtype. The predicted binding of all ligands was highly similar, with the exception of the analog containing a 5 methylene unit spacer. However, in vitro competition binding assays revealed that the ligands possessed dissimilar binding affinities, with a K_i range of more than an order of magnitude (K_i = 0.50 to >10 μM), and only SEN12333 itself exhibited functional activity at the α7 nAChR.

Neuronal nicotinic acetylcholine receptors (nAChRs) are a family of ligand-gated cation channels distributed throughout the central and peripheral nervous systems (CNS and PNS, respectively), and have generated much interest as potential therapeutic targets for the treatment of cognitive disorders.[1–3] Multiple nAChR subtypes are known to exist, with each subtype comprising a homo- or heteropentameric combination of twelve possible subunits; α2–α10 and β2–β4. The two most abundant nAChRs in the human brain are the α4β2 and α7 subtypes, the latter distinguished from other subtypes by its unique pharmacology and relatively extreme Ca^{2+} permeability.[4,5]

The α7 subtype has been implicated in schizophrenia, with specific regions of the postmortem brains of schizophrenic patients showing a reduction in α7 nAChR mRNA expression and a concomitantly reduced density of α7 nAChR protein.[6–8] Moreover, polymorphisms within the α7 nAChR subunit gene *CHRNA7* have been linked to auditory gating deficits in schizophrenia, an aberrance that is normalized by nicotine, possibly accounting for the high rate of tobacco use among schizophrenic patients.[9–11] Selective α7 nAChR agonists have shown excellent in vivo efficacy in the normalization of auditory gating in rats, indicating potential utility in the treatment of the cognitive deficits associated with schizophrenia.[12–14]

Reduced expression of α7 nAChR protein has also been observed in the hippocampus of Alzheimer's disease (AD) patients.[15,16] A component of the neuritic plaques that characterize AD and are thought to contribute to neurodegeneration, β-amyloid (Aβ) peptides, were found to interact with α7 nAChRs with picomolar affinity.[17,18] The exogenous nAChR agonist nicotine shows protective effects against the neurotoxicity of Aβ peptides, and this neuroprotection can be blocked by selective α7 nAChR antagonists.[19] Moreover, inhibition of α7 mRNA and protein expression using siRNA transfection exacerbated the toxicity of Aβ peptides in neuroblastoma SH-SY5Y cells.[20] Consistent with the aforementioned findings, selective stimulation of α7 nAChRs was shown to attenuate Aβ-induced cell death, suggesting a therapeutic role for α7 nAChR agonists in the treatment of AD.[19,20]

Although α7 nAChRs represent a promising target for therapeutic intervention in schizophrenia and AD, few structural classes of selective α7 nAChR agonists are known, with reported ligands predominantly based on anabaseine, quinuclidine, or diazabicyclic scaffolds. One of the first functionally α7-selective agents described was the partial agonist, dimethoxybenzilidene anabasein (DMXB-A, **1**, Fig. 1), a natural product derivative with only micromolar potency at α7 nAChRs and off-target activity at α4β2 nAChRs

* Corresponding author.
E-mail address: michael.kassiou@sydney.edu.au (M. Kassiou).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.02.052

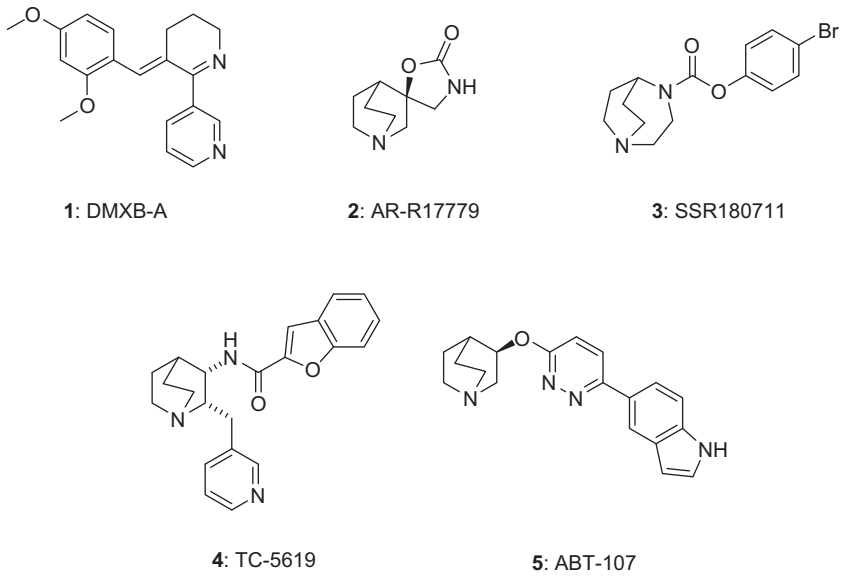


Figure 1. Selected α7 nAChR agonists evaluated in preclinical and clinical studies.

and 5-HT_3 receptors.[21,22] DMXB-A subsequently entered a proof-of-concept trial where it improved neurocognitive measures in non-smoking schizophrenic patients,[23] and has progressed to Phase II studies.[3]

The potential therapeutic applications of α7 agonists have generated much interest within the pharmaceutical industry. An early example disclosed by AstraZeneca was the quinuclidine-derived spiro-oxazolidinone, AR-R17779 (**2**), a potent full agonist that demonstrates several hundred-fold in vitro selectivity for rat α7 over rat α4β2 nAChRs.[24] AR-R17779 has undergone extensive pharmacological profiling in vivo, and improves learning and memory in several rat models, consistent with the anticipated cognition-enhancing effects of selective α7 nAChR agonists.[25,26] However, even minor structural changes to AR-R17779 attenuated α7 affinity, limiting its use as a lead for the further development of α7 agonists.[24]

Sanofi-Aventis has also reported the diazabicyclic SSR180711 (**3**) as a potent partial agonist of recombinant human α7 nAChRs, with greater than 250-fold selectivity over other nAChR subtypes, and negligible affinity for 100 other receptors.[27,28] SSR180711 was able to ameliorate the cognitive deficits induced by repeated phencyclidine administration in mice and, like many α7 nAChR agonists, has shown promise in animal models of the cognitive aspects schizophrenia.[29,30]

Selective α7 nAChR agonists represent promising candidates for the alleviation of cognitive dysfunction in schizophrenia (CDS), and neuroprotection in AD.[31–33] Indeed, many α7 nAChR agonists are progressing through clinical trials and proving efficacious for the treatment of CDS, including TC-5619 (**4**, Phase II), ABT-107 (**5**, Phase I), and MEM-3454 (structure undisclosed, Phase II).[3] However, known α7 ligands display relatively little structural diversity, having been developed through lead optimization of few chemotypes, and most possess cross-reactivity with other sites.

High-throughput screening by Siena Biotech and Wyeth identified piperazine **6** (Fig. 2) as a novel chemotype with weak partial agonist activity at α7 nAChRs,[34] and investigation of the piperazine, biaryl, and amide regions of **6** led to the discovery of SEN12333 (**7**).[35] SEN12333 is a potent and selective α7 nAChR agonist, exhibiting high selectivity for α7 over other nAChR subtypes, 5-HT_3 receptors, and hERG.[35,36] In addition to its promising in vitro profile, **7** also showed reasonable bioavailability and good brain permeation in vivo.[36] Preliminary evaluation of SEN12333 in animal models of episodic memory revealed its ability to reverse both scopolamine- and MK-801-induced amnesia.[35,36]

The development of SEN12333 was focused on improving both potency and drug-like properties. As a result, only limited structure–activity relationships are available for this class of α7 nAChR ligands. Alteration of the morpholine group of **7** revealed that a reasonable diversity of heterocycles was tolerated, with small, aliphatic azacycles being optimal. Similarly, replacement of the arylanilide with other aromatic moieties had little effect on α7 nAChR activity, but biaryls were generally preferred over monoaromatic groups. The butyl chain tethering the morpholine and pyridylanilide groups allows a large degree of conformational freedom, and little is known about the optimal orientation of these two pharmacophic units.

To determine the most favorable distance between the morpholine ring and the pyridylanilide group in SEN12333, analogs of **7** containing contracted or elongated alkyl tethers were synthesized. The desired ligands contained an alkyl linker of 1, 2, 3, or 5 methylene units (**8a–d**, Scheme 1), were synthesized from the corresponding ω-bromoalkanoic acids (**9a–d**, respectively). The commercially available acids were converted to the corresponding acid chlorides using oxalyl chloride, and subsequent treatment with 4-bromoaniline in the presence of a suitable base gave anilides **10a–d** in acceptable yield. Alkylation of morpholine by bromoalkanes **10a–d** was achieved in the presence of catalytic iodide to give compounds **11a–d** in good yields. Subjecting bromoarenes **11a–d** to Suzuki coupling with 3-pyridyl boronic acid gave the desired ligands **8a–d**. SEN12333 (**7**, $n = 4$) was synthesized analogously, starting from commercially available 5-bromopentanoic acid (**9e**), for direct comparison with the new analogs.

Molecular modeling was undertaken to predict possible variation of binding modes for ligands **7** and **8a–d**. Firstly, a homology model of the α7 nAChR was generated by using the 'prime' suite in Maestro.[37] The crystal structure of the acetylcholine binding protein (AChBP)[38] from *L. stagnalis* (PDB code: 1UW6) was used as a template for generating the model. The sequence of α7 nAChR (accession code: AAA83561) as obtained from NCBI was aligned on the template. Five subunits of the α7 nAChR were individually made and merged to form an α7 nAChR pentamaric model. The OPLS_2005 all-atom force field was used for energy scoring of

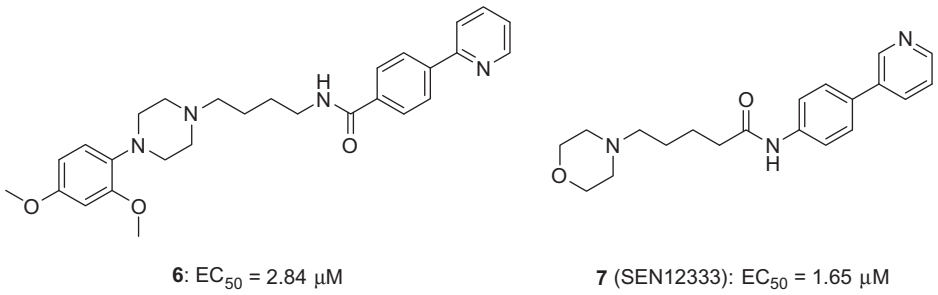
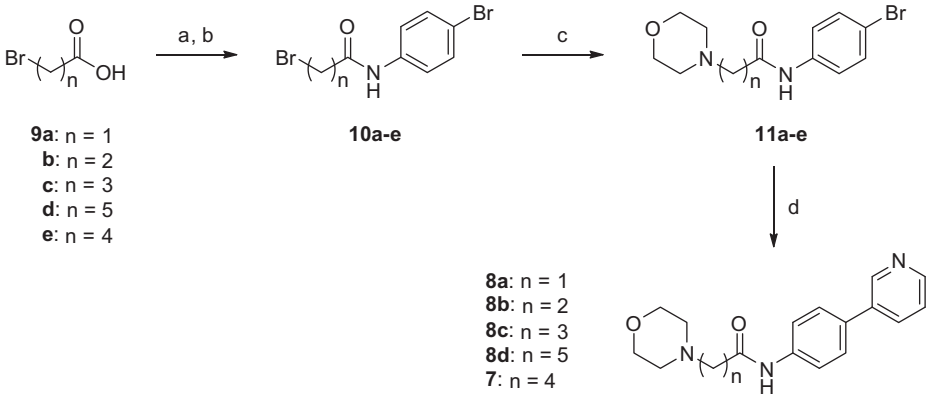


Figure 2. Activity of SEN12333 and its parent structure, **6**, at α7 nAChRs.



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, rt, 45 min; (b) 4-bromoaniline, Et_3N or K_2CO_3, CH_2Cl_2, −78 °C to rt, 1 h, 54–94% over 2 steps; (c) morpholine, NaI, Et_3N, DMF, reflux, 16 h, 64–85%; (d) $Pd(PPh_3)_4$, pyridine-3-boronic acid, MeCN–0.4 M aq Na_2CO_3 (50:50), reflux, 18 h, 67–83%.

the protein, and surface generalized Born (SGB) continuum solvation model for treating solvation energies and effects. The predicted model was then prepared for docking by using protein preparation wizard, wherein hydrogens were added, bond orders assigned, and disulfide bonds created. Finally the corrected structure was optimized by restrained minimization using 'impref minimization' by selecting hydrogens only so that heavy atoms were left untouched.

Docking studies were conducted by using 'Glide' software as provided in Maestro.[39] A docking model was generated by forming a receptor grid around nicotine, the ligand from the template, which was included in the model as a reference for the active site between two adjacent α7 monomers. The ligands under study were minimized using OPLS_2005 forcefield, with water as solvent and 'constant dielectric' as electrostatic treatment, in the Macromodel module of Maestro. Finally they were protonated at pH 7.4 and docked flexibly in to the active site using extra-precision (XP) mode.[40]

To understand the ligand–receptor interactions of α7 nAChR receptor agonists, three known ligands with agonist activity were docked into the α7 nAChR model; AR-R177796, acetylcholine, and nicotine. Interestingly, all three ligands showed comparable interactions with the receptor (Fig. 3). The protonated nitrogen of these ligands was found to make cation–π interactions with one of the five aromatic amino acids that comprise the wall of the hydrophobic pocket (Chain A: Trp77 and Chain B: Tyr115, Trp171, Tyr210, and Tyr217). The other important common feature is that all compounds made a hydrogen bond interaction with hydroxyl group of Ser172 (ChainB). These two interactions seem to be very important for the agonistic activity of α7 nAChR ligands.

Compounds **7** and **8a–d** were docked into the α7 nAChR active site to predict the effect of chain length variation on α7 nAChR activity. As seen in Figure 4, all five ligands docked in a similar way, with the exception of **8d** ($n = 5$), which docked in an alternative orientation to accommodate the elongated chain. The docking poses and the XP[40] docking summary (see Table S1) show that all ligands except **8a** ($n = 1$) make cation–π interactions with the receptor, all but **8d** form a hydrogen bond with Gln139, and none interact with Ser172. The docking results predict an increase in activity within this series when chain length is increased from 4 (SEN12333, **7**) to 5 methylene units (**8d**), as indicated by the G-score increase from −10.59 to −11.13. Compound **8a** ($n = 1$) was predicted to be least active, with a G-score of −0.67, having a relative large, negatively contributing 'penalty' for polar atom burial, desolvation, and intra-ligand contacts.

To confirm the validity of the modeling, **7** and **8a–d** were subjected to competition binding measurements against racemic [^{3}H]epibatidine. The binding assays were performed using membrane homogenates of stably transfected HEK293 cells expressing rat α7, α4β2, or α3β4 nAChR subtype.[41–43] The binding affinities of these analogs are shown in Table 1. Contrary to the predictions of the docking studies, **7** and **8a–d** displayed K_i values ranging from 0.50 μM to greater than 10 μM, a difference of more than two orders of magnitude. The highest affinity was displayed by SEN12333 itself (**5**, $K_i = 0.50$ μM). Extending the distance of the alkyl tether of SEN12333 by a single methylene unit, to give **8d**, resulted in more than an order of magnitude reduction in α7 nAChR binding ($K_i > 10$ μM), contradicting the improved affinity anticipated by the docking studies. Compared to SEN12333, the ethylene- and propylene-linked congeners (**8b** and **8c**) also showed diminished α7 nAChR binding ($K_i > 10$ μM in both cases). However, **8a** ($K_i = 3.9$ μM), containing the shortest linker, exhibited α7 affinity less than 8 times lower than that of SEN12333 itself, despite the contrary predictions of the docking studies.

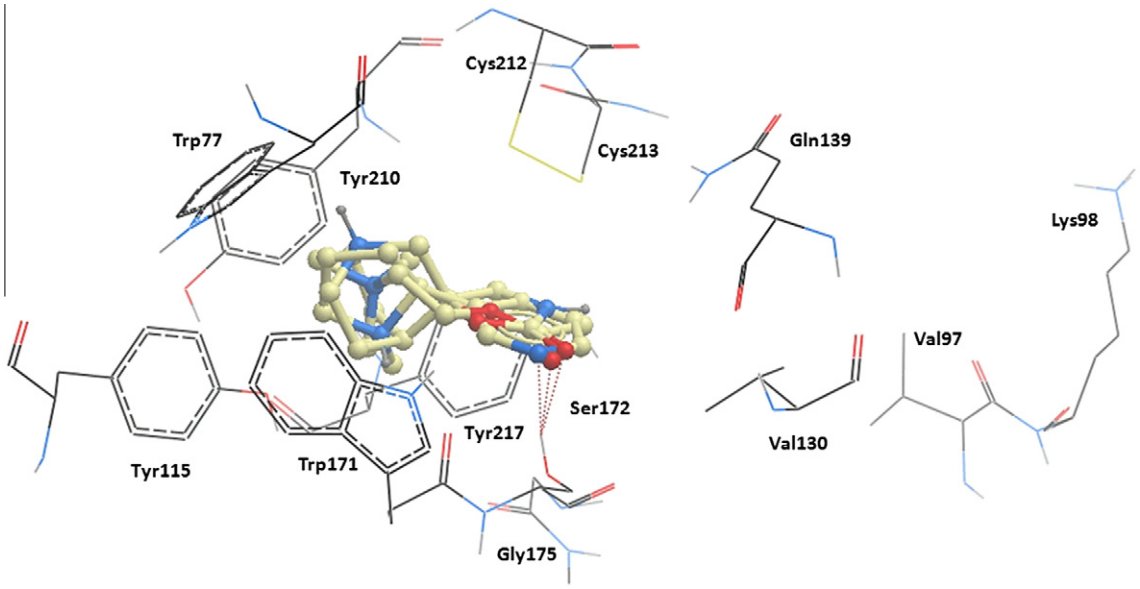


Figure 3. AR-R17779, acetylcholine, and nicotine docked into the active site of the α7 nAChR model. Hydrogen bonding with Ser172 is also shown. Image drawn using ICM browser.

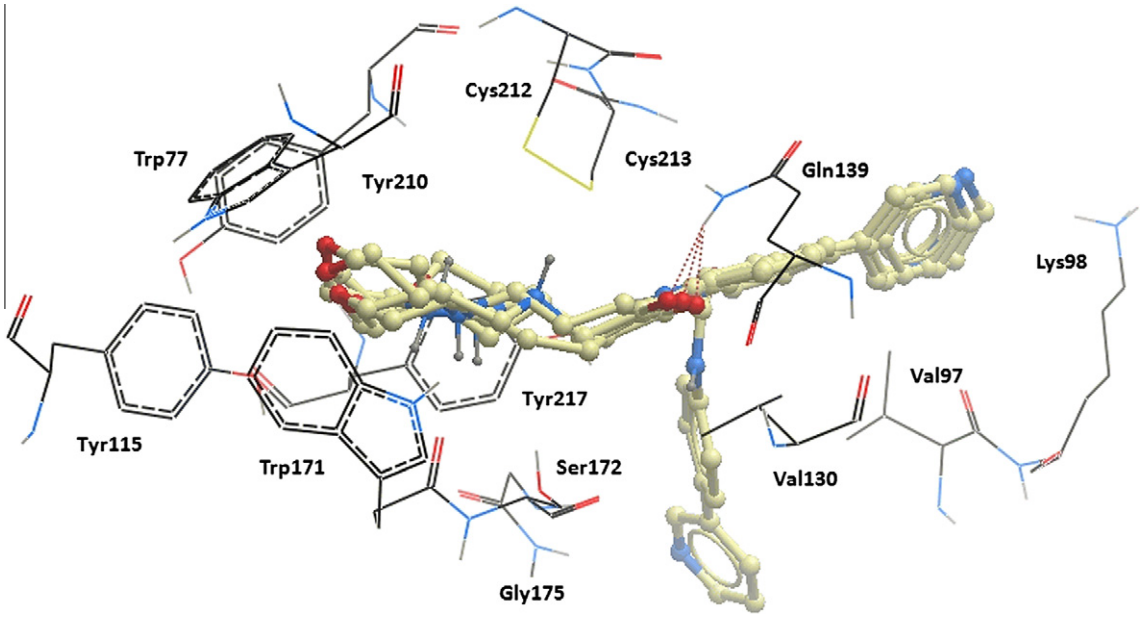


Figure 4. Compounds **7** and **8a–d** docked into the active site of the α7 nAChR model. Hydrogen bonding with Gln139 is also shown. Image drawn using ICM browser.

Table 1
Binding affinities of **7** and **8a–d** for multiple nAChR subtypes

Compound	K_i[a] (μM)		
	α7	α4β2	α3β4
8a	3.9 ± 1.5	>10	>10
8b	>10	>10	>10
8c	>10	>10	>10
8d	>10	>10	>10
7 (SEN12333)	0.50 ± 0.03	>10	>10

[a] K_i values represent the mean ± SEM of three independent experiments.

Compounds **8a–d** and SEN12333 (**7**) were also evaluated on rat recombinant α7 receptors expressed in *Xenopus* oocytes. Only SEN12333 exhibited partial agonist activity, activating the receptor by approximately 11% compared to the maximal ACh response with an EC_{50} of 9.5 μM (95% confidence interval 3.4–26) (Fig. 5). The remaining analogs did not exhibit any agonist or antagonist activity when tested at 100 μM (data not shown).

Contraction and elongation of the alkyl chain in SEN12333 produces distinct changes in α7 affinity, and optimal binding is conferred by a 4 methylene unit linker, as found in SEN12333 itself. However, the variation of binding affinity is not predicted by molecular docking to a α7 nAChR homology model, demonstrating the limited predictive utility of this model for the rational design of α7 receptor agonists. A 4-carbon chain appears to offer ideal interfunctional group distances for interaction with α7 nAChRs, and future work could focus on the functionalization or conformational restriction of this tether.

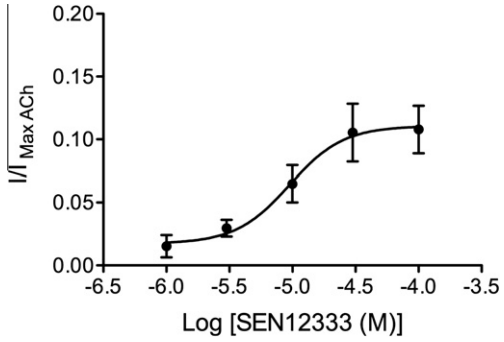


Figure 5. Effect of SEN12333 (**7**) on rat recombinant α7 receptors expressed in *Xenopus* oocytes. SEN12333 (**7**) exhibited partial agonist activity, activating the receptor by approximately 11% compared to the maximal ACh response. The EC_{50} value was 9.5 μM (95% CI 3.4–26). Data are mean ± SEM from 4 oocytes.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.052.

References and notes

1. Broad, L. M.; Sher, E.; Astles, P. C.; Zwart, R.; O'Neill, M. J. *Drugs Future* **2007**, *32*, 161.
2. Dani, J. A.; Bertrand, D. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 699.
3. D'Hoedt, D.; Bertrand, D. *Expert Opin. Ther. Targets* **2009**, *13*, 395.
4. Seguela, P.; Wadiche, J.; Dineley-Miller, K.; Dani, J. A.; Patrick, J. W. *J. Neurosci.* **1993**, *13*, 596.
5. Berg, D. K.; Conroy, W. G. *J. Neurobiol.* **2002**, *53*, 512.
6. Guan, Z.-Z.; Zhang, X.; Blennow, K.; Nordberg, A. *NeuroReport* **1999**, *10*, 1779.
7. Marutle, A.; Zhang, X.; Court, J.; Piggott, M.; Johnson, M.; Perry, R.; Perry, E.; Nordberg, A. *J. Chem. Neuroanat.* **2001**, *22*, 115.
8. Martin-Ruiz, C. M.; Haroutunian, V. H.; Long, P.; Young, A. H.; Davis, K. L.; Perry, E. K.; Court, J. A. *Biol. Psychiatry* **2003**, *54*, 1222.
9. Freedman, R.; Coon, H.; Myles-Worsley, M.; Orr-Urtreger, A.; Olincy, A.; Davis, A.; Polymeropoulos, M.; Holik, J.; Hopkins, J.; Hoff, M.; Rosenthal, J.; Waldo, M. C.; Reimherr, F.; Wender, P.; Yaw, J.; Young, D. A.; Breese, C. R.; Adams, C.; Patterson, D.; Adler, L. E.; Kruglyak, L.; Leonard, S.; Byerley, W. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 587.
10. Leonard, S.; Breese, C.; Adams, C.; Benhammou, K.; Gault, J.; Stevens, K.; Lee, M.; Adler, L.; Olincy, A.; Ross, R.; Freedman, R. *Eur. J. Pharmacol.* **2000**, *393*, 237.
11. De Luca, V.; Wong, A. H. C.; Muller, D. J.; Wong, G. W. H.; Tyndale, R. F.; Kennedy, J. L. *Neuropsychopharmacology* **2004**, *29*, 1522.
12. O'Neill, H.; Rieger, K.; Kem, W.; Stevens, K. *Psychopharmacology* **2003**, *169*, 332.
13. Freedman, R.; Olincy, A.; Buchanan, R. W.; Harris, J. G.; Gold, J. M.; Johnson, L.; Allensworth, D.; Guzman-Bonilla, A.; Clement, B.; Ball, M. P.; Kutnick, J.; Pender, V.; Martin, L. F.; Stevens, K. E.; Wagner, B. D.; Zerbe, G. O.; Soti, F.; Kem, W. R. *Am. J. Psychiatry* **2008**, *165*, 1040.
14. Acker, B. A.; Jacobsen, E. J.; Rogers, B. N.; Wishka, D. G.; Reitz, S. C.; Piotrowski, D. W.; Myers, J. K.; Wolfe, M. L.; Groppi, V. E.; Thornburgh, B. A.; Tinholt, P. M.; Walters, R. R.; Olson, B. A.; Fitzgerald, L.; Staton, B. A.; Raub, T. J.; Krause, M.; Li, K. S.; Hoffmann, W. E.; Hajos, M.; Hurst, R. S.; Walker, D. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3611.
15. Guan, Z.-Z.; Zhang, X.; Ravid, R.; Nordberg, A. *J. Neurochem.* **2000**, *74*, 237.
16. Mousavi, M.; Hellström-Lindahl, E.; Guan, Z. Z.; Shan, K. R.; Ravid, R.; Nordberg, A. *Neuroscience* **2003**, *122*, 515.
17. Wang, H.-Y.; Lee, D. H. S.; D'Andrea, M. R.; Peterson, P. A.; Shank, R. P.; Reitz, A. B. *J. Biol. Chem.* **2000**, *275*, 5626.
18. Wang, H.-Y.; Lee, D. H. S.; Davis, C. B.; Shank, R. P. *J. Neurochem.* **2000**, *75*, 1155.
19. Kihara, T.; Shimohama, S.; Sawada, H.; Kimura, J.; Kume, T.; Kochiyama, H.; Maeda, T.; Akaike, A. *Ann. Neurol.* **1997**, *42*, 159.
20. Qi, X.-L.; Nordberg, A.; Xiu, J.; Guan, Z.-Z. *Neurochem. Int.* **2007**, *51*, 377.
21. Briggs, C. A.; Anderson, D. J.; Brioni, J. D.; Buccafusco, J. J.; Buckley, M. J.; Campbell, J. E.; Decker, M. W.; Donnelly-Roberts, D.; Elliott, R. L.; Gopalakrishnan, M.; Holladay, M. W.; Hui, Y. H.; Jackson, W. J.; Kim, D. J.; Marsh, K. C.; O'Neill, A.; Prendergast, M. A.; Ryther, K. B.; Sullivan, J. P.; Arneric, S. P. *Pharmacol. Biochem. Behav.* **1997**, *57*, 231.
22. Kem, W.; Soti, F.; Wildeboer, K.; LeFrancois, S.; MacDougall, K.; Wei, D.-Q.; Chou, K.-C.; Arias, H. R. *Mar. Drugs* **2006**, *4*, 255.
23. Olincy, A.; Harris, J. G.; Johnson, L. L.; Pender, V.; Kongs, S.; Allensworth, D.; Ellis, J.; Zerbe, G. O.; Leonard, S.; Stevens, K. E.; Stevens, J. O.; Martin, L.; Adler, L. E.; Soti, F.; Kem, W. R.; Freedman, R. *Arch. Gen. Psychiatry* **2006**, *63*, 630.
24. Mullen, G.; Napier, J.; Balestra, M.; DeCory, T.; Hale, G.; Macor, J.; Mack, R.; Loch, J.; Wu, E.; Kover, A.; Verhoest, P.; Sampognaro, A.; Phillips, E.; Zhu, Y.; Murray, R.; Griffith, R.; Blosser, J.; Gurley, D.; Machulskis, A.; Zongrone, J.; Rosen, A.; Gordon, J. *J. Med. Chem.* **2000**, *43*, 4045.
25. Levin, E. D.; Bettegowda, C.; Blosser, J.; Gordon, J. *Behav. Pharmacol.* **1999**, *10*, 675.
26. van Kampen, M.; Selbach, K.; Schneider, R.; Schiegel, E.; Boess, F.; Schreiber, R. *Psychopharmacology (Berl.)* **2004**, *172*, 375.
27. Biton, B.; Bergis, O. E.; Galli, F.; Nedelec, A.; Lochead, A. W.; Jegham, S.; Godet, D.; Lanneau, C.; Santamaria, R.; Chesney, F.; Léonardon, J.; Granger, P.; Debono, M. W.; Bohme, G. A.; Sgard, F. *Neuropsychopharmacology* **2007**, *32*, 1.
28. Pichat, P.; Bergis, O. E.; Terranova, J.-P.; Urani, A.; Duarte, C.; Santucci, V.; Gueudet, C.; Voltz, C.; Steinberg, R.; Stemmelin, J.; Oury-Donat, F.; Avenet, P.; Griebel, G.; Scatton, B. *Neuropsychopharmacology* **2007**, *32*, 17.
29. Hashimoto, K.; Ishima, T.; Fujita, Y.; Matsuo, M.; Kobashi, T.; Takahagi, M.; Tsukada, H.; Iyo, M. *Biol. Psychiatry* **2008**, *63*, 92.
30. Barak, S.; Arad, M.; De Levie, A.; Black, M. D.; Griebel, G.; Weiner, I. *Neuropsychopharmacology* **2009**, *34*, 1753.
31. Martin, L. F.; Kem, W. R.; Freedman, R. *Psychopharmacology* **2004**, *174*, 54.
32. Martin, L. F.; Freedman, R. In *International Review of Neurobiology*; Anissa, A. D., Olivier, G., Eds.; Academic Press, 2007; Vol. 78, pp 225–246.
33. Toyohara, J.; Hashimoto, K. *Open Med. Chem. J.* **2010**, *4*, 37.
34. Gaviraghi, G.; Ghiron, C.; Bothmann, H.; Roncarati, R.; Terstappen, G. C. PCT Int. Appl. WO2006008133A2, 2006.
35. Haydar, S. N.; Ghiron, C.; Bettinetti, L.; Bothmann, H.; Comery, T. A.; Dunlop, J.; La Rosa, S.; Micco, I.; Pollastrini, M.; Quinn, J.; Roncarati, R.; Scali, C.; Valacchi, M.; Varrone, M.; Zanaletti, R. *Bioorg. Med. Chem.* **2009**, *17*, 5247.
36. Roncarati, R.; Scali, C.; Comery, T. A.; Grauer, S. M.; Aschmi, S.; Bothmann, H.; Jow, B.; Kowal, D.; Gianfriddo, M.; Kelley, C.; Zanelli, U.; Ghiron, C.; Haydar, S.; Dunlop, J.; Terstappen, G. C. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 459.
37. Prime, version 2.2, Schrödinger, LLC, New York, NY, 2009.
38. Celie, P. H. N.; van Rossum-Fikkert, S. E.; van Dijk, W. J.; Brejc, K.; Smit, A. B.; Sixma, T. K. *Neuron* **2004**, *41*, 907.
39. Glide, version 5.6, Schrödinger, LLC, New York, NY, 2009.
40. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. *J. Med. Chem.* **2006**, *49*, 6177.
41. Xiao, Y.; Kellar, K. J. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 98.
42. Xiao, Y.; Baydyuk, M.; Wang, H.; Davis, H. E.; Kellar, K. J. *Bioorg. Med. Chem. Lett.* **1845**, *2004*, 14.
43. Xiao, Y.; Abdrakhmanova, G. R.; Baydyuk, M.; Hernandez, S.; Kellar, K. J. *Acta Pharmacol. Sin.* **2009**, *30*, 842.