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Synthesis and evaluation of N^1-alkylindole-3-ylalkylammonium compounds as nicotinic acetylcholine receptor ligands

Edwin G. Pérez [a,c,*], Bruce K. Cassels [b,c], Christoph Eibl [d,†], Daniela Gündisch [d,†]

[a] Facultad de Química, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, Casilla 306, Correo 22, Santiago, Chile
[b] Department of Chemistry, Faculty of Sciences, University of Chile, Casilla 653, Santiago, Chile
[c] Millennium Institute for Cell Dynamics and Biotechnology, Plaza Ercilla 847, Santiago, Chile
[d] Institute of Pharmacy, Department of Pharmaceutical Chemistry, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany

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ABSTRACT

In this study thirty-three novel indole derivatives were designed and synthesized based on the structure of deformylflustrabromine B (**1**), a metabolite isolated from the marine bryozoan *Flustra foliacea* L. The syntheses were carried out using standard methodologies and in good yields. The molecules were tested for their affinities for the α4β2*, α3β4*, α7* and $(\alpha1)_2\beta1\gamma\delta$ nicotinic acetylcholine receptor (nAChR) subtypes. Binding assays showed that, among these ligands, compound **7c** exhibited the highest affinity with K_i = 136.1, 93.9 and 862.4 nM for the α4β2*, α3β4*, and α7* nAChRs subtypes, respectively. These results indicated that the indole core might be a useful scaffold for the development of new potent and selective nAChR ligands.

1. Introduction

Nicotinic acetylcholine receptors (nAChR) are prototypical ligand-gated ion channels that mediate fast synaptic transmission.[1] These receptors are pentameric proteins comprising either five copies of the same (or different) α subunit(s) or combinations of two different types of subunits (α and β) in the neuronal subtypes, or two α1, one β1, one γ and one δ (or ε) subunit in the neuromuscular subtype, symmetrically arranged around a central ion pore.[2] Nine different types of α subunits (α2–α10) and three kinds of β subunits (β2–β4) have been cloned and characterized as constituents of neuronal nAChR. The physiological ligand of nAChR is acetylcholine (ACh); however, tobacco components such as nicotine (Nic) and [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] (NNK) are known to be high-affinity agonists of nAChR, and nicotine is the primary addictive component of tobacco smoke.[3–5] Binding of ligands such as ACh, Nic or NNK induces a conformational change in the receptor that opens the ion channel to transmit signals at neuromuscular junctions and synapses in the central and peripheral nervous systems.[2] Charged amino acids line the channel pore and select the ions that pass through the channel.[3]

Natural compounds acting on the central nervous system have played an important role in drug discovery and are the source of numerous therapeutic agents,[6] and the cholinergic system is a target frequently mentioned in the literature.[7–9] Among them, bungarotoxins, conotoxins, methyllycaconitine, anatoxin-a and epibatidine have often been discussed as molecular probes for the receptor but rarely as potential drugs because of their toxicity and/or low selectivity, while nicotine and cytisine have been used to facilitate smoking cessation in various formulations (e.g., tablets, gum, spray, patches and herbal preparations). Finally, varenicline, inspired in the structure of cytisine, was launched on the market in 2006 and is commercialized as a smoking cessation agent.[10]

Although marine natural products appear frequently in the literature as potent agents acting on the CNS, they have usually been overlooked and have only recently arisen as possibly valuable drug leads. Among them, those with an indole moiety represent a rich group with tremendous potential for the design and development of drugs for the treatment of various psychiatric diseases and disorders.[11,12]

Tryptamine [2-(1*H*-indol-3-yl)ethanamine] derivatives deformylflustrabromine B (**1**) and deformylflustrabromine (**2**), isolated from the widespread marine bryozoan *Flustra foliacea* L., showed weak binding affinities at α7 and α4β2 nAChRs, respectively,[13] and deformylflustrabromine (**2**) was identified as a positive allosteric modulator (PAM) of α4β2 nAChR.[14,15] Quite recently it was shown that compound **2** not only potentiates the responses to both partial and full agonists but has no effect when it is co-applied with antagonists.[16] Deformylflustrabromine B (**1**) did not increase

* Corresponding author.
E-mail address: eperezh@uc.cl (E.G. Pérez).
† Present address: Department of Pharmaceutical Sciences, College of Pharmacy, University of Hawaii at Hilo, 34 Rainbow Drive, Hilo, HI 96720, USA.

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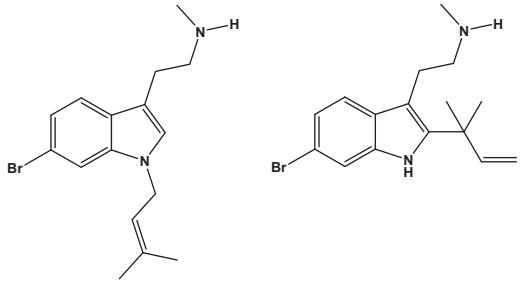
responses when co-applied with ACh on α4β2 nAChR, but instead inhibited ACh-induced responses in α7 and α4β2 receptors.[15] The fact that **1** displaces radioligands from these nAChR subtypes indicates that its inhibitory activity might be due, at least in part, to orthosteric binding. As this compound might be an antagonist at these two nAChR subtypes, in this study we synthesized a series of N^1-alkylindole-3-ylalkylamine homologues of **1**, and their methiodides, and evaluated them for their binding affinities at the three major neuronal subtypes of nAChR (α4β2, α7, and α3β4) and, for selected compounds, on the muscle subtype.



1 2

2. Results and discussion

2.1. Chemistry

6-Bromo-N^{10}-Boc-N^{10}-methyltryptamine (**3a**) and its debrominated derivative (**3b**) were synthesized as previously described,[17,18] and N^1-alkylated using NaH as base and the corresponding alkyl halides to produce **4a–d**. These compounds were *N*-Boc-deprotected using TFA, and the secondary amines (**5a–d)** were converted to the tertiary amines (**6a–c)** and quaternized to **7a–d** using $(CHO)_n$/$NaBH_4$, and MeI, respectively (Scheme 1).

To extend the tryptamine series, we further synthesized N^{10},N^{10}-dimethyltryptamines **6d-f** and N^{10},N^{10},N^{10}-trimethyltryptaminium iodides **7e–g**, alkylated on N^1 with longer alkyl chains (C_8, C_{10}, C_{12}), to determine the importance of the substitution on the basic nitrogen and the optimal length of the chain attached to the indole nitrogen. To obtain these compounds, the synthetic route involved an initial coupling using indole (**8**) and oxalyl chloride to produce the corresponding glyoxalyl chloride which was treated with gaseous dimethylamine to generate *N*,*N*-dimethylindole-3-ylglyoxylamide (**9**). This amide was then N^1-alkylated (**10a–c**) followed by reduction with $LiAlH_4$ to afford N^1-alkyl-N^{10},N^{10}-dimethyltryptamines **6d–f**. The corresponding trimethylammonium iodides (**7e–g**) were obtained by adding MeI (Scheme 2).

To obtain compound **7h**, an N^{10}-cyclic tryptamine (Scheme 3), the intermediate chloroamide (**11**), produced as described above using pyrrolidine instead of dimethylamine, was reduced with $LiAlH_4$, the tryptamine analogue **12** was N^1-alkylated with prenyl bromide, and finally the methylammonium iodide was obtained by adding MeI.

The synthesis of N^1-prenylgramines (N^1-prenylindol-3-yl-*N*,*N*-dimethylmethanamines **15a–b**) and their methiodides (**16a–b**) involved three steps (Scheme 4). Treatment of indole-3-carboxaldehydes **13a–b** with NaH and prenyl bromide in dry THF gave N^1-prenylindole-3-carboxaldehydes **14a–b**, and subsequent reductive amination using dimethylamine and $NaBH_4$ in MeOH followed by quaternization using MeI produced the desired compounds.

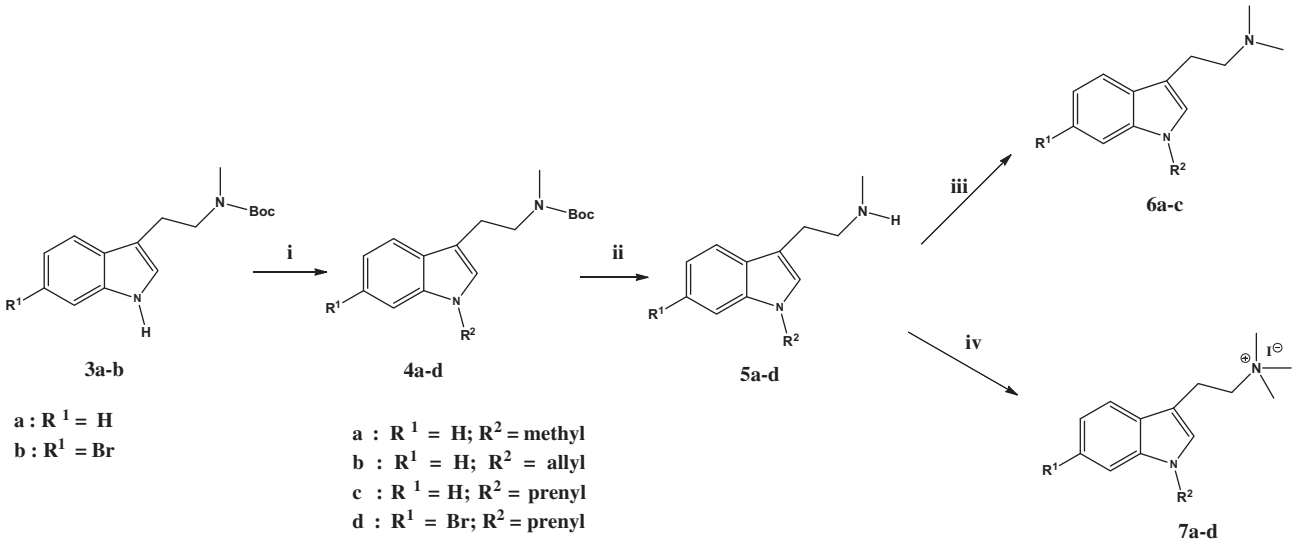
The synthesis of N^1-alkylated gramines **15c–e** with longer alkyl chains (C_8, C_{10}, C_{12}) was effected via a Mannich reaction of N^1-alkylated indoles **17c–e** with dimethylamine and formaldehyde. Trimethylammonium iodides **16c–e** were obtained as mentioned above (Scheme 5).

The synthetic steps followed to obtain N^1-alkylhomotryptamines (3-(1*H*-indol-3-yl)propanamines **21a–c**) and their methiodides (**22a–c**) are depicted in Scheme 6. *N*,*N*-Dimethyl-3-indolepropanamide (**19**) prepared from commercially available indole-3-propionic acid (**18**) was N^1-alkylated with the appropriate alkyl bromides (C_8, C_{10}, C_{12}), and these intermediates (**20a–c**) were reduced with $LiAlH_4$ as described above to afford the desired homotryptamines. The trimethylammonium iodides were obtained by adding MeI.

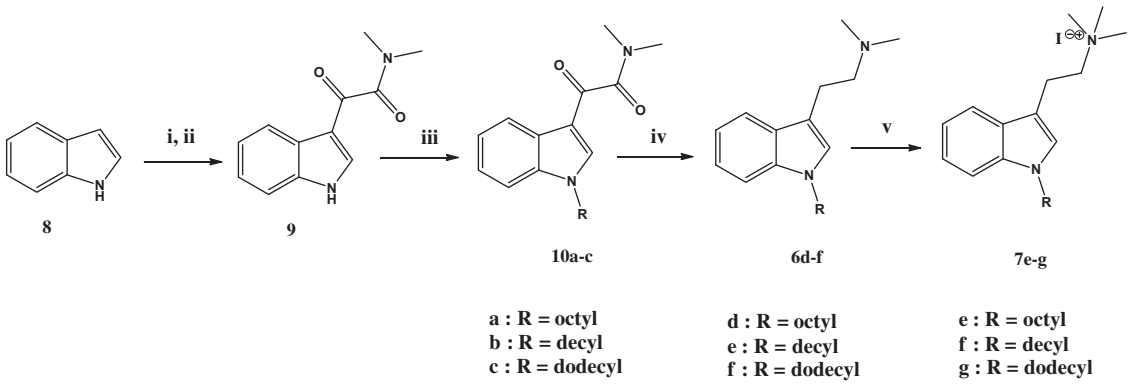
The synthesis of N^1-octylisogramine (N^1-octylindol-2-yl-*N*,*N*-dimethylmethanamine, **26**) was accomplished by amidation of indole-2-carboxylic acid (**23**) with dimethylamine to produce **24** which was N^1-alkylated with NaH and octyl bromide to produce **25**. This compound was reduced with $LiAlH_4$ affording the expected dimethylaminomethyl derivative (**26**). Trimethylammonium iodide **27** was obtained as mentioned before. Scheme 7.

2.2. Biological results and discussion

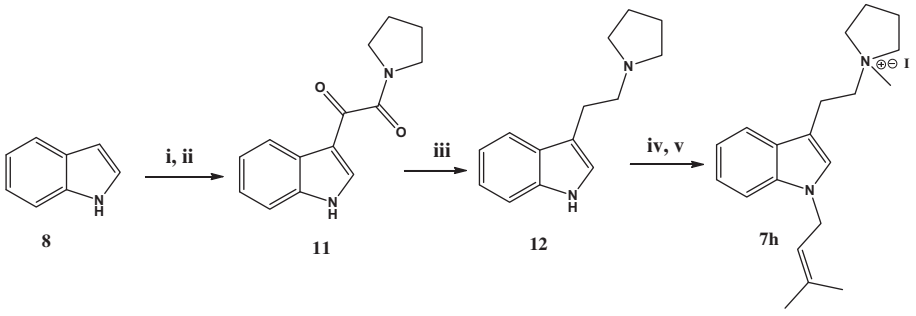
The presence of a bromine atom at C6 of the indole ring, the length of the chain joining the indole C3 to the basic nitrogen,



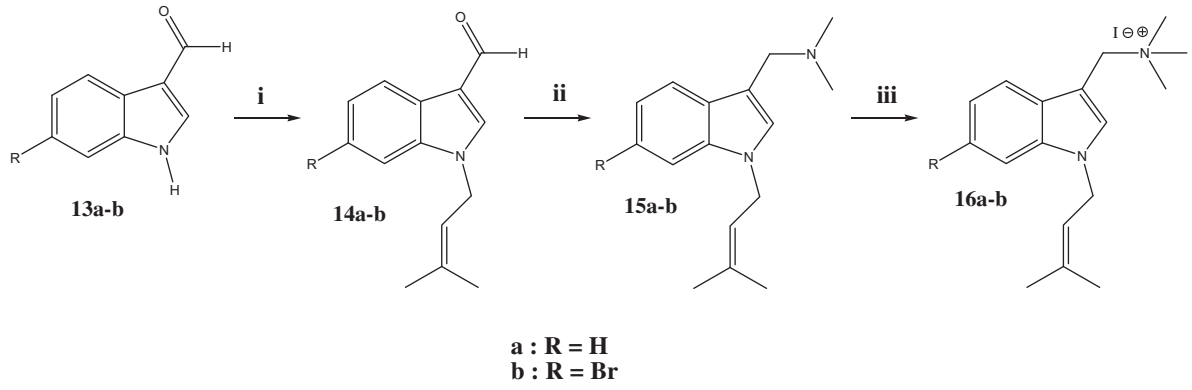
Scheme 1. Reagents and conditions: (i) NaH, THF, 0 °C to rt, 30 min, R^2Br, rt, 16 h; (ii) TFA, DCM, rt, 2 h; (iii) $(CHO)_n$, $NaBH_4$, MeOH, rt, 2 h; (iv) MeI, acetone, rt, 16 h.



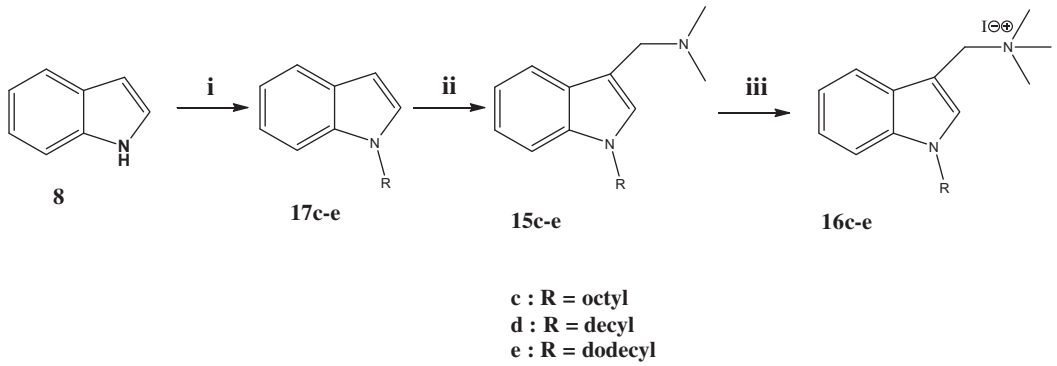
Scheme 2. Reagents and conditions: (i) $(CO)_2Cl_2$, Et_2O, 0 °C; (ii) $Me_2NH_{(g)}$, DCM, rt, 80% two steps; (iii) NaH, THF, 0 °C to rt, 30 min, RBr, rt, 16 h; (iv) $LiAlH_4$, THF, reflux, 4 h; (v) MeI, acetone, rt, 16 h.



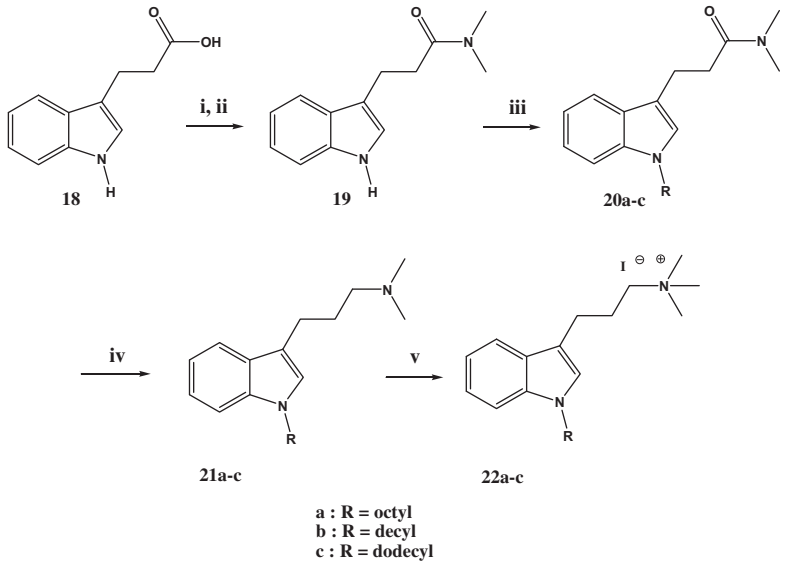
Scheme 3. Reagents and conditions: (i) $(CO)_2Cl_2$, Et_2O, 0 °C; (ii) pyrrolidine, DCM, rt, 85% two steps; (iii) $LiAlH_4$, THF, reflux, 4 h, 79%; (iv) NaH, THF, 0 °C to rt, 30 min, 1-bromo-3-methyl-2-butene, rt, 16 h; (v) MeI, acetone, 16 h, 80% two steps.



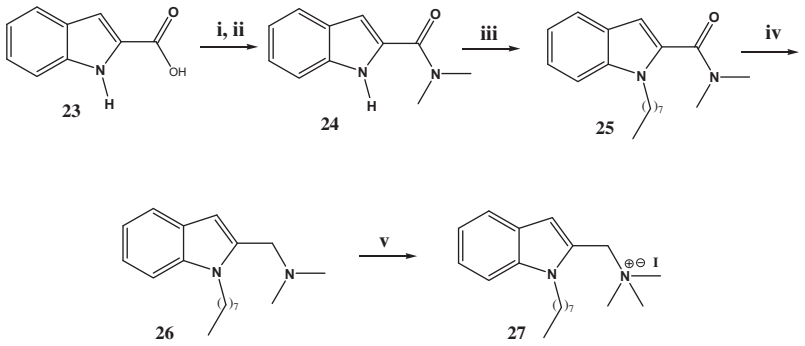
Scheme 4. Reagents and conditions: (i) NaH, THF, 0 °C to rt, 30 min, 1-bromo-3-methyl-2-butene rt, 16 h; (ii) $Me_2NH_{(g)}$, 2 h, $NaBH_4$, MeOH, rt, 2 h; (iii) MeI, acetone, rt, 16 h.



Scheme 5. Reagents and conditions: (i) Bu_4NBr, NaOH, H_2O, toluene, RBr, rt, 16 h; (ii) Me_2NH_{aq}, HCHO, $AcOH/H_2O$, rt, 24 h; (iii) MeI, acetone, rt, 16 h.



Scheme 6. Reagents and conditions: (i) CDI, DMF, 0 °C, 1 h; (ii) $Me_2NH_{(g)}$, THF, 2 h, 86% two steps; (iii) NaH, THF, 0 °C to rt, 30 min, RBr, rt, 16 h; (iv) $LiAlH_4$, THF, reflux, 4 h; (v) MeI, acetone, rt, 16 h.



Scheme 7. Reagents and conditions: (i) $SOCl_2$, THF, rt, 16 h; (ii) Me_2NH_{aq}, rt,10 min, 80% two steps; (iii) NaH, THF, 0 °C to rt, 30 min, 1-bromooctane, rt, 16 h, 73%; (iv) $LiAlH_4$, THF, reflux, 4 h, 95%; (v) MeI, acetone, rt,16 h, 87%.

the substitution on the latter and the length of the alkyl group (C_4 to C_{12}) on the indole nitrogen were systematically varied. Radioligand receptor binding assays were carried out to determine the compounds' abilities to compete for [^{3}H]epibatidine and [^{3}H]metyllycaconitine (MLA) binding sites using different tissue preparations (Table 1) and to gain insight into structure-affinity relationships.

Membrane fractions from rat forebrain, calf adrenals, and *Torpedo californica* electroplax were used to study the interactions with the α4β2∗, α3β4∗, and $(\alpha1)_2\beta1\gamma\delta$ nAChR subtypes by displacement of [^{3}H]epibatidine. Test compounds were incubated with membrane fractions from rat forebrain to evaluate their affinities for α7∗ nAChR by competition with [^{3}H]MLA (Table 1).[19–21]

Most of the compounds synthesized had negligible binding affinities for all three neuronal nAChR subtypes. However there were some exceptions, among which moderate-to-high affinities (K_i as low as 93.9 nM, almost the same as nicotine) were seen for α4β2∗ and α3β4∗ nAChR. As noted previously, compound **1** (**=5d**) showed low micromolar affinity for α7∗ nAChR, and no radioligand displacement could be detected up to 50 μM for the α4β2∗ subtype. In our hands, binding of **5d** to α3β4∗ receptors was not appreciably stronger than to α4β2∗ nAChR.

Interestingly, dimethyltryptamine derivatives **6a–c** have low micromolar affinities for the α3β4∗ subtype in spite of their lack of affinity for α4β2∗ and α7∗ nAChR. Although in the original publication on compounds **1** and **2** their affinities for the α3β4∗ nAChR were not determined, our present findings indicate that early studies on this ganglionic receptor subtype should not be neglected in view of the possibility of new compounds acting on the periphery instead of, or in addition to the CNS. The activity of the dimethyltryptamine analogues increased slightly with increasing length of the alkyl chain bound to the indole nitrogen from methyl to dimethylallyl (prenyl, 3-methyl-2-butenyl), but when the chain was lengthened to octyl, the K_i value rose above 10 μM (Table 1). We found that *N,N,N*-trimethyltryptaminium derivatives **7b** and **7c**, with an allyl (2-propenyl) or a dimethylallyl chain on the indole nitrogen, have nanomolar affinities for all three neuronal nAChR subtypes. Unlike their dimethyltryptamine counterparts, they bind almost equally strongly to α4β2∗ and α3β4∗ nAChR and significantly, though much more weakly, to α7∗ nAChR, and **7c** even shows low micromolar affinity for the muscle subtype, It is intriguing that introduction of a bromine atom at C6 of the isopentenyl compound **7d** leads to a preference for α4β2∗ over α3β4∗ receptors, mainly due to a more than 60-fold fall in affinity for the latter subtype. Apparently the dimethylallyl group on the indole nitrogen is close to optimal for α3β4∗ nAChR affinity because both shorter (methyl, **7a**; allyl, **7b**) and longer (octyl, **7e**) chains showed higher K_i values, and decyl and dodecyl derivatives

Table 1
Radioligand binding affinities of tryptamine (**5** = *N*-methyl, **6** = *N,N*-dimethyl, **7** = *N,N,N*-trimethyltryptaminium), gramine (**15** = *N,N*-dimethyl, **16** = *N,N,N*-trimethylammonium), homotryptamine (**21** = *N,N*-dimethyl, **22** = *N,N,N*-trimethylammonium), and isogramine (**26**, **27**) derivatives for different nAChR subtypes

Compound	α4β2* rat brain [b] K_i (nM) (± SEM)[a]	α3β4* calf adrenal[b] K_i (nM) (± SEM)[a]	α7* rat brain[c] K_i (nM) (± SEM)[a]	$(\alpha1)_2\beta1\gamma\delta$ *T. californica* electroplax[b] K_i (nM) (± SEM)[a]
Nicotine	0.85 (0.16)	75.3 (8.9)	129 (14)	n.d.
5a	>10,000	>10,000	n.d.	n.d.
5b	>10,000	>10,000	n.d.	n.d.
5c	>10,000	>10,000	>10,000	n.d.
5d = 1	>10,000	>10,000	17,000 [d]	n.d.
6a	>10,000	3,676	n.d.	n.d.
6b	>10,000	2,846	>10,000	n.d.
6c	>10,000	1,823	>10,000	n.d.
6d	>10,000	>10,000	>10,000	>10,000
6e	>10,000	>10,000	n.d.	n.d.
6f	>10,000	>10,000	n.d.	n.d.
7a	>10,000	2,011	>10,000	>10,000
7b	517.1	365.5	6795.5	>10,000
7c	136.1	93.9	862.4	2359.3
7d	412.0	5,334	1534.2	>10,000
7e	527.0	>10,000	n.d.	n.d.
7f	>10,000	>10,000	n.d.	n.d.
7g	>10,000	>10,000	n.d.	n.d.
7h	>10,000	n.d.	n.d.	n.d.
15a	>10,000	>10,000	n.d.	n.d.
15b	1380	>10,000	n.d.	n.d.
15c	>10,000	>10,000	>10,000	n.d.
15d	>10,000	>10,000	>10,000	n.d.
15e	>10,000	>10,000	>10,000	n.d.
16a	435.0	>10,000	>10,000	n.d.
16b	202.5	>1,000	>1,000	n.d.
16c	705.6	3404	>5000	n.d.
16d	>10,000	>10,000	>10,000	n.d.
16e	>10,000	>10,000	>10,000	n.d
21a	>10,000	>10,000	n.d.	n.d.
21b	>10,000	>10,000	>10,000	n.d
21c	>10,000	>10,000	>10,000	n.d
22a	243.5	>10,000	>1,000	n.d.
22b	>10,000	>10,000	>10,000	n.d
22c	>10,000	>10,000	>10,000	n.d
26	>10,000	>10,000	>10,000	n.d.
27	>10,000	>10,000	>10,000	n.d.

[a] Values are the means from at least 3–5 independent assays.
[b] By displacement of [^{3}H]epibatidine.
[c] By displacement of [^{3}H]methyllycaconitine.
[d] Ref.[7]. n.d. = not determined.
* Naturally expressed nAChRs which may contain other subunits in addition to those indicated.

7f-7g are practically inactive at this receptor subtype. Although most of the secondary and tertiary (including cyclic) amines are inactive at submicromolar concentrations, some trimethyltryptaminium compounds have affinities up to three orders of magnitude higher than those of the natural products **1** and **2**. This is in agreement with the trends reported by Dwoskin et al. for several series of amine and ammonium compounds and other antagonists such as hexamethonium and decamethonium.[22]

Of the five gramine derivatives tested (**15a–15e**) only **15b**, which is the single brominated member of this subgroup, proved active, with a low micromolar K_i, at α4β2 nAChR. Of the five corresponding *N*-methylgraminiums (**16a–16e**), three (**16a–16c**) have submicromolar affinities for α4β2 nAChR, and here again the brominated **16b** is the most potent. However, **16c** was the only one to show micromolar affinity for α3β4 nAChR. Neither one of the isogramine derivatives (**26**, **27**) showed any activity.

None of the homotryptamines (**21a–21c**) showed any activity. Of their quaternary salts, only **22a** exhibited fairly high affinity for the α4β2 receptor subype. This compound bears an octyl chain on the indole nitrogen.

3. Conclusions

In summary, this preliminary work allowed the very modest nAChR affinity (K_i >10,000 nM) of the natural lead compound deformylflustrabromine B to be raised to levels close to that of nicotine (i.e. K_i value at the α3β4 nAChR subtype for **7c**, 93.9 nM; for nicotine, 75.3 nM). Most of the active compounds showed some slight selectivity for the α4β2∗ subtype. Unfortunately, the relative paucity of positive results makes it difficult to discern any structure–activity relationship. However, the indole core, attached to a short aminoalkyl substituent and bearing a medium-length substituent on N1, has provided unprecedented examples of arylalkylamine derivatives with good nAChR affinity. Further modification, for instance by substitution on the benzene ring of the indole moiety, is now an attractive approach in the quest for subtype-selective nicotinic ligands.

4. Experimental section

4.1. Chemistry

4.1.1. General information

Melting points were determined on a Reichert Galen III hot plate microscope apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AMX 300, Bruker Avance 400, or Bruker Avance 500 instruments at 300, 400 or 500 MHz, respectively. ^{13}C NMR spectra were recorded on the same instruments at 75, 100 or 125 MHz. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane in $CDCl_3$ or DMSO-d_6 and coupling constants (J) are given in Hertz. Precoated silica gel 60 plates (Merck 60 F_{254} 0.2 mm) were used for TLC. TLC spots were visualized by spraying with Dragendorff's reagent or by exposing to iodine vapor.

4.1.2. N^1,N^{10}-Dimethyl-N^{10}-Boc-tryptamine (4a)

To a suspension of sodium hydride (0.044 g, 1.7 mmol) in anhydrous THF (10 mL) was added N^{10}-methyl-N^{10}-Boc-tryptamine (**3a**, 0.387 g, 1.5 mmol) at 0 °C. The mixture was stirred for 30 min at rt, and then MeI (0.240 g, 1.7 mmol) dissolved in THF (5 mL) was added at room temperature. The reaction mixture was allowed to stir for 16 h, and then quenched with water (10 mL). The suspension was extracted with EtOAc (3 × 15 mL), the organic layer was dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed *in vacuo*. Chromatography on silica gel (hexane:EtOAc 60:40) gave 0.326 g (80%) of the title compound as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.60 (d, 1H, J = 5.5 Hz), 7.27 (d, 1H, J = 8.0 Hz), 7.20 (t, 1H, J = 7.5 Hz), 7.09 (t, 1H, J = 7.0 Hz), 6.82 (s, 1H), 3.72 (s, 3H), 3.47 (t, 2H, J = 7.5 Hz), 2.94 (t, 2H, J = 7.5 Hz), 2.86 (s, 3H,), 1.33 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.7, 137.0, 127.8, 126.6, 121.5, 118.76, 118.70, 111.7, 109.1, 79.10, 49.76, 34.18, 32.53, 28.28 (3C), 23.83.

4.1.3. N^1,N^{10}-Dimethyltryptamine

To a solution of N^1,N^{10}-dimethyl-N^{10}-Boc-tryptamine (**4a**) (0.3 g, 1.1 mmol) in DCM (2 mL) at room temperature was added TFA (0.2 mL). The deep red mixture was stirred for 2 h and then quenched with a saturated solution of $NaHCO_3$ (2 mL). The mixture was extracted with DCM (3 × 5 mL), and the organic layer was dried over anhydrous $MgSO_4$ and filtered. Chromatography (DCM:MeOH:TEA 9:0.5:0.5) afforded 0.17 g (85%) of the title product as a clear liquid. ^{1}H NMR (oxalate salt, 500 MHz, DMSO-d_6): δ 8.85 (s, 1H), 7.58 (d, 1H, J = 8.0 Hz), 7.39 (d, 1H, J = 8.0 Hz), 7.19 (s, 1H), 7.15 (t, 1H, J = 7.0 Hz), 7.03 (t, 1H, J = 7.0 Hz), 3.72 (s, 3H), 3.13 (t, 2H, J = 7.5 Hz), 3.02 (t, 2H, J = 7.5 Hz), 2.28 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 164.7, 136.8, 127.8, 127.2, 121.5,

118.7, 118.6, 109.8, 108.8, 48.8, 32.6, 32.4, 21.6. Anal. Calcd for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.11; H, 6.77; N, 9.96.

4.1.4. N^1,N^{10},N^{10}-Trimethyltryptamine (6a)

To a suspension of paraformaldehyde (0.045 g, 1.5 mmol) in MeOH (10 mL) at room temperature was added N^1,N^{10}-dimethyltryptamine (**5a**, 0.056 g, 0.3 mmol) and the mixture was stirred for 0.5 h. Then to the above solution was added $NaBH_4$ (0.06 g, 1.5 mmol) at room temperature, and the resulting mixture was stirred for another 2 h and quenched with a saturated solution of $NaHCO_3$ (5 mL). The mixture was extracted with DCM (3 × 20 mL), and the organic layer was dried over anhydrous $MgSO_4$ and filtered. Chromatography (DCM:MeOH:TEA 9:0.5:0.5) gave 0.04 g (75%) of the title product as a clear liquid. ^{1}H NMR (oxalate salt, 500 MHz, DMSO-d_6): δ 10.10 (s, 1H), 7.61 (d, 1H, J = 8.0 Hz), 7.39 (d, 1H, J = 8.0 Hz), 7.19 (s, 1H), 7.16 (t, 1H, J = 7.0 Hz), 7.03 (t, 1H, J = 7.0 Hz), 3.73 (s, 3H), 3.24 (t, 2H, J = 7.5 Hz), 3.07 (t, 2H, J = 7.5 Hz), 2.80 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 136.8, 127.7, 127.2, 121.5, 118.71, 118.65, 118.6, 109.8, 108.7, 57.00, 42.32 (2C), 32.43, 20.16. Anal. Calcd for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.98; H, 7.01; N, 9.67.

4.1.5. N^1,N^{10},N^{10},N^{10}-Tetramethyltryptaminium iodide (7a)

To a solution of N^1,N^{10}-dimethyltryptamine (**5a**, 0.05 g, 0.26 mmol) in acetone (5 mL), iodomethane (0.14 g, 1.0 mmol) was added and the reaction mixture was stirred for 16 h. Then, Et_2O was added and the solid collected by filtation to afford 0.08 g (88%) of a white solid: mp 220 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.29 (s, 1H), 7.58 (d, 1H, J = 8.0 Hz), 7.40 (d, 1H, J = 8.0 Hz), 7.22 (s, 1H), 7.16 (t, 1H, J = 7.0 Hz), 7.05 (t, 1H, J = 7.0 Hz), 3.74 (s, 3H), 3.17 (t, 2H, J = 7.5 Hz), 3.01 (t, 2H, J = 7.5 Hz), 2.61 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 136.9, 127.9, 127.2, 121.5, 118.7, 118.5, 109.9, 108.4, 48.78, 32.72, 32.49, 21.62. Anal. Calcd for $C_{14}H_{21}IN_2$: C, 48.85; H, 6.15; I, 36.87. Found: C, 49.02; H, 6.03; I, 36.80

4.1.6. 2-(1*H*-Indol-3-yl)-*N*,*N*-dimethyl-2-oxoacetamide (9)

To a solution of indole (**8**, 6.00 g, 51.2 mmol) in dry Et_2O (50 mL) was added dropwise oxalyl chloride (7.80 g, 61.9 mmol, 1.2 eq) at 0 °C with stirring. After 30 min, the yellow solid was filtered and washed with cold Et_2O (50 mL). A suspension of this yellow solid in dry DCM was treated with dry gaseous dimethylamine for 10 min and the mixture was stirred for 3 h at rt, the solvent was removed under reduced pressure, and water (200 mL) was added. The aqueous suspension was extracted with EtOAc (3 × 150 mL), and the organic phase was dried over anhydrous $MgSO_4$ and filtered. The product, crystallized as a white solid in EtOH, weighed 8.37 g (80% two steps): mp 158–160 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.25 (s, 1H), 8.10 (d, 1H, J = 8.0 Hz), 8.09 (s, 1H), 7.52 (dd, 1H, J_1 = 1.5 Hz, J_2 = 6.5 Hz), 7.25 (m, 2H), 2.98 (s, 3H), 2.90 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 186.8, 167.5, 137.04, 137.01, 125.0, 123.6, 122.6, 121.0, 113.1, 112.7, 36.90, 33.54.

4.1.7. *N*,*N*-Dimethyl-2-(1-octyl-1*H*-indol-3-yl)-2-oxoacetamide (10a)

Prepared in a similar fashion as described above (**4a**) using **9** (2.00 g, 6.08 mmol) and replacing MeI with 1-bromooctane (1.40 g, 7.3 mmol). Chromatography (hexane:EtOAc 8:2) afforded 2.48 g (77%) of the title compound as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (t, 1H, J = 5.6 Hz), 7.93 (s, 1H), 7.61 (m, 3H), 4.16 (t, 2H, J = 7.6 Hz), 3.14 (s, 3H), 3.10 (s, 3H), 1.91 (m, 2H), 1.31 (m, 10H), 0.90 (t, 3H, J = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.5, 167.7, 138.2, 137.0, 126.5, 123.8, 123.1, 122.5, 113.3, 110.2, 47.44, 37.53, 34.48, 31.73, 29.80, 29.09 (2C), 26.88, 22.59, 14.06.

4.1.8. *N*,*N*-Dimethyl-2-(1-octyl-1*H*-indol-3-yl)ethanamine (N^1-octyl-N^{10},N^{10}-dimethyltryptamine, 6d)

To a suspension of $LiAlH_4$ (2.00 g, 52.6 mmol) in dry THF (200 mL) at 0 °C, was added dropwise a solution of *N*,*N*-dimethyl-2-(1-octyl-1*H*-indol-3-yl)-2-oxoacetamide (**10a**, 2.00 g, 6.07 mmol) in dry THF. The mixture was refluxed with stirring for 4 h and was cooled to rt and then in an ice-water bath. Aqueous THF (1:1) was added carefully, the solid was removed by filtration and the volatiles were evaporated under reduced pressure. Chromatography (DCM:MeOH 9:1) gave 1.17 g (64%) of the desired product as a clear oil. ^{1}H NMR (oxalate salt, 300 MHz, DMSO-d_6): δ 7.76 (d, 1H, J = 7.6 Hz), 7.47 (d, 1H, J = 8.4 Hz), 7.41 (s, 1H), 7.16 (t, 1H, J = 7.2 Hz), 7.06 (t, 1H, J = 7.2), 5.86 (d, 1H, J = 3.6 Hz), 5.48 (d, 1H, J = 10.4 Hz), 4.14 (t, 2H, J = 9.0 Hz), 3.81 (dd, 1H, J_1 = 10.4 Hz J_2 = 13.6 Hz), 3.51 (d, 1H, J = 13.2 Hz), 3.25 (s, 6H), 1.72 (t, 2H, J = 6.4 Hz), 1.24 (m, 10H), 0.84 (t, 3H, J = 4.8 Hz). ^{13}C NMR (oxalate salt, 75 MHz, DMSO-d_6): δ 165.0 136.6, 126.8, 125.8, 121.9, 120.1, 119.3, 115.0, 110.5, 70.33, 62.46, 53.95 (2C), 45.94, 31.67, 30.37, 29.08 (2C), 26.75, 22.50, 14.39. Anal. Calcd for $C_{22}H_{34}N_2O_4$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.60; H, 8.88; N, 7.19.

4.1.9. *N*,*N*,*N*-Trimethyl-2-(1-octyl-1*H*-indol-3-yl)ethanaminium iodide (N^1-octyl-N^{10},N^{10},N^{10}-trimethyltryptaminium iodide, 7e)

Prepared in a similar fashion as described above (**7a**) by replacing N^1,N^{10}-dimethyltryptamine with *N*,*N*-dimethyl-2-(1-octyl-1*H*-indol-3-yl)ethanamine (**6d**, 0.5 g, 1.7 mmol) to afford 0.66 g (90%) of a white solid: mp 223–226 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.76 (d, 1H, J = 7.6 Hz), 7.47 (d, 1H, J = 8.4 Hz), 7.41 (s, 1H), 7.16 (t, 1H, J = 7.2 Hz), 7.06 (t, 1H, J = 7.2), 5.86 (d, 1H, J = 3.6 Hz), 5.48 (d, 1H, J = 10.4 Hz), 4.14 (t, 2H, J = 9.0 Hz), 3.81 (dd, 1H, J_1 = 10.4 Hz J_2 = 13.6 Hz), 3.51 (d, 1H, J = 13.2 Hz), 3.27 (s, 9H), 1.72 (t, 2H, J = 6.4 Hz), 1.24 (m, 10H), 0.84 (t, 3H, J = 4.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 136.6, 126.8, 125.8, 121.9, 120.1, 119.3, 115.0, 110.5, 70.33, 62.46, 53.95 (3C), 45.94, 31.67, 30.37, 29.08 (2C), 26.75, 22.50, 14.39. Anal. Calcd for $C_{21}H_{35}IN_2$: C, 57.01; H, 7.97; N, 6.33. Found: C, 56.93; H, 8.07; N, 6.42.

4.1.10. 1-(1*H*-Indol-3-yl)-2-(pyrrolidin-1-yl)ethane-1,2-dione (11)

Prepared in a similar fashion as described above (**9**) using indole (**8**, 2.0 g, 17.1 mmol) and replacing dimethylamine with pyrrolidine (3.5 g, 50 mmol) giving 3.38 g (82%, two steps) of a white solid: mp 124–126 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.22 (s, 1H), 8.16 (s, 1H), 8.11 (d, 1H, J = 8.5 Hz), 7.52 (dd, 1H, J_1 = 1.5 Hz, J_2 = 6.5 Hz), 7.25 (m, 2H), 3.47 (t, 2H, J = 7.0 Hz), 3.38 (t, 4H, J = 7.0 Hz), 1.84 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 186.5, 165.4, 137.4, 137.0, 125.3, 123.6, 122.6, 121.1, 112.8, 112.7, 46.65, 45.06, 25.66, 23.65.

4.1.11. 3-(2-(Pyrrolidin-1-yl)ethyl)-1*H*-indole (12)

Prepared in a similar fashion as described above (**6d**) by replacing *N*,*N*-dimethyl-2-(1-octyl-1*H*-indol-3-yl)-2-oxoacetamide with 1-(1*H*-indol-3-yl)-2-(pyrrolidin-1-yl)ethane-1,2-dione (**11**, 2.0 g, 8.26 mmol) to afford 1.4 g (79%) of a clear liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 1H) 7.61 (d, 1H, J = 7.5 Hz), 7.31 (d, 1H, J = 7.5 Hz), 7.16 (t, 1H, J = 8.5 Hz), 7.09 (t, 1H, J = 8.5 Hz), 6.98 (d, 1H, J = 2.0 Hz), 3.00 (m, 2H), 2.81 (m, 2H), 2.63 (m, 4H), 1.82 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.2, 127.4, 121.8, 121.4, 119.1, 118.8, 114.4, 111.0, 57.17, 54.18, 25.01, 23.44.

4.1.12. 1-Methyl-1-(2-(1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)-ethyl)pyrrolidinium iodide (7h)

Prepared in a similar fashion as described above (**4a**) using 3-(2-(pyrrolidin-1-yl)ethyl)-1*H*-indole (**12**, 1.0 g, 4.7 mmol) and replacing MeI with 1-bromo-3-methyl-2-butene (1.1 g, 5.2 mmol). This compound was quaternized without prior purification in the next reaction as described above (**5a**) by replacing N^1,N^{10}-dimethyltryptamine with the recently prepared product to yield 1.58 g (80% two steps) of a deliquescent solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.65 (d, 1H, J = 7.6 Hz), 7.42 (d, 1H, J = 7.5 Hz), 7.27 (s, 1H), 7.16 (t, 1H, J = 7.6), 7.06 (t, 1H, J = 7.6), 5.31 (m, 1H), 4.73 (d, 2H, J = 7.0 Hz), 3.60 (m, 6H,), 3.19 (m, 2H,), 3.16 (s, 3H), 2.13 (m, 4H), 1.82 (s, 3H), 1.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 136.2, 136.0, 127.6, 126.8, 121.9, 120.8, 119.1 (2C), 110.4, 108.6, 63.97, 63.92, 62.48, 48.04, 43.81, 25.95, 25.87, 21.60, 19.81, 18.32. Anal. Calcd for $C_{20}H_{29}IN_2{\cdot}3H_2O$: C, 50.21; H, 7.37; N, 5.86. Found: C, 50.11; H, 7.22, N, 5.95.

4.1.13. 1-(3-Methylbut-2-enyl)-1*H*-indole-3-carbaldehyde (14a)

Prepared in a similar fashion as described above (**4a**) using 1*H*-indole-3-carbaldehyde (**13a**, 2.0 g, 13.8 mmol) and replacing MeI with 1-bromo-3-methyl-2-butene (3.1 g, 15 mmol). Chromatography using DCM afforded 2.7 g (92%) of the title compound as pale yellow crystals: mp 80–82 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.11 (s, 1H), 7.98 (s, 1H),7.81 (d, 1H, J = 5.7 Hz), 7.21 (d, 1H, J = 5.7 Hz), 6.94 (m, 2H), 5.33 (m, 1H), 4.79 (d, 2H, J = 7.2 Hz), 1.78 (s, 3H), 1.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.5, 139.0, 137.8, 137.3, 125.6, 123.8, 122.9, 122.0, 118.0, 117.8, 110.2, 44.75, 25.73, 18.17.

4.1.14. *N*,*N*-Dimethyl(1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)-methanamine (N^1-prenylgramine, 15a)

Through a solution of 1-(3-methylbut-2-enyl)-1*H*-indole-3-carbaldehyde (**14a**, 1.0 g, 4.7 mmol) in MeOH (25 mL) was bubbled dry dimethylamine (DMA) for 10 min and the mixture was stirred for 1 h. $NaBH_4$ (0.6 g, 16 mmol) was added carefully over 10 min and the mixture was stirred for additional 3 h. Then the solution was carefully diluted with water and extracted with DCM (3 × 100 mL). The combined organic layers were washed twice with brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated. The mixture was purified by chromatography (DCM:MeOH 9:1) to give 0.7 g (62%) of a clear liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, 1H, J = 7.8 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.20 (t, 1H, J = 7.5 Hz), 7.11 (t, 1H, J = 7.2 Hz), 7.10 (s, 1H), 5.36 (m, 1H), 4.66 (d, 2H, J = 6.9 Hz), 3.67 (s, 2H), 2.30 (s, 6H), 1.81 (s, 3H), 1.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 136.3, 136.1, 128.6, 127.4, 121.4, 119.9, 119.2 (2C), 110.6, 109.6, 54.14, 44.97 (2C), 44.08, 25.75, 18.08. Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.04; H, 7.28; N, 8.43. Found: C, 64.88; H, 7.40; N, 8.51.

4.1.15. *N*,*N*,*N*-Trimethyl(1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)-methanaminium iodide (*N*-methyl-N^1-prenylgraminium iodide, 16a)

Prepared in a similar fashion as described above (**7a**) by replacing N^1,N^{10}-dimethyltryptamine with *N*,*N*-dimethyl(1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)methanamine (**15a**, 0.3 g, 1.2 mmol), to yield 0.4 g (87%) of a white solid: mp 212–215 °C (dec).^{1}H NMR (400 MHz, DMSO-d_6): δ 7.84 (d, 1H, J = 7.6 Hz), 7.69 (s, 1H), 7.50 (d, 1H, J = 8.0 Hz), 7.23 (t, 1H, J = 7.2 Hz), 7.17 (t, 1H, J = 7.6 Hz), 5.36 (t, 1H, J = 6.8 Hz), 4.83 (d, 2H, J = 7.2 Hz), 4.68 (s, 2H), 3.04 (s, 9H), 1.83 (s, 3H), 1.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 136.6, 136.3, 133.2, 128.7, 122.4, 120.8, 120.3, 119.3, 111.1, 101.6, 60.89, 51.72, 51.68 (2C), 44.36, 25.84, 18.40. Anal. Calcd for $C_{17}H_{25}IN_2$: C, 53.13; H, 6.56; N, 7.29. Found: C, 53.02; H, 6.65; N, 7.23.

4.1.16. *N*,*N*-Dimethyl(1-octyl-1*H*-indol-3-yl)methanamine (N^1-octylgramine, 15c)

To a solution of aqueous formaldehyde (37%, 1.1 g, 14 mmol) in AcOH (15 mL) was added aqueous DMA (40%, 5.0 g, 45 mmol) followed by 1-octyl-1*H*-indole (**17c**, 3.00 g, 13.1 mmol). The reaction mixture was stirred at rt for 24 h and was then diluted with 2 N NaOH to raise the pH to > 10. The aqueous solution was extracted with DCM (3 × 50 mL), the combined organic extracts were dried over anhydrous Na_2SO_4 and volatiles removed under reduced pressure. Chromatography (DCM:MeOH 9:1) gave the desired product, 2.85 g (76%), as a clear liquid. ^{1}H NMR (oxalate salt, 400 MHz, DMSO-d_6): δ 7.73 (d, 1H, J = 8.0 Hz), 7.57 (s, 1H), 7.49 (d, 1H, J = 8.4 Hz), 7.20 (t, 1H, J = 7.2 Hz), 7.12 (t, 1H, J = 8.4 Hz), 4.38 (s, 2H), 4.16 (t, 2H, J = 6.8 Hz), 2.71 (s, 6H), 1.74 (t, 2H, J = 6.8 Hz), 1.19 (m, 10H), 0.80 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.42, 136.3, 132.1, 128.0, 122.4, 120.3, 119.2, 110.8, 102.7, 51.51 (2C), 46.01, 41.61, 31.52 (2C), 29.98, 29.40, 28.80, 26.53, 22.42, 14.33. Anal. Calcd for $C_{21}H_{32}N_2O_4$: C, 66.99; H, 8.57; N, 7.44. Found: C, 66.90; H, 8.66; N, 7.41.

4.1.17. *N*,*N*,*N*-Trimethyl(1-octyl-1*H*-indol-3-yl)methanaminium iodide (*N*-methyl-N^1-octylgraminium iodide, 16c)

Prepared in a similar fashion as described above (**7a**) by replacing N^1,N^{10}-dimethyltryptamine with *N*,*N*-dimethyl(1-octyl-1*H*-indol-3-yl)methanamine (**15c**, 1.0 g, 3.5 mmol), to afford 1.3 g (86%) of a white solid: mp 198–200 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (d, 1H, J = 7.6 Hz), 7.74 (s, 1H), 7.57 (d, 1H, J = 8.0 Hz), 7.23 (t, 1H, J = 7.2 Hz), 7.17 (t, 1H, J = 7.6 Hz), 4.70 (s, 2H), 4.24 (t, 2H, J = 6.8 Hz), 3.05 (s, 9H), 1.78 (t, 2H, J = 6.8 Hz), 1.22 (m, 10H), 0.83 (t, 3H, J = 6.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 136.3, 133.6, 128.6, 122.4, 120.7, 119.3, 111.0, 101.4, 60.84, 51.65 (2C), 51.61, 46.23, 31.62, 30.03, 29.05, 28.98, 26.64, 22.49, 14.40. Anal. Calcd for $C_{20}H_{33}IN_2$: C, 56.07; H, 7.76; N, 6.54. Found: C, 55.96; H, 7.79; N, 6.63.

4.1.18. 3-(1*H*-Indol-3-yl)-*N*,*N*-dimethylpropanamide (19)

To a solution of 3-indolylpropionic acid (4.00 g, 21.2 mmol) in dry THF (500 mL) was added carbonyldiimidazole (DCI, 3.56 g, 22 mmol) and the mixture was stirred under nitrogen for 1.5 h. Then, the mixture was treated with gaseous DMA for 10 min and stirred for 16 h. The mixture was diluted with water (300 mL), extracted with EtOAc (4 × 200 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Chromatography (EtOAc:-MeOH 95:5) gave the desired product 4.20 g (92%) as a white solid: mp 137–139 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.41 (s broad, 1H), 7.61 (d, 1H, J = 8.0 Hz), 7.35 (d, 1H, J = 8.0 Hz), 7.18 (t, 1H, J = 7.2 Hz), 7.11 (t, 1H, J = 7.6 Hz), 7.01 (s, 1H), 3.14 (t, 2H, J = 7.6 Hz), 2.96 (s, 3H), 2.90 (s, 3H), 2.71 (t, 2H, J = 8.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.9, 136.4, 127.3, 121.9, 121.8, 119.2, 118.7, 115.4, 111.3, 37.24, 35.47, 34.25, 20.96.

4.1.19. *N*,*N*-Dimethyl-3-(1-octyl-1*H*-indol-3-yl)propanamide (20a)

Prepared in a similar fashion as described above (**4a**) using 3-(1*H*-indol-3-yl)-*N*,*N*-dimethylpropanamide (**19**, 1.00 g, 4.63 mmol) and replacing MeI with 1-bromooctane (1.07 g, 5.55 mmol). Chromatography using EtOAc afforded 1.32 g (87%) of the title compound as a pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (d, 1H, J = 8.0 Hz), 7.30 (d, 1H, J = 8.0 Hz), 7.19 (t, 1H, J = 7.2 Hz), 7.09 (t, 1H, J = 7.6 Hz), 6.94 (s, 1H), 4.05, (t, 2H, J = 7.2 Hz), 3.11 (t, 2H, J = 7.6 Hz), 2.95 (s, 3H), 2.90 (s, 3H), 2.69 (t, 2H, J = 8.4 Hz), 1.80 (t, 2H, J = 6.8 Hz), 1.28 (m, 10H), 0.87 (t, 3H, J = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 136.3, 127.7, 125.5, 121.3, 118.9, 118.6, 114.0, 109.4, 46.21, 37.24, 35.43, 34.49, 31.81, 30.35, 29.26, 29.20, 27.07, 22.64, 20.89, 14.05.

4.1.20. *N,N*-Dimethyl-3-(1-octyl-1*H*-indol-3-yl)propan-1-amine (21a)

Prepared in a similar fashion as described above (**6d**) by replacing *N,N*-dimethyl-2-(1-octyl-1*H*-indol-3-yl)-2-oxoacetamide with *N,N*-dimethyl-3-(1-octyl-1*H*-indol-3-yl)propanamide (**20a**, 0.8 g, 2.4 mmol) giving 0.57 g (73%) of a clear liquid. ^{1}H NMR (oxalate salt, 300 MHz, DMSO-d_6): δ 8.51 (d, 1H, J = 7.4 Hz), 7.33 (d, 1H, J = 7.8 Hz), 7.15 (s, 1H), 7.05 (t, 1H, J = 7.6 Hz), 6.94 (t, 1H, J = 7.4 Hz), 4.05 (t, 2H, J = 6.8 Hz), 3.01 (t, 2H, J = 7.6 Hz), 2.74 (s, 3H), 2.71 (s, 3H), 2.52 (t, 2H, J = 7.6 Hz), 2.03 (m, 2H), 1.73 (t, 2H, J = 6.8 Hz), 1.18 (m, 10H), 0.80 (t, 3H, J = 6.4 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.6, 136.6, 127.2, 125.9, 121.0, 118.5, 118.2, 112.0, 109.6, 65.35, 54.86, 52.21, 45.23, 31.10, 29.81, 28.53 (2C), 26.24, 23.05, 21.95, 21.40, 13.84. Anal. Calcd for $C_{23}H_{36}N_2O_4$: C, 68.29; H, 8.97; N, 6.92. Found: C, 68.36; H, 8.92; N, 7.02.

4.1.21. *N,N,N*-Trimethyl-3-(1-octyl-1*H*-indol-3-yl)propan-1-aminium iodide (22a)

Prepared in a similar fashion as described above (**7a**) by replacing N^1,N^{10},N^{10}-trimethyltryptamine with *N,N*-dimethyl-3-(1-octyl-1*H*-indol-3-yl)propan-1-amine (**21a**, 0.2 g, 0.64 mmol), to afford 0.3 g (88%) of a white solid: mp 167–169 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.51 (d, 1H, J = 6.0 Hz), 7.34 (d, 1H, J = 6.3 Hz), 7.19 (s, 1H), 7.08 (t, 1H, J = 5.4 Hz), 6.96 (t, 1H, J = 7.2), 4.06 (t, 2H, J = 5.1 Hz), 3.35 (m, 2H), 3.03 (s, 9H), 2.68 (t, 2H, J = 5.7 Hz), 2.03 (m, 2H), 1.68 (t, 2H, J = 5.1 Hz), 1.20 (m, 10H), 0.78 (t, 3H, J = 4.8 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 136.5, 127.7, 126.4, 121.5, 119.0, 118.8, 112.5, 110.1, 52.77 (2C), 52.73, 45.78, 31.72, 30.36, 29.78, 29.56, 29.42,26.78, 23.60, 22.53, 21.96, 14.39. Anal. Calcd for $C_{22}H_{37}IN_2$: C, 57.89; H, 8.17; N, 6.14. Found: C, 57.98; H, 8.12; N, 6.07.

4.1.22. *N,N*-Dimethyl-1*H*-indole-2-carboxamide (24)

To a solution of indole-2-carboxylic acid (**23**, 2.00 g, 12.4 mmol) in dry THF (20 mL) was added $SOCl_2$ (5 mL) and the mixture was stirred at rt for 16 h. Then, the volatiles were evaporated and the viscous liquid was dissolved in DCM (20 mL). To this solution was added 40% aqueous DMA (5.6 g, 50 mmol) and the mixture was stirred for 10 min. Water (20 mL) was added, and the mixture was extracted with DCM (4 × 25 mL), and the combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The product was crystallized in MeOH to produce 1.86 g (80%) of brown crystals: mp 77–78 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.46 (s broad, 1H), 7.55 (d, 1H, J = 8.0 Hz), 7.38 (d, 1H, J = 8.0 Hz), 7.13 (t, 1H, J = 7.2 Hz), 6.98 (t, 1H, J = 7.6 Hz), 6.81 (s, 1H), 3.20 (s, 3H), 3.05 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.1, 136.2, 130.7, 127.53, 127.49, 123.7, 121.8, 120.1, 112.53, 112.48, 105.0.

4.1.23. *N,N*-Dimethyl-1-octyl-1*H*-indole-2-carboxamide (25)

Prepared in a similar fashion as described above (**4a**) using *N,N*-dimethyl-1*H*-indole-2-carboxamide (**24**, 1.00 g, 5.32 mmol) and replacing MeI with 1-bromooctane (1.13 g, 5.55 mmol). Chromatography using EtOAc afforded 1.17 g (73%) of the title compound as a clear liquid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68 (d, 1H, J = 8.0 Hz), 7.42 (d, 1H, J = 8.0 Hz), 7.32 (t, 1H, J = 7.2 Hz), 7.17 (t, 1H, J = 7.6 Hz), 6.65 (s, 1H), 4.35 (t, 1H, J = 7.6 Hz), 3.22 (s, 6H), 1.81 (m, 2H), 1.31 (m, 10H, J = 4.4 Hz), 0.92 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 137.1, 131.6, 126.6, 123.0, 121.6, 120.0, 110.2, 103.9, 44.33 (2C), 31.81, 30.45, 29.25 (2C), 26.98, 22.65, 14.10.

4.1.24. *N,N*-Dimethyl(1-octyl-1*H*-indol-2-yl)methanamine (26)

Prepared in a similar fashion as described above (**11a**) by replacing *N,N*-dimethyl-2-(1-octyl-1*H*-indol-3-yl)-2-oxoacetamide with *N,N*-dimethyl-1-octyl-1*H*-indole-2-carboxamide (**25**, 1.00 g, 3.33 mmol). Chromatography (DCM:MeOH 9:1) yielded 0.9 g (95%) of a clear liquid. ^{1}H NMR (oxalate salt, 400 MHz, DMSO-d_6): δ 7.51 (d, 1H, J = 7.6 Hz), 7.43 (d, 1H, J = 8.4 Hz), 7.13 (t, 1H, J = 7.6 Hz), 7.00 (t, 1H, J = 7.2 Hz), 6.60 (s, 1H), 4.23 (m, 4H), 2.60 (s, 6H), 1.58, (m, 2H), 1.19 (m, 10H), 0.79 (t, 3H, J = 6.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.5, 138.1, 137.2, 127.3, 122.3, 120.9, 119.9, 110.8, 104.9, 43.37, 42.91, 42.83, 31.63, 30.30, 29.17, 29.06, 26.65, 22.49, 14.37. Anal. Calcd for $C_{21}H_{32}N_2O_4$: C, 66.99; H, 8.57; N, 7.44. Found: C, 66.90; H, 8.55; N, 7.60.

4.1.25. *N,N,N*-Trimethyl(1-octyl-1*H*-indol-2-yl)methanaminium iodide (27)

Prepared in a similar fashion as described above (**8a**) by replacing N^1,N^{10},N^{10}-trimethyltryptamine with *N,N*-dimethyl(1-octyl-1*H*-indol-2-yl)methanamine (**26**, 0.5 g, 1.7 mmol), to afford 0.65 g (87%) of a white solid: mp 189–192 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.60 (d, 1H, J = 8.0 Hz), 7.52 (d, 1H, J = 8.4 Hz), 7.21 (t, 1H, J = 7.6 Hz), 7.06 (t, 1H, J = 7.6 Hz), 6.80 (s, 1H), 4.78 (s, 2H), 4.30 (t, 1H, J = 7.2 Hz) 3.08 (s, 9H), 1.55, (m, 2H), 1.15 (m, 10H), 0.78 (t,3H, J = 6.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 139.8, 138.1, 137.6, 127.2, 123.3, 121.6, 120.5, 111.6, 109.0, 59.43, 52.18 (2C), 43.56, 31.59, 30.11, 29.18, 29.03, 26.45, 22.47, 14.37. Anal. Calcd for $C_{20}H_{33}IN_2$: C, 56.07; H, 7.76; N, 6.54. Found: C, 56.14; H, 7.85; N, 6.53.

4.2. Biological assays

4.2.1. Materials

(±)-[^{3}H]Epibatidine (33–56.2 Ci/mmol) was obtained from PerkinElmer/NEN Life Science Products (Cologne, Germany).[^{3}H]MLA (20–100 Ci/mmol) was purchased from Biotrend (Cologne, Germany). All other chemicals used were obtained from Sigma-Aldrich (Deisenhofen, Germany). Frozen Sprague–Dawley rat brains were purchased from Pel-Freez Biologicals (Rogers, AR, USA.), *Torpedo californica* electric organs were purchased from Marinus, Inc. (CA, USA), and calf adrenals were obtained from a local slaughterhouse.

4.2.2. Membrane preparation and binding assays

Crude membrane fractions (P2) from rat brains were isolated as previously described[23,24] and P1 membrane fractions were obtained from calf adrenals and *Torpedo californica* electroplax. All assays were carried out as previously described in HEPES-salt solution (HSS) containing HEPES (15 mM), NaCl (120 mM), KCl (5.4 mM), $MgCl_2$ (0.8 mM), and $CaCl_2$ (1.8 mM) and at 22 °C performed in duplicate. Briefly, nonspecific binding was determined in the presence of 300 μM (−)-nicotine if (±)-[^{3}H]epibatidine was used as a radioligand ($\alpha4\beta2*$, $\alpha3\beta4*$, $\alpha12\beta1\gamma\delta$). Also, each assay sample of a total volume of 0.5 ml contained 60 μg of membrane protein, 0.5 nM (±)-[^{3}H]epibatidine, and 0.2 ml of a test compound. After 90 min incubations were terminated by vacuum filtration through Whatman GF/B glass fiber filters, presoaked in 1% poly(ethyleneimine) using a Brandel 24-channel cell harvester. The radioactivity was measured using a liquid scintillation counter (Tri-Carb 2100 TR, Packard, Dreieich, Germany). For $\alpha7*$ binding assays, nonspecific binding was measured in the presence of 1 μM methyllycaconitine (MLA). Each assay sample contained 50 μl of the test compound, 100 μl [^{3}H]MLA to achieve a final concentration of 2 nM, and 100 μl resuspended membranes. After 180 min (at 22 °C) incubation was terminated by rapid filtration under vacuum through Whatman GF/B filters presoaked in 1% poly(ethyleneimine). The radioactivity bound to the filters was measured using a liquid scintillation counter.

4.2.3. Data analysis

Competition binding data were analyzed using nonlinear regression methods. K_i values were Calcd using the Cheng-Prusoff[25] equation based on the measured IC_{50} values and K_d = 10 pM for binding of (±)-[^{3}H]epibatidine and K_d = 1 nM for [^{3}H]MLA. The K_d values were obtained from five independent experiments performed on the same membrane preparations that were used for the competition assays.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.04.050.

References and notes

1. Lippiello, P. M.; Bencherif, M.; Hauser, T. A.; Jordan, K. G.; Letchworth, S. R.; Mazurov, A. A. *Expert Opin. Drug Discov.* **2007**, *2*, 1185.
2. Romanelli, M. N.; Gratteri, P.; Guandalini, L.; Martini, E.; Bonaccini, C.; Gualtieri, F. *ChemMedChem* **2007**, *2*, 746.
3. Jensen, A. A.; Frølund, B.; Liljefors, T.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2005**, *48*, 4705.
4. Edelstein, S. J.; Changeux, J. P. In, Advances in Protein Chemistry, Vol 51, 1998; Vol. 51, 121.
5. Bertrand, D.; Gopalakrishnan, M. *Biochem. Pharmacol.* **2007**, *74*, 1155.
6. Butler, M. S. *Natural Product Reports* **2005**, *22*, 162.
7. Souccar, C.; Salamanca, A. L. V.; Tanae, M. M.; Lima-Landman, M. T. R.; Lapa, A. J. *Journal of Molecular Neuroscience* **2010**, *40*, 138.
8. Cassels, B. K.; Bermúdez, I.; Dajas, F.; Abin-Carriquiry, J. A.; Wonnacott, S. *Drug Discovery Today* **2005**, *10*, 1657.
9. Stromgaard, K. *Chem. Rec.* **2005**, *5*, 229.
10. Coe, J. W.; Brooks, P. R.; Vetelino, M. G.; Wirtz, M. C.; Arnold, E. P.; Huang, J. H.; 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.; O'Neill, B. T. *J. Med. Chem.* **2005**, *48*, 3474.
11. Kochanowska-Karamyan, A. J.; Hamann, M. T. *Chemical Reviews* **2010**, *110*, 4489.
12. Gul, W.; Hamann, M. T. *Life Sci.* **2005**, *78*, 442.
13. Peters, L.; Wright, A. D.; Kehraus, S.; Gündisch, D.; Tilotta, M. C.; König, G. M. *Planta Med.* **2004**, *70*, 883.
14. Sala, F.; Mulet, J.; Reddy, K. P.; Bernal, J. A.; Wikman, P.; Valor, L. M.; Peters, L.; König, G. M.; Criado, M.; Sala, S. *Neurosci. Lett.* **2005**, *373*, 144.
15. Kim, J. S.; Padnya, A.; Weltzin, M.; Edmonds, B. W.; Schulte, M. K.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4855.
16. Weltzin, M. M.; Schulte, M. K. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 917.
17. Benson, S. C.; Lee, L.; Yang, L.; Snyder, J. K. *Tetrahedron* **2000**, *56*, 1165.
18. Austin, J. F.; Kim, S. G.; Sinz, C. J.; Xiao, W. J.; MacMillan, D. W. C. *Proc. Nat. Acad. Sci. U.S.A.* **2004**, *101*, 5482.
19. Chefer, S. I.; Horti, A. G.; Koren, A. O.; Gündisch, D.; Links, J. M.; Kurian, V.; Dannals, R. F.; Mukhin, A. G.; London, E. D. *NeuroReport* **1999**, *10*, 2715.
20. Gohlke, H.; Gündisch, D.; Schwarz, S.; Seitz, G.; Tilotta, M. C.; Wegge, T. *J. Med. Chem.* **2002**, *45*, 1064.
21. Mukhin, A. G.; Gündisch, D.; Horti, A. G.; Koren, A. O.; Tamagnan, G.; Kimes, A. S.; Chambers, J.; Vaupel, D. B.; King, S. L.; Picciotto, M. R.; Innis, R. B.; London, E. D. *Mol. Pharmacol.* **2000**, *57*, 642.
22. Dwoskin, L. P.; Joyce, B. M.; Zheng, G.; Neugebauer, N. M.; Manda, V. K.; Lockman, P.; Papke, R. L.; Bardo, M. T.; Crooks, P. A. *Biochem. Pharmacol.* **2007**, *74*, 1271.
23. Gündisch, D.; London, E. D.; Terry, P.; Hill, G. R.; Mukhin, A. G. *NeuroReport* **1999**, *10*, 1631.
24. Imming, P.; Klaperski, P.; Stubbs, M. T.; Seitz, G.; Gündisch, D. *Eur. J. Med. Chem.* **2001**, *36*, 375.
25. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.