DOI: 10.1002/cmdc.200700073

Chemical Medicine: Novel 10-Substituted Cytisine Derivatives with Increased Selectivity for α4β2 Nicotinic Acetylcholine Receptors

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Neuronal nicotinic acetylcholine receptors (nAChR) are widely distributed in the central and peripheral nervous systems.[1–4] These receptors are crucial to many normal physiological functions, and they are involved in a wide range of diseases.[5–7] For these reasons nAChRs are important targets for drug discovery.[8–10]

Neuronal nAChRs are composed of subunits (α2-α10, β2-β4) that form pentameric ligand-gated cation channels.[11] Different combinations of subunits define the nAChR subtypes.[12,13] Among all heteromeric nAChR subtypes in the vertebrate brain, the α4β2 receptor is the predominant subtype.[14–16] In contrast, the α3β4 nAChR is the major subtype in ganglia.[17–19] The α4β2 receptors are involved in important brain functions, including memory, cognition, arousal, relaxation, and pleasure.[2,15,20,21] Several lines of evidence suggest that the α4β2 nAChRs mediate addiction to nicotine in tobacco smoking.[20–22]

In addition to acetylcholine and (−)-nicotine (**1**), many nAChR ligands have been discovered from natural sources, including epibatidine (**2**) and (−)-cytisine (**3**). The structures of nicotine, epibatidine, cytisine, and other natural nicotinic ligands have led to the development of a host of other synthetic compounds that act on neuronal nicotinic receptors.[8,10] Although many of the synthetic analogues of these natural ligands exhibit excellent overall selectivity to neuronal nAChRs over other families of receptors, the development of ligands highly selective for a certain nAChR subtype over other subtypes has been slow because of the large number of nAChR subtypes together with the relatively subtle differences in their structures.

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(−)-Cytisine (**3**) is a plant alkaloid isolated originally from the gold chain tree (*Laburnum anagyroides*).[23] This ligand binds to α4β2 nAChRs with a much higher affinity than it binds to α3β4 receptors in in vitro ligand binding assays.[24–27] However, when its effects on nAChR channel function were measured in heterologous expression models, cytisine is a full or nearly full agonist at α3β4 nAChRs but a partial agonist at β2-containing receptors, stimulating less than 50% of the maximal response elicited by acetylcholine or nicotine.[28–33] Cytisine has been marketed in central and eastern Europe for aiding smoking cessation for more than 40 years though its effectiveness and safety have not been conclusively established through randomized, double-blind, placebo-controlled clinical trials.[34–36] As cytisine shows poor penetration of the blood-brain barrier this may be one of the reasons that cytisine failed to show a robust effect in the clinic.[37,38] In spite of these clinical shortcomings, the interesting pharmacological profile of cytisine has led to a number of medicinal chemistry efforts in recent years to create analogues with an improved subtype selectivity or PK parameters.[8,10,39–42] Most of these reported structural modifications involve the introduction of substituents at the alicyclic nitrogen (position 3), or at the 9- or 11- positions of the pyridone ring (Figure 1).

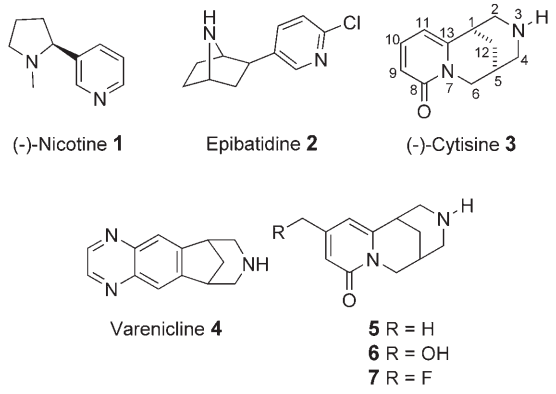


Figure 1. nAChR ligands.

Varenicline (**4**), a partial α4β2 nAChR agonist developed through synthetic efforts that used cytisine as the starting template, has been marketed as a smoking cessation drug since August 2006.[43–46] Much like cytisine, varenicline also acts as nearly a full agonist at α3β4 nAChRs.[47] In randomized, double-blind, placebo-controlled clinical trials, 23% of participants in the varenicline group were continuously abstinent at 52 weeks after starting on a 12-week treatment, compared with 10.3% in the placebo group and 14.6% in a bupropion group.[48] Though varenicline was generally well tolerated during the 12-week treatment, 29.4% of participants reported nausea. These and other clinical trials demonstrate that varenicline is reasonably efficacious for smoking cessation and that it has an acceptable safety profile. On the other hand, these data also show the need for developing improved smoking cessation drugs with higher efficacy and a better safety profile.[49]

We recently reported the first synthesis of two cytisine derivatives (**5** and **6**) bearing substitution at the 10-position of the pyridone ring.[50] These two cytisine analogues showed much higher selectivity for the $\alpha4\beta2$ nAChR subtype in binding assays than cytisine. In continuation of our efforts to further improve upon subtype selectivity and, in particular, to find ligands with improved Clog*P* values to facilitate brain entry, we expanded the structure activity relationship (SAR) study of these 10-substituted cytisine derivatives. Herein we report preliminary molecular modeling studies, synthesis, and pharmacological properties of the new cytisine derivatives *rac*-**7** and *rac*-**16**–**23**.

At the start of this study, we carried out preliminary modeling investigations to explore the type of structural modifications that could be made to cytisine (**3**) that would be compatible with its predicted binding site. Nicotine, epibatidine, and (−)-cytisine were docked individually to the binding site of $\alpha4\beta2$ and $\alpha3\beta4$ using GOLD docking software.[51–58] GOLDScore was used as a fitness function and all docking parameters were set to default values. Homology models of the rat $\alpha3\beta4$ (PDB entry 1OLJ) and $\alpha4\beta2$ (PDB entry 1OLE) nicotinic receptors used for the docking studies have been published by Le Novere et al.[59] The models were built using the model of the chick $\alpha7$-pentamer as the template, whereas the $\alpha7$-pentamer was created from the X-ray structure of AChBP.[59,60] Docking and visualization were performed on a RedHat EL WS 4.0 Linux workstation.

In both proteins $\alpha4\beta2$ and $\alpha3\beta4$, the docking poses of nicotine, epibatine, and (−)-cytisine (Figure 2a, only the docked pose of cytisine in $\alpha4\beta2$ is shown) are similar to those reported earlier by us[61] and other research groups[59,62,63] and are consistent with X-ray findings.[1] Figure 2b shows that C10 in (−)-cytisine is close to the opening of the binding site. C10 is also positioned so that it can be modified with an additional substituent without clashing with the binding site residues. The distance between C10 of cytisine and the gorge located on the outer surface of the $\alpha4$ and $\beta2$ subunits varies between approximately 7 Å near Cys 190 and Phe 117 and 10 Å near Glu 193. The amino acid residues closest to the C10 position of cytisine are the protonated amino group of Lys 77 and isopropyl group of Val 109 (Figure 2a).

The overall geometries of the $\alpha4\beta2$ and $\alpha3\beta4$ binding sites were found to be suitable for the introduction of either small substituents able to fit within the cavity or longer, flexible substituents able to turn towards the gorge region (shown in Figure 2b) and thereby avoid unfavorable clashes with the binding site. We also found that bulky groups attached in a rigid fashion to the pyridone ring of 3 were likely to clash with the binding site. On the other hand, C10 substituents that are long enough to reach the surface of the protein may actually contain large end groups, as these may bind to the surface of the protein. Such end group modifications may possibly be used to further improve the activity and selectivity of the ligands. Interestingly, a similar narrow channel connecting the ligands with the protein surface was found near C4 of (−)-cytisine. On the basis of these findings, ligands containing either flexible acyclic alkyl substituents or flexible substitutents with a bulky end group were suggested for further synthesis.

To synthesize precursors **12** and **13** of *rac*-**7** and *rac*-**16**–**23**, we followed the synthetic route summarized in Scheme 1 for synthesizing the precursors **12** and **13**.[50] Starting from *rac*-**6** as starting material, we synthesized several alkyl and benzyl ether derivatives by functionalization of the hydroxyl group together with the possible introduction of substitution at the alicyclic nitrogen (Scheme 2–3). Thus, the propyl ether derivative **16** was prepared by reaction of allyl bromide with the *N*-benzyl cytisine derivative **12** to yield the *O*-allyl ether derivative **15**. Hydrogenation followed by *N*-Boc deprotection using trifluoroacetic acid (TFA) gave the 10-propyloxymethyl cytisine analog **16**.

Following the steps of Scheme 3, we synthesized cyclohexylmethyl and benzyl ether derivatives **17**–**20** starting from **13**. N-alkylation of *rac*-**17** and *rac*-**18** gave the N-substituted cytisine derivatives **21**–**23**. The conversion of the hydroxymethyl group to fluoromethyl *rac*-**7**, was achieved using diethylaminosulphurtrifluoride (DAST). The chemistry used for the syntheses of these compounds was efficient and straightforward; the products were obtained in good yields. The best binding poses of the synthesized ligands were predicted by GOLDScore and were found to be consistent with the orientation of cytisine shown in Figure 2. The correlation between the experimental K_i values and the docking scores for the $\alpha3\beta4$ and $\alpha4\beta2$ nAChRs was poor (not shown), which is not surprising for docking based on homology models. It is well accepted that homology models are generally more useful in guiding synthetic chemistry efforts than for making high quality affinity predictions.

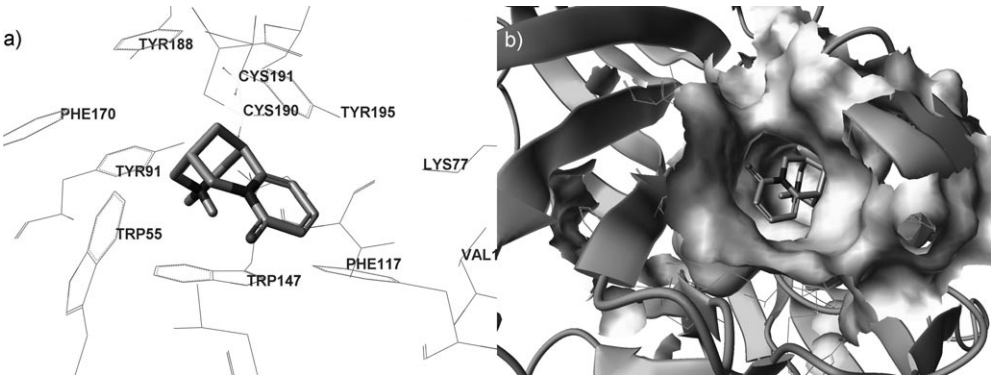
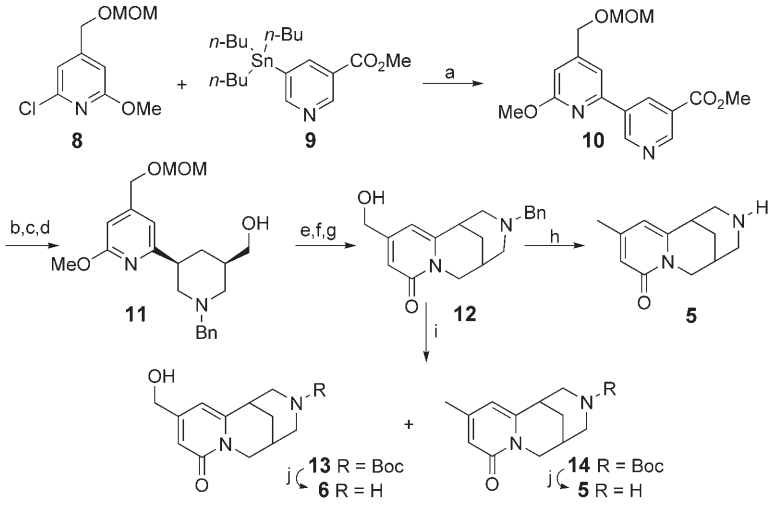
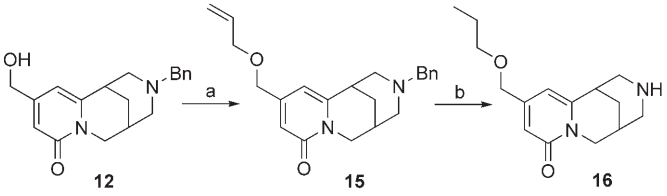


Figure 2. (−)-Cytisine docked to its calculated binding site in $\alpha4\beta2$. a) The amino acids located within a 6 Å radius of the $\alpha4\beta2$ nAChR subtype of the docked ligands; b) solvent accessible area in the binding site of $\alpha4\beta2$.

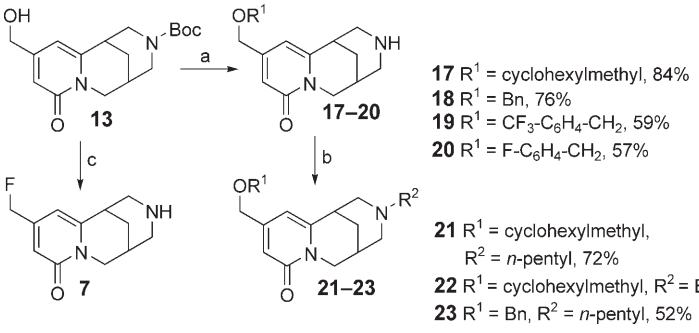
In vitro binding affinities (K_i values) of nine new cytisine C10 analogues **7** and **16**–**23** were measured at six defined nAChR subtypes expressed in stably transfected cell lines, as well as at native nAChRs expressed in



Scheme 1. Reagents and conditions: a) $Pd(PPh_3)_4$, DMF, 130 °C, 15 h, 79%; b) $LiAlH_4$, THF, −20 °C, 3.5 h, 59%; c) BnBr, CH_3CN, reflux, 2 h; d) H_2 (1 atm), PtO_2, Et_3N, MeOH, RT, 15 h, *cis*:*trans* = 5:1; *cis*, 67%; e) MsCl, Et_3N, DCM, 0 °C, 30 min, 84%; f) Toluene, reflux, 3 h, 83%; g) TFA, RT, 3 h, 91%; h) H_2 (1 atm), 10% Pd-C (1 equiv *w*/*w*), MeOH, RT, 15 h; i) H_2 (1 atm), 20% $Pd(OH)_2$-C (0.1 equiv), $(Boc)_2O$, MeOH, reflux, 30 min, 92%; j) TFA, CH_2Cl_2, RT, 1 h, 87–93%.



Scheme 2. Reagents and conditions: a) NaH, TBAI, allyl bromide, DMF, 0 °C–RT, 3.5 h, 97%; b) 1. H_2 (1 atm), 20% Pd $(OH)_2$-C, $(Boc)_2O$, MeOH, 72 °C, 5 min., 2. TFA, DCM, RT, 69%.



Scheme 3. Reagents and conditions: a) 1. NaH, TBAI, cyclohexylmethyl bromide or benzyl bromide, DMF, 0 °C–RT, 2. TFA, DCM, RT, (76–84%); b) 1-bromo pentane or ethyl bromide, acetone, reflux, 16 h, 52–72%; c) 1. DAST, DCM, −78 °C to RT, 3 h, 2. TFA, DCM, RT, 61%.

rat forebrain, which are mainly α4β2 receptors. As shown in Table 1, compounds **7**, **16**–**20** showed high binding affinities (with low nM K_i values) to α4β2 nAChRs. The three N-substituted analogues, **21**–**23**, however, showed markedly reduced binding affinities to the α4β2 receptors. All nine new analogues showed very low binding affinities (with μM K_i values) to α3β4 nAChRs. Compared to that of cytisine, compounds **7** and **17**–**20** had much higher selectivity for the α4β2 nAChR subtype over the α3β4 subtype. It is worth to note that three new analogues, **7**, **19**, and **20**, showed even higher selectivity for the α4β2 subtype over the α3β4 subtype than that of varenicline (**4**). To the best of our knowledge, the 6400-fold selectivity for α4β2 nAChRs over the α3β4 subtype in binding assays, makes the 4-CF_3 substituted benzyl ether derivative **19** the most selective cytisine derivative that has been reported to date. We note here that this high degree of subtype selectivity was not predicted from the initial modeling studies, which may reflect the poor quality of the model used for the α3β4 subtype.

As shown in Table 1, all nine new cytisine derivatives possess higher calculated Clog*P* values. Therefore, the five highly selective ligands, **7** and **17**–**20**, may be able to penetrate the blood-brain-barrier better than (−)-cytisine does.

Though they have high binding affinities to the α4β2 nAChRs, the five new selective cytisine derivatives, **7** and **17**–**20**, did not show any agonist activity at the α4β2 nAChRs in preliminary experiments. When applied simultaneously with nicotine to cells expressing α4β2 receptors, these ligands showed very low potencies in inhibiting channel activation by nicotine. Similarly, when tested at the α3β4 subtype, these cytisine analogues showed no agonist activity and very low potencies in inhibiting nicotine stimulated channel activation.

Interestingly, the in vitro pharmacological properties of compounds **7** and **17**–**20** are similar to those of sazetidine-A, a silent desensitizer of α4β2 nAChRs.[65] Sazetidine-A has very high affinity to α4β2 nAChRs in binding assays. However, in functional assays in vitro, sazetidine-A neither activates the channel function of nAChRs when applied alone nor inhibits nAChR channel function when applied simultaneously with nicotine, but sazetidine-A does block α4β2 nAChR channel function very potently when it is preincubated for 10 min with nAChRs. In fact, under these conditions, it is one of the most potent inhibitors of heteromeric nAChR that we have studied. It is likely that a silent desensitizer of the α4β2 nAChRs, such as sazetidine-A, may exhibit some in vivo effects that are similar to those caused by nicotine.[65] It will thus be valuable to test these cytisine analogues for their ability to desensitize the α4β2 nAChRs in cell models.

In summary, preliminary modeling studies, the chemical synthesis, and the SAR of nine new 10-substituted cytisine analogues have been described. The fluoromethyl derivative *rac*-**7** and the cyclohexylmethyl and benzyl ether derivatives *rac*-**17**–**20** were found to exhibit better pharmacological profiles than that of the parent natural product. Compared to (−)-cytisine, these ligands have similar or slightly lower binding affinities for the α4β2 nAChRs. However, their binding affinities for the α3β4 subtype are at least 30-fold lower than that of cytisine. Thus, these analogues are

Table 1. Comparison of binding affinities at nAChR subtypes and calculated lipophilicities of cytisine C10 analogues to those of (−)-cytisine, (−)-nicotine, and varenicline.[a]

Compd.	K_i [nM][b]							Selectivity	Lipophilicity
	α2β2	α2β4	α3β2	α3β4	α4β2	α4β4	Forebrain	(K_i Ratio:α3β4/α4β2)	(Calculated Clog*P*)[64]
rac-**5**[20]	7.5	180	540	6700	1.9	38	20	3500	1.15
rac-**6**[20]	32	300	467	10,000	11	68	38	910	−0.32
rac-**7**	2.2	200	250	8200	3.2	35	11	2600	1.09
rac-**16**	13	130	1300	3800	48	34	110	79	1.36
rac-**17**	28	610	3600	25 000	33	130	110	760	3.14
rac-**18**	18	270	790	12 000	15	39	38	800	2.09
rac-**19**	14	680	1300	43 000	6.7	100	65	6400	3.05
rac-**20**	14	350	950	23 000	5.9	210	46	3900	2.29
rac-**21**	1700	20 000	23 000	91 000	2100	2100	8200	43	5.32
rac-**22**	440	11 000	7400	10 000	520	1400	3100	19	3.84
rac-**23**	630	6400	14 000	25 000	330	2100	1000	76	4.26
(−)-Nicotine (**1**)[c]	12	110	47	440	10	40	12	44	1.00
(−)Cytisine (**3**)[c]	1.1	5.4	37	220	1.5	2.1	1.9	150	0.60
Varenicline (**4**)[c]				83	0.11			760	1.03

[a] Competition binding assays were carried out in membrane homogenates of stably transfected cells or rat forebrain tissue as described previously.[27] The nAChRs were labeled with [^{3}H] epibatidine. [b] The K_d values of [^{3}H]-epibatidine used for calculating K_i values were 0.02 for α2β2, 0.08 for α2β4, 0.03 for α3β2, 0.3 for α3β4, 0.04 for α4β2, 0.09 for α4β4, and 0.05 for rat forebrain. [c] K_i values of *rac*-**5**, *rac*-**6**, (−)-nicotine, (−)-cytisine, and varenicline from previous publications are shown here for comparison.[27,46,50]

much more selective for the α4β2 nAChRs over the α3β4 receptors than cytisine. Despite their high affinities for the α4β2 nAChRs, they do not have any agonist activity at this receptor subtype, nor do they show agonist activity at the α3β4 receptors. Furthermore, these compounds showed very low potencies in inhibiting nicotine activated channel function at both nAChR subtypes. Taken together, the overall in vitro pharmacological properties of these cytisine analogues indicate that these highly selective nicotinic ligands may prove to be members of a new class of nicotinic ligands, the silent desensitizers, which we discovered recently.[65] We anticipate that some of these new cytisine analogues may prove to be safe and efficacious therapeutics for treating CNS diseases, including aiding smoking cessation. Psychogenics Inc. analytical system reveals compound **18** to possess therapeutic potential for psychiatric disorders.

Experimental Section

For a detailed experimental procedure, Please see the Supporting Information.

Acknowledgements

We thank National Institute of Health (Grant R01 DA017980) for their support of this work.

Keywords: α4β2 receptor · natural products · nicotinic ligands · smoking cessation · subtype selectivity

[1] A. Karlin, *Nat. Rev. Neurosci.* **2002**, *3*, 102.
[2] K. J. Kellar, M. I. Davila-Garcia, Y. Xiao, *Nicotine Tob. Res.* **1999**, *1*(Suppl 2), S117.
[3] N. Le Novère, P. J. Corringer, J. P. Changeux, *J. Neurobiol.* **2002**, *53*, 447.
[4] R. J. Lukas, J. P. Changeux, N. Le Novere, E. X. Albuquerque, D. J. Balfour, D. K. Berg, D. Bertrand, V. A. Chiappinelli, P. B. Clarke, A. C. Collins, J. A. Dani, S. R. Grady, K. J. Kellar, J. M. Lindstrom, M. J. Marks, M. Quik, P. W. Taylor, S. Wonnacott, *Pharmacol. Rev.* **1999**, *51*, 397.
[5] J. A. Dani, D. Bertrand, *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 699.
[6] E. D. Levin, F. J. McClernon, A. H. Rezvani, *Psychopharmacology* **2006**, *184*, 523.
[7] J. Lindstrom, *Mol. Neurobiol.* **1997**, *15*, 193.
[8] J. W. Daly, *Cell. Mol. Neurobiol.* **2005**, *25*, 513.
[9] C. Gotti, M. Zoli, F. Clementi, *Trends Pharmacol. Sci.* **2006**, *27*, 482.
[10] A. A. Jensen, B. Frolund, T. Liljefors, P. Krogsgaard-Larsen, *J. Med. Chem.* **2005**, *48*, 4705.
[11] N. Unwin, *J. Mol. Biol.* **2005**, *346*, 967.
[12] C. Gotti, L. Riganti, S. Vailati, F. Clementi, *Curr. Pharm. Des.* **2006**, *12*, 407.
[13] N. S. Millar, *Biochem. Soc. Trans.* **2003**, *31*, 869.
[14] C. M. Flores, S. W. Rogers, L. A. Pabreza, B. B. Wolfe, K. J. Kellar, *Mol. Pharmacol.* **1992**, *41*, 31.
[15] D. Paterson, A. Nordberg, *Prog .Neurobiol.* **2000**, *61*, 75.
[16] P. Whiting, J. Lindstrom, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 595.
[17] C. M. Flores, R. M. DeCamp, S. Kilo, S. W. Rogers, K. M. Hargreaves, *J. Neurosci.* **1996**, *16*, 7892.
[18] V. I. Skok, *Auton. Neurosci.* **2002**, *97*, 1.
[19] N. Wang, A. Orr-Urtreger, A. D. Korczyn, *Prog. Neurobiol.* **2002**, *68*, 341.
[20] L. M. Marubio, A. M. Gardier, S. Durier, D. David, R. Klink, M. M. Arroyo-Jimenez, J. M. McIntosh, F. Rossi, N. Champtiaux, M. Zoli, J. P. Changeux, *Eur. J. Neurosci.* **2003**, *17*, 1329.
[21] A. R. Tapper, S. L. McKinney, R. Nashmi, J. Schwarz, P. Deshpande, C. Labarca, P. Whiteaker, M. J. Marks, A. C. Collins, H. A. Lester, *Science* **2004**, *306*, 1029.
[22] M. R. Picciotto, M. Zoli, R. Rimondini, C. Lena, L. M. Marubio, E. M. Pich, K. Fuxe, J. P. Changeux, *Nature* **1998**, *391*, 173.
[23] H. R. Ing, *J. Chem. Soc.* **1932**, 2778.
[24] L. A. Pabreza, S. Dhawan, K. J. Kellar, *Mol. Pharmacol.* **1991**, *39*, 9.
[25] C. Romano, A. Goldstein, *Science* **1980**, *210*, 647.
[26] R. D. Schwartz, K. J. Kellar, *Science* **1983**, *220*, 214.
[27] Y. Xiao, K. J. Kellar, *J. Pharmacol. Exp. Ther.* **2004**, *310*, 98.
[28] L. E. Chavez-Noriega, A. Gillespie, K. A. Stauderman, J. H. Crona, B. O. Claeps, K. J. Elliott, R. T. Reid, T. S. Rao, G. Velicelebi, M. M. Harpold, E. C. Johnson, J. Corey-Naeve, *Neuropharmacology* **2000**, *39*, 2543.
[29] J. B. Eaton, J. H. Peng, K. M. Schroeder, A. A. George, J. D. Fryer, C. Krishnan, L. Buhlman, Y. P. Kuo, O. Steinlein, R. J. Lukas, *Mol. Pharmacol.* **2003**, *64*, 1283.

[30] C. W. Luetje, J. Patrick, *J. Neurosci.* **1991**, *11*, 837.
[31] E. L. Meyer, Y. Xiao, K. J. Kellar, *Mol. Pharmacol.* **2001**, *60*, 568.
[32] R. L. Papke, S. F. Heinemann, *Mol. Pharmacol.* **1994**, *45*, 142.
[33] K. A. Stauderman, L. S. Mahaffy, M. Akong, G. Velicelebi, L. E. Chavez-Noriega, J. H. Crona, E. C. Johnson, K. J. Elliott, A. Gillespie, R. T. Reid, P. Adams, M. M. Harpold, J. Corey-Naeve, *J. Pharmacol. Exp. Ther.* **1998**, *284*, 777.
[34] K. Cahill, L. Stead, T. Lancaster, *Cochrane Database Syst. Rev.* **2007**, CD006103.
[35] J. F. Etter, *Arch. Intern. Med.* **2006**, *166*, 1553.
[36] P. Tutka, W. Zatonski, *Pharmacol. Rep.* **2006**, *58*, 777.
[37] R. B. Barlow, G. M. Thompson, N. C. Scott, *Br. J. Pharmacol.* **1969**, *37*, 555.
[38] C. Reavill, B. Walther, I. P. Stolerman, B. Testa, *Neuropharmacology* **1990**, *29*, 619.
[39] C. Canu Boido, F. Sparatore, *Farmaco* **1999**, *54*, 438.
[40] R. W. Fitch, Y. Kaneko, P. Klaperski, J. W. Daly, G. Seitz, D. Geundisch, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1221.
[41] E. Marriere, J. Rouden, V. Tadino, M. C. Lasne, *Org. Lett.* **2000**, *2*, 1121.
[42] J. Rouden, A. Ragot, S. Gouault, D. Cahard, J.-C. Plaquevent, M. C. Lasne, *Tetrahedron: Asymmetry* **2002**, *13*, 1299.
[43] J. W. Coe, *Org. Lett.* **2000**, *2*, 4205.
[44] J. W. Coe, P. R. Brooks, M. G. Vetelino, M. C. Wirtz, E. P. Arnold, J. Huang, S. B. Sands, T. I. Davis, L. A. Lebel, C. B. Fox, A. Shrikhande, J. H. Heym, E. Schaeffer, H. Rollema, Y. Lu, R. S. Mansbach, L. K. Chambers, C. C. Rovetti, D. W. Schulz, F. D. Tingley, 3rd, B. T. O'Neill, *J. Med. Chem.* **2005**, *48*, 3474.
[45] B. T. O'Neill, D. Yohannes, M. W. Bundesmann, E. P. Arnold, *Org. Lett.* **2000**, *2*, 4201.
[46] H. Rollema, L. K. Chambers, J. W. Coe, J. Glowa, R. S. Hurst, L. A. Lebel, Y. Lu, R. S. Mansbach, R. J. Mather, C. C. Rovetti, S. B. Sands, E. Schaeffer, D. W. Schulz, F. D. Tingley, 3rd, K. E. Williams, *Neuropharmacology* **2007**, *52*, 985.
[47] K. B. Mihalak, F. I. Carroll, C. W. Luetje, *Mol. Pharmacol.* **2006**, *70*, 801.
[48] D. E. Jorenby, J. T. Hays, N. A. Rigotti, S. Azoulay, E. J. Watsky, K. E. Williams, C. B. Billing, J. Gong, K. R. Reeves, *J. Am. Med. Assoc.* **2006**, *296*, 56.
[49] R. C. Klesges, K. C. Johnson, G. Somes, *J. Am. Med. Assoc.* **2006**, *296*, 94.
[50] S. K. Chellappan, Y. Xiao, W. Tueckmantel, K. J. Kellar, A. P. Kozikowski, *J. Med. Chem.* **2006**, *49*, 2673.
[51] G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, *267*, 727.
[52] M. L. Verdonk, G. Chessari, J. C. Cole, M. J. Hartshorn, C. W. Murray, J. W. Nissink, R. D. Taylor, R. Taylor, *J. Med. Chem.* **2005**, *48*, 6504.
[53] J. C. Cole, C. W. Murray, J. W. Nissink, R. D. Taylor, R. Taylor, *Proteins Struct. Funct. Genet.* **2005**, *60*, 325.
[54] M. L. Verdonk, V. Berdini, M. J. Hartshorn, W. T. Mooij, C. W. Murray, R. D. Taylor, P. Watson, *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 793.
[55] M. L. Verdonk, J. C. Cole, M. J. Hartshorn, C. W. Murray, R. D. Taylor, *Proteins Struct. Funct. Genet.* **2003**, *52*, 609.
[56] R. Taylor, *Acta Crystallogr. Sect. D* **2002**, *58*, 879.
[57] J. W. Nissink, C. Murray, M. Hartshorn, M. L. Verdonk, J. C. Cole, R. Taylor, *Proteins Struct. Funct. Genet.* **2002**, *49*, 457.
[58] G. Jones, P. Willett, R. C. Glen, *J. Mol. Biol.* **1995**, *245*, 43.
[59] N. Le Novere, T. Grutter, J. P. Changeux, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3210.
[60] K. Brejc, W. J. van Dijk, R. V. Klaassen, M. Schuurmans, J. van Der Oost, A. B. Smit, T. K. Sixma, *Nature* **2001**, *411*, 269.
[61] H. Yuan, P. A. Petukhov, *Bioorg. Med. Chem.* **2006**, *14*, 7936.
[62] M. Schapira, R. Abagyan, M. Totrov, *BMC Struct. Biol.* **2002**, *2*, 1.
[63] V. Costa, A. Nistri, A. Cavalli, P. Carloni, *Br. J. Pharmacol.* **2003**, *140*, 921.
[64] W. M. Meylan, P. H. Howard, *J. Pharm. Sci.* **1995**, *84*, 83.
[65] Y. Xiao, H. Fan, J. L. Musachio, Z. L. Wei, S. K. Chellappan, A. P. Kozikowski, K. J. Kellar, *Mol. Pharmacol.* **2006**, *70*, 1454.

Received: March 30, 2007
Revised: April 30, 2007
Published online on May 25, 2007

