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Deconstruction of the $\alpha 4\beta 2$ Nicotinic Acetylcholine (nACh) Receptor Positive Allosteric Modulator *des*-Formylflustrabromine (dFBr)

Nadezhda German¹, Jin-Sung Kim¹, Atul Jain¹, Malgorzata Dukat¹, Anshul Pandya^{2,a}, Yilong Ma³, Maegan Weltzin², Marvin K. Schulte², and Richard A. Glennon^{1,*}

¹Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University; Richmond, VA 23298

²Department of Chemistry and Biochemistry, Institute of Arctic Biology, University of Alaska Fairbanks; Fairbanks, AK 99775-7000

³Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK 99775-7000, USA

Abstract

des-Formylflustrabromine (dFBr; **1**), perhaps the first selective positive allosteric modulator of $\alpha 4\beta 2$ neuronal nicotinic acetylcholine (nACh) receptors, was deconstructed to determine which structural features contribute to its actions on receptors expressed in *Xenopus* oocytes using 2-electrode voltage clamp techniques. Although the intact structure of **1** was found optimal, several deconstructed analogs retained activity. Neither the 6-bromo substituent nor the entire 2-position chain is required for activity. In particular, reduction of the olefinic side chain of **1**, as seen with **6**, not only resulted in retention of activity/potency but in enhanced selectivity for $\alpha 4\beta 2$ versus $\alpha 7$ nACh receptors. Pharmacophoric features for the allosteric modulation of $\alpha 4\beta 2$ nACh receptors by **1** were identified.

Introduction

The neurotransmitter acetylcholine (ACh) has been implicated in several neurological and neuropsychiatric disorders, such as Alzheimer's disease, autism, and schizophrenia, and might play a role in cognition, memory, and pain.^{1–3} Receptors for ACh are of two broad types: muscarinic receptors and nicotinic receptors.^{1,4} Muscarinic receptors (m1-m5) are G-protein coupled, whereas nicotinic acetylcholine (nACh) receptors are associated with ion channels and are composed of five membrane-spanning subunits.^{1,4} Neuronal nACh receptors, an object of the present study, consist of either α or α and β subunits; multiple neuronal nACh receptor types are possible because several isoforms of α and β subunits exist ($\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$).⁴ The two most prevalent neuronal nACh receptor subtypes in human brain are the heteromeric $\alpha 4\beta 2$ receptors and the homomeric $\alpha 7$ receptors. A large number of nACh receptor agonists and antagonists have been identified [reviewed: 4], but a persistent problem is their low (or lack of) selectivity amongst the various nACh receptor subtypes.

*Corresponding author: Department of Medicinal Chemistry, School of Pharmacy, Box 980540 Virginia Commonwealth University, Richmond, VA 23298., Phone: (804)-828-8487, glennon@vcu.edu.

^aCurrent Address: Laboratory of Neurobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services; Research Triangle Park, NC 27709, USA

Apart from direct-acting agonists and competitive antagonists that bind at the same (i.e., orthosteric) site as ACh, an alternate approach to influence or modulate the actions of ACh is *via* allosteric modulation of its receptors.^{e.g. 2,3,5} Positive allosteric modulators, for example, can enhance the actions of an endogenous ligand (e.g. a neurotransmitter such as ACh) by interaction at a binding site distinct from the orthosteric site. By definition, endogenous neurotransmitters and other orthosteric ligands normally do not interact with high affinity at an allosteric binding site(s) and, conversely, allosteric ligands do not bind with high affinity at the orthosteric site. Consequently, due to lack of suitable design templates, it is virtually impossible, in the absence of any other available information (e.g. on some other allosteric agent that might bind in a similar fashion), to design allosteric agents *de novo*. Yet, the availability of allosteric agents can be advantageous because they typically fail to desensitize receptors, are usually active only in the presence of the orthosteric agonist, and might achieve selectivity of effect if different subtypes of receptors possess different allosteric binding sites.³

Several years ago, a positive allosteric modulator of $\alpha 4\beta 2$ nACh receptors was serendipitously discovered: *des*-formylfluorabromine (dFBr; **1**).^{6,7} Initially isolated from a North Sea marine organism,⁶ **1** was later synthesized as a water soluble salt.⁸ In addition to increasing whole cell current when co-applied with effective concentrations of ACh to $\alpha 4\beta 2$ -containing preparations, **1** failed to potentiate the actions of ACh at $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 4\beta 4$, and $\alpha 7$ nACh receptors expressed in *Xenopus* oocytes.⁷⁻⁹ As such, **1** can now serve as a template for the development of newer, more selective agents.

Because of its actions as a positive allosteric modulator, *des*-formylfluorabromine (**1**) represents a novel lead structure; however, nothing is known about its structure-activity relationships for producing this effect. In order to understand which structural features of *des*-formylfluorabromine (**1**) are required for, or contribute to, this action, the structure of **1** was “deconstructed”. Other goals are to identify a pharmacophore for the action of **1** as a positive allosteric modulator at $\alpha 4\beta 2$ nACh receptors and, more long-term, to identify structures that retain this action but more readily lend themselves to convenient scale-up synthesis in order to have available sufficient quantities of compound for *in vivo* behavioral (and other) studies. We began our investigation by attempting to determine exactly what it is, structurally, that makes *des*-formylfluorabromine (**1**) a positive allosteric modulator at $\alpha 4\beta 2$ nACh receptors.

Chemistry

Compound **1** (as its HCl salt) was prepared exactly as previously reported.⁸ That is, 6-bromoindole (**13**; Scheme 1) was subjected to a Speeter/Anthony synthesis (i.e., reaction of an indole with oxalyl chloride, treatment of the glyoxylyl chloride product with methylamine followed by reduction of the resulting glyoxylamide with a hydride reducing agent – dimethylethylamine-alane in the case of **1**) to afford tryptamine **3**; protection of the basic amine of **3** with a Boc group (i.e., **14**) followed by prenylation (to **16**), and acidic deprotection provided **1**. The primary amine counterpart of **1** (i.e., **2**) was prepared in the same manner by prenylation of *N*-Boc-protected 6-bromotryptamine, rather than 6-bromo-*N*-methyltryptamine (**14**) used in the synthesis of **1**, followed by de-protection.

Compound **14**,⁸ however, was a useful intermediate for the synthesis of several other desired targets (Scheme 1). Treatment of **14** with *tert*-butyl hypochlorite followed by allyl tributyltin and boron trifluoride afforded intermediate **15**. Reduction of the double bond of **15** using sodium borohydride and cobalt chloride,¹⁰ followed by de-protection, provided **4**.

De-protection of **15** without the intervening reduction step should have afforded **5**. It appears, however, that some double-bond migration occurred from the 2'-position to the 1'-position. That is, although elemental analysis for **5** was correct, the ¹H-NMR spectrum of the product showed several minor spurious peaks, such as, for example, a small doublet of doublets at δ 1.85 (presumably a C-CH₃ signal). Elemental analysis and integration of the ¹H-NMR signals indicated that two different positional isomers with the same elemental composition were obtained with 86% of the product being the desired target **5** and 14% being the more conjugated 1'-alkenyl derivative. Neither isomer could be obtained in pure form. Compound **15** was also employed in the synthesis of **10** (Scheme 1); olefinic reduction of **15** and hydrogenolysis of the halogen afforded **10** following de-protection.

The double bond of **16**⁸ was reduced using the same (i.e., sodium borohydride and cobalt chloride) reduction procedure described above (to yield **17**), and de-protection of **17** afforded the desired **6** (Scheme 1).

The *des*-bromo analog of **1** (i.e. **7**) was obtained from **18** (Scheme 2). Compound **18** was prepared in modest yield according to a reported procedure.¹¹ However, upon changing the reaction solvent from dioxane to methylene chloride, the product could be obtained in 80% yield as a white solid (mp 86–89 °C) with a 3-h reaction time relative to that reported with dioxane (15 h). Compound **8** was prepared from the same intermediate (i.e. **19**) except that the double bond was catalytically reduced prior to de-protection.

Compounds **9** and **12** were prepared in a similar fashion (shown for **12** in Scheme 3). That is, the requisite indole (e.g. **23**) was treated with 1-dimethylamino-2-nitroethylene to afford nitroalkene **24** which was readily reduced to primary amine **25**; acylation of **25** using ethyl chloroformate followed by reduction of the resulting carbamate **26** using lithium aluminum hydride provided the desired target **12**. Compound **9** was prepared from 2-*sec*-butylindole (**21**) in like manner. The tertiary butyl derivative **11** was prepared via a classical Speeter/Anthony synthesis from 2-*tert*-butylindole.

Results and Discussion

A near-maximal effect in the present functional assays was consistently observed at an ACh concentration of 10 μ M. The deconstructed analogs of **1** were examined using two-electrode voltage clamp recordings with $\alpha 4\beta 2$ nACh receptors expressed in *Xenopus* oocytes in the presence and absence of this concentration of ACh. None of the analogs produced an effect in the absence of ACh. Several of the deconstructed analogs of **1** potentiated the effect

achieved by ACh (Table 1). As with **1** itself, all deconstructed analogs that potentiated the action of ACh produced an inhibitory effect at high concentrations (representative tracings are shown in Figures 1 and 2). The mechanism underlying this action requires further investigation; however, data obtained with **1** suggest that the mechanism of the inhibitory component might be due to open channel blockade.⁹

At concentrations slightly higher than those required to potentiate its ACh-enhancing action in the $\alpha 4\beta 2$ receptor preparation, **1** inhibited (but failed to potentiate) the action of ACh in an $\alpha 7$ nACh receptor preparation.⁸ As an initial measure of selectivity, the compounds prepared in this investigation were also examined at $\alpha 7$ nACh receptors in functional assays (Table 2).

Effect of deconstructed analogs of **1** on $\alpha 4\beta 2$ nACh receptor function

The pEC₅₀ for **1** (6.48; Table 1; EC₅₀ ca. 0.32 μ M) for the potentiation of 100 QM ACh was quite comparable to what we originally reported (pEC₅₀ ca. 6.8; EC₅₀ = 0.12 μ M);⁸ the small difference likely reflects experimental variation and the much greater number of determinations that now have been performed with **1**. The *N*-des-methyl primary amine counterpart of **1** (i.e., **2**; pEC₅₀ = 6.29; Table 1) was found to be about half as potent as **1** as a positive allosteric modulator at $\alpha 4\beta 2$ receptors. Hence, all subsequent compounds retained the *N*-methyl group associated with **1**.

The next several compounds addressed the role of the indole 2-position substituent. Complete elimination of the 2-position substituent (i.e., *N*-methyl-6-bromotryptamine; **3**) resulted in a compound that failed to enhance the action of ACh at the highest concentrations at which it was evaluated (Table 1). Similar results were obtained with the simple *n*-propyl analog **4** (Table 1). Either unsaturation and/or at least one of the *gem* dimethyl groups is important for the ability of **1** to behave as a positive allosteric modulator at $\alpha 4\beta 2$ nACh receptors. The propenyl compound **5**, lacking the *gem* dimethyl groups of **1**, also lacked potentiating activity, whereas the saturated *gem* dimethyl analog of **1**, **6** (pEC₅₀ = 6.86), was at least as potent, if not twice as potent, as **1**. Evidently, the *gem*-dimethyl groups, but not the unsaturation associated with the chain, is required for allosteric action.

Before continuing to investigate 2-position substitution, the necessity of the 6-bromo group was examined. *des*-Bromo **1** and *des*-bromo **6** (i.e., **7** and **8**; pEC₅₀ = 5.14 and 5.52, respectively) (Table 1) retained the allosteric action of their parents but were substantially less potent. Apparently, the bromo group contributes to potency, but its presence is not

required for the compounds to behave as positive allosteric modulators at $\alpha 4\beta 2$ nACh receptors.

Further deconstruction of **7** and **8** provided analogs **9–12**. Again suggesting that the *gem*-dimethyl groups are important, the racemic monomethyl counterpart of **8** (i.e., **9**) (Table 1) was inactive (as a positive allosteric modulator). This is further supported by the inactivity of **10**. Interestingly, although the inactive compound **12** possesses a *gem*-dimethyl group, compound **11** ($pEC_{50} = 5.40$) is active (Table 1). It would seem that it is not simply the presence of the *gem*-dimethyl group but, rather, the quaternary nature of this carbon atom that contributes to activity.

Effect of deconstructed analogs of **1** on blocking the functional effects of ACh at $\alpha 4\beta 2$ nACh receptors

As previously shown for **1**,⁸ high concentrations of all the deconstructed compounds blocked the action of 10 μ M ACh at $\alpha 4\beta 2$ nACh receptors. For those compounds acting as positive allosteric modulators, pIC_{50} values could not be determined with accuracy. pIC_{50} values for compounds not acting as positive allosteric modulators ranged from 5.45 for **5** to 4.14 for **4**; additional results are shown in Table 1. The mechanism(s) whereby these analogs are able to functionally antagonize the actions of ACh at $\alpha 4\beta 2$ receptors remains to be elucidated.

Effect of deconstructed analogs of **1** on $\alpha 7$ nACh receptor function

As an initial measure of selectivity, compounds were examined for their ability to potentiate the electrophysiological effects of ACh at $\alpha 7$ receptors. None proved to be active at the highest concentrations examined. However, all of the examined compounds inhibited the action of 10 μ M ACh at $\alpha 7$ receptors (Table 2). Interestingly, whereas **1** attenuated the action of ACh at $\alpha 7$ receptors at a concentration of only six times greater than that required for potentiation of ACh at $\alpha 4\beta 2$ receptors, its reduced counterpart **6** was 190- fold selective for the latter.

Conclusion

Deconstruction of the structure of the positive allosteric modulator **1** has shown, although several “deconstructed” analogs retained activity, that the intact structure of **1** seems optimal as a positive allosteric modulator at $\alpha 4\beta 2$ nACh receptors. The reduced counterpart of **1** (i.e., **6**), retains the action and potency of **1**; however, **6** offers no synthetic advantage in that it was synthesized by reduction of **1**. Also learned was that a) *N*-demethylation of **1** to its primary amine reduces potency by 50%, b) the 6-bromo group of **1** (and **6**) contributes to potency, not action, c) the presence of the *gem*-dimethyl groups plays an important role in conferring activity, and d) it is likely that the quaternary nature of the branched carbon atom, not simply the presence of *gem*-dimethyl groups, defines this action. Finally, as with **1**,⁸ analogs **2–11** were capable of attenuating the action of ACh at $\alpha 7$ receptors; however, compound **6** was considerably more selective than **1** as a positive allosteric modulator at $\alpha 4\beta 2$ receptors. This might be an advantage for functional studies where both receptor types are present. In summary, structural features of **1** important for its action and potency as a positive allosteric modulator at $\alpha 4\beta 2$ nACh receptors were determined, pharmacophoric features were identified, and **6** was found to be at least as potent as, and more selective than, **1** in this regard.

Experimental Section

Chemistry

Melting points were taken in glass capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were recorded either with a Varian EM-390 or Bruker 400 MHz spectrometer and peak position are given in parts per million (δ) downfield from tetramethylsilane as an internal standard. Purity of compounds (>95%) was established by elemental analysis; microanalyses were performed by Atlantic Microlab (GA) for the indicated elements, and the results are within 0.4% of theoretical values. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F₂₅₄ Merck plates, and chromatographic separations were performed using Aldrich Silica gel 60 columns unless otherwise stated.

6-Bromo-2-(1,1-dimethylallyl)tryptamine Hydrochloride (2)

Following the addition of *tert*-BuOCl (0.10 g, 0.9 mmol) to a stirred solution of *tert*-butyl-2-(6-bromo-1*H*-indol-3-yl)ethylcarbamate¹² (0.25 g, 0.7 mmol) and Et₃N (0.90 g, 0.9 mmol) in THF (10 mL) at -78°C , stirring was allowed to continue for 45 min. Freshly prepared prenyl 9-BBN (1.5 mmol)¹³ was added in a dropwise manner over a 20-min period while maintaining temperature at -78°C . The reaction mixture was allowed to warm to room temperature and stirring was continued for an additional 2 h. Aqueous NaOH (3M, 3 mL) and H₂O₂ (30%, 3 mL) were added in a dropwise fashion and stirring continued for another 1 h. The reaction mixture was diluted with Et₂O (100 mL), the organic portion was washed with H₂O (3 $\times$ 30 mL), brine (40 mL), and dried (Na₂SO₄). The solution was evaporated to dryness and the residue was purified by chromatography using hexanes/EtOAc (10:1) as eluent to afford 0.10 g (33%) of a white foam.

Gaseous HCl was bubbled into a stirred solution of the above compound (0.09 g, 0.22 mmol) in dry EtOAc (10 mL) at 0°C ; stirring was allowed to continue for 24 h and the solvent was evaporated to yield a white solid. Recrystallization from EtOAc/MeOH provided 0.03 g (33%) of **2** as white crystals: mp $256\text{--}258^\circ\text{C}$; ^1H NMR (DMSO-*d*₆) δ 1.49 (s, 6H, 2CH₃), 2.85 (t, 2H, CH₂), 3.05 (t, 2H, CH₂), 5.05–5.10 (m, 2H, CH), 6.10–6.17 (m, 1H, CH), 7.12 (dd, 1H, ArH), 7.48–7.51 (m, 2H, ArH), 7.98 (br s, 3H, NH₃⁺), 10.76 (s, 1H, NH). Anal. Calcd for (C₁₅H₂₀BrN₂·HCl·0.25 H₂O) C, H, N.

N-Methyl-6-bromo-2-*n*-propyltryptamine Oxalate (4)

According to the procedure described for preparation of **17**, compound **15** (0.50 g, 1.27 mmol) was treated with NaBH₄ (0.10 g, 2.54 mmol) and CoCl₂·6H₂O (0.3 g, 1.27 mmol) to give 0.40 g (78%) of the reduced compound as a white solid: mp $157\text{--}159^\circ\text{C}$; ^1H NMR (CDCl₃) δ 1.01 (t, $J = 7.2$ Hz, 3H, CH₃), 1.42 (s, 9H, *t*-Bu), 1.62–1.77 (m, 2H, CH₂), 2.63–2.78 (m, 2H, CH₂), 2.80–2.96 (m, 2H, CH₂), 2.88 (s, 3H, N-CH₃), 3.33–3.46 (m, 2H, CH₂), 2.20 (dd, $J = 1.5, 8.4$ Hz, 1H, ArH), 7.35–7.47 (m, 2H, ArH), 7.86 (br s, 1H, NH).

The reduced product was deprotected as follows: TFA (0.8 mL) was added to a solution of the above compound (0.13 g, 0.33 mmol) in CH₂Cl₂ (5 mL) at room temperature and the reaction mixture was allowed to stir for 0.5 h. An additional 10 mL of CH₂Cl₂ was added; the solution was cooled to 0°C , treated with a saturated solution of NaHCO₃, and extracted with EtOAc (3 $\times$ 10 mL). The combined organic portion was dried (Na₂SO₄) and the solvent was removed. The crude product was purified by column chromatography (CH₂Cl₂/MeOH/NH₄OH; 9:1:0.1) to give 0.10 g (98%) of the free base of **4** as a brown oil: ^1H NMR (CDCl₃) δ 0.96 (t, $J = 7.2$ Hz, 3H, CH₃), 1.59–1.74 (m, 2H, CH₂), 2.52 (s, 3H, N-CH₃), 2.63–2.73 (m, 2H, CH₂), 2.86–3.03 (m, 4H, 2CH₂), 4.65 (br s, 1H, amine NH), 7.16 (dd, $J = 1.8, 8.4$ Hz, 1H, ArH), 7.35–7.43 (m, 2H, ArH), 8.25 (br s, 1H, NH). The free base in Et₂O

(5 mL) was treated with ethereal oxalic acid. The precipitated oxalate salt was collected by filtration, washed with anhydrous Et₂O (3 × 5 mL), and recrystallized from absolute EtOH/anhydrous Et₂O to afford 0.05 g (37%) of the salt as a off-white solid: mp 211–213 °C; ¹H NMR (DMSO-*d*₆) δ 0.91 (t, *J* = 7.2 Hz, 3H, CH₃), 1.58–1.72 (m, 2H, CH₂), 2.60 (s, 3H, N-CH₃), 2.63–2.69 (m, 2H, CH₂), 2.91–3.04 (m, 4H, 2CH₂), 7.09 (dd, *J* = 1.5, 8.4 Hz, 1H, ArH), 7.41–7.48 (m, 2H, ArH), 11.09 (br s, 1H, NH). Anal. Calcd for (C₁₄H₁₉BrN₂·C₂H₂O₄) C, H, N.

***N*-Methyl-2-allyl-6-bromotryptamine Oxalate (5)**

According to the procedure described for preparation of **4**, compound **15** (0.07 g, 0.17 mmol) was treated with TFA (0.5 mL) to give 0.04 g (74%) of the free base **5** as a pale-yellow oil which was treated with ethereal oxalic acid to afford the oxalate salt. Recrystallization from absolute EtOH/anhydrous Et₂O afforded 0.02 g (24%) of product as a pale-yellow solid: mp 198–203 °C (dec.); ¹H NMR (DMSO-*d*₆) δ 2.62 (s, 3H, N-CH₃), 2.91–3.08 (m, 4H, 2CH₂), 3.50 (d, *J* = 6.0 Hz, 2H, allylic CH₂), 5.08–5.20 (m, 2H, vinylic H), 5.99 (m, 1H, vinylic H), 7.13 (br d, *J* = 8.7 Hz, 1H, ArH), 7.43–7.53 (m, 2H, ArH), 11.09 (br s, 1H, NH). Anal. Calcd for (C₁₄H₁₇BrN₂·C₂H₂O₄) C, H, N. Note: subsequent studies showed this product to be an isomeric mixture of positional isomers (see text).

***N*-Methyl-6-bromo-2-(1,1-dimethylpropyl)tryptamine Oxalate (6)**

Sodium borohydride (0.02 g, 0.37 mmol) was added in portions to a solution of **16**⁸ (0.08 g, 0.18 mmol) and CoCl₂·6H₂O (0.04 g, 0.18 mmol) in EtOH (2 mL) at 0 °C. The reaction mixture was allowed to stir under a N₂ atmosphere at room temperature for 1 h and quenched by addition of H₂O (5 mL). The aqueous solution was extracted with Et₂O (3 × 10 mL). The combined organic portion was dried (Na₂SO₄) and solvent was removed under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc; 5:1) to give 0.06 g (76%) of **17** as a white solid: mp 154–157 °C; ¹H NMR (CDCl₃) δ 0.79 (t, *J* = 7.2 Hz, 3H, CH₃), 1.47 (s, 6H, 2CH₃), 1.52 (s, 9H, *t*-Bu), 1.78 (q, *J* = 7.2 Hz, 2H, CH₂), 2.93 (s, 3H, N-CH₃), 3.02–3.09 (m, 2H, CH₂), 3.36–3.46 (m, 2H, CH₂), 7.19 (dd, *J* = 1.5, 8.4 Hz, 1H, ArH), 7.41–7.48 (m, 2H, ArH), 7.92 (br s, 1H, NH).

The reduced product (0.08 g, 0.18 mmol), was treated with TFA (0.45 mL) to give the free base of **6** (0.06 g, 96%) as a brown oil which was converted to the oxalate salt: mp 192–194 °C following recrystallization from absolute EtOH/anhydrous Et₂O; ¹H NMR (DMSO-*d*₆) δ 0.68 (t, *J* = 7.2 Hz, 3H, CH₃), 1.39 (s, 6H, 2CH₃), 1.70 (q, *J* = 7.2 Hz, 2H, CH₂), 2.63 (s, 3H, N-CH₃), 2.90–3.01 (m, 2H, CH₂), 3.06–3.16 (m, 2H, CH₂), 7.10 (dd, *J* = 1.5, 8.7 Hz, 1H, ArH), 7.44 (d, *J* = 1.5 Hz, 1H, ArH), 7.48 (d, *J* = 8.7 Hz, 1H, ArH), 10.70 (br s, 1H, NH). Anal. Calcd for (C₁₆H₂₃BrN₂·C₂H₂O₄) C, H, N.

***N*-Methyl-2-(1,1-dimethylallyl)tryptamine Hydrochloride (7)**

tert-Butyl hypochlorite (0.19 mL, 2.15 mmol) was added in a dropwise manner to a stirred solution of **18**¹¹ (0.49 g, 1.8 mmol) and Et₃N (0.30 mL, 2.2 mmol) in anhydrous THF (7.0 mL) at –78 °C. The clear solution was allowed to stir for an additional 0.5 h before freshly prepared prenyl 9-BBN¹³ (0.68 g, 3.59 mmol) solution in THF was added in a dropwise manner. After 30 min the reaction mixture was allowed to warm to room temperature, and stirring was continued for 1 h. The addition of 3M NaOH (1.8 mL) and 30% H₂O₂ (1.8 mL) was followed by stirring for 1 h. The reaction mixture was diluted with Et₂O (30 mL), the organic layer was washed with 3M NaCl solution (3 × 50 mL), dried (Na₂SO₄), and solvent was removed under reduced pressure. The resultant residue was subjected to flash chromatography (hexanes/EtOAc; 10:1) to give 0.30 g (40%) of **19** as an oil which crystallized upon standing: mp 141–143 °C; ¹H NMR (CDCl₃): δ 1.51 (s, 9H, Boc), 1.58 (s, 6H, 2CH₃), 2.94 (s, 3H, N-CH₃), 2.93–3.10 (m, 2H, CH₂), 3.38–3.52 (m, 2H, CH₂), 5.18–

5.22 (m, 2H, vinylic H), 6.16 (dd, $J = 10.5, 17.1$ Hz, 1H, vinylic H), 7.16 (m, 2H, ArH), 7.30–7.35 (m, 1 H, ArH), 7.61 (m, 1H, ArH), 7.91 (br s, 1H, NH).

Gaseous HCl was bubbled through a solution of **19** (0.10 g, 0.29 mmol) in anhydrous EtOAc (10 mL). The salt was recrystallized from MeOH/anhydrous Et₂O to afford 0.04 g (50%) of **7** as an off-white solid: mp 223–225 °C; ¹H NMR (DMSO-*d*₆) δ 1.56 (s, 6H, 2CH₃), 2.61 (s, 3H, N-CH₃), 2.90–3.00 (m, 2H, CH₂), 3.02–3.12 (m, 2H, CH₂), 5.12 (dd, $J = 1.2, 17.0$ Hz, 2H, vinylic H), 6.16 (dd, $J = 10.5, 17.0$ Hz, 1H, vinylic H), 6.88–7.08 (m, 2H, ArH), 7.30 (d, $J = 8.0$ Hz, 1H, ArH), 7.59 (d, $J = 8.0$ Hz, 1H, ArH), 10.61 (br s, 1H, NH). Anal. Calcd for (C₁₆H₂₂N₂·HCl) C, H, N.

***N*-Methyl-2-(1,1-dimethylpropyl)tryptamine Hydrochloride (8)**

A catalytic amount of 10% Pd/C (0.02 g) was added to **19** (0.09 g, 0.26 mmol) in MeOH (15 mL) and hydrogenated at ca. 50 psi for 3 h. The catalyst was removed by filtration, and the solvent was removed under reduced pressure. Compound **20** (0.09 g, 92%; mp 172–174 °C) was obtained as an off-white solid and used without further purification.

Gaseous HCl was bubbled through a solution of **20** (0.09 g, 0.25 mmol) in dry EtOAc (10 mL). The salt was recrystallized from MeOH/anhydrous Et₂O to afford 0.04 g (50%) of **8** as an off-white solid: mp 234–235 °C; ¹H NMR (DMSO-*d*₆) δ 0.69 (t, $J = 7.5$ Hz, 3H, CH₃), 1.41 (t, $J = 12$ Hz, 6H, 2CH₃), 1.73 (q, $J = 7.5$ Hz, 2H, CH₂), 2.58 (s, 3H, N-CH₃), 2.87–3.00 (m, 2H, CH₂), 3.05–3.23 (m, 2H, CH₂), 6.88–7.08 (m, 2H, ArH), 7.30 (d, $J = 7.5$ Hz, 1H, ArH), 7.59 (d, $J = 7.5$ Hz, 1H, ArH), 10.51 (br s, 1H, NH). Anal. Calcd for (C₁₆H₂₄N₂·HCl·0.25 H₂O) C, H, N.

***N*-Methyl-2-sec-butyltryptamine Oxalate (9)**

Ethyl chloroformate (0.06 mL, 0.65 mmol) was added in a dropwise manner to a solution of **22** (0.14 g, 0.65 mmol) in CHCl₃ (5 mL) at 0 °C, followed by addition of aqueous 4M NaOH (0.17 mL, 0.65 mmol). The reaction mixture was allowed to stir at room temperature for 2 h and diluted with CHCl₃ (20 mL). The organic portion was separated, washed with H₂O (20 mL), dried (Na₂SO₄), and solvent was removed under reduced pressure to afford 0.14 g (75%) of *N*-Boc-**22** as a brown oil: ¹H NMR (CDCl₃) δ 0.95 (t, $J = 6$ Hz, 3H, CH₃), 1.21–1.61 (m, 6H, 2CH₃), 1.62–2.12 (m, 2H, CH₂), 3.03 (m, 3H, CH₂, CH), 3.32–3.70 (m, 2H, CH₂), 3.95–4.30 (m, 2H, CH₂), 7.13–7.21 (m, 2H, ArH), 7.38 (d, $J = 9$ Hz, 1H, ArH), 7.62 (d, $J = 9$ Hz, 1H, ArH), 8.33 (br s, 1H, NH). The crude reaction product was directly used in the next step.

A solution of the above product (0.14 g, 0.48 mmol) in anhydrous THF (5 mL) was added in a dropwise manner to a stirred suspension of LiAlH₄ (0.11 g, 3 mmol) in dry THF (10 mL) at 0 °C. The stirred mixture was heated at reflux under a N₂ atmosphere for 2 h, cooled to 0 °C, and successively quenched with MeOH (1 mL), H₂O (1.5 mL), NaOH (3M, 1 mL) and diluted with CH₂Cl₂ (10 mL). The organic portion was dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was purified on a silica gel column using CH₂Cl₂/MeOH (9:1) → CH₂Cl₂/MeOH/Et₃N (9:1:0.1) as eluent to afford the amine as a brown oil. The oxalate salt was prepared and recrystallized from MeOH/anhydrous Et₂O to give 0.08 g (48%) of **9** as a beige solid: mp 180–181 °C; ¹H NMR (DMSO-*d*₆) δ 0.79 (t, $J = 6.9$ Hz, 3H, CH₃), 1.29 (d, $J = 6.9$ Hz, 3H, CH₃), 1.57–1.72 (m, 2H, CH₂), 2.57–2.67 (m, 4H, 2CH₂), 2.62 (s, 3H, CH₃), 2.93–3.00 (m, 1H, CH), 6.95–7.08 (m, 2H, ArH), 7.29 (d, $J = 7.5$ Hz, ArH), 7.50 (d, $J = 7.5$ Hz, 1H, ArH), 10.79 (s, 1H, NH₃⁺). Anal. Calc for (C₁₅H₂₂N₂·C₂H₄O₄·0.25 H₂O) C, H, N.

N-Methyl-2-(*n*-propyl)tryptamine Oxalate (10)

A suspension of **4** (0.13 g, 0.33 mmol) in absolute EtOH (10 mL) was hydrogenated in the presence of 10% Pd/C (0.03 g) under a H₂ atmosphere (45 psi) at room temperature for 1 h. The catalyst was removed by filtration and the filtrate was evaporated to give 0.10 g (100%) of a cream-colored solid: mp 142–144 °C; ¹H NMR (CDCl₃) δ 1.01 (t, *J* = 7.5 Hz, 3H, CH₃), 1.44 (s, 9H, 3CH₃), 1.64–1.80 (m, 2H, CH₂), 2.74 (t, *J* = 7.8 Hz, 2H, CH₂), 2.82–3.00 (m, 2H, CH₂), 2.90 (s, 3H, N-CH₃), 3.43 (t, *J* = 7.8 Hz, 2H, CH₂), 7.10 (dt, *J* = 1.5, 6.9 Hz, 1H, ArH), 7.14 (dt, *J* = 1.5, 6.9 Hz, 1H, ArH), 7.30 (dd, *J* = 1.5, 6.9 Hz, 1H, ArH), 7.56 (m, 1H, ArH), 7.83 (br s, 1H, NH).

Using the procedure described for **4**, the amine (0.1 g, 0.31 mmol) was treated with TFA (0.8 mL) to give 0.06 g (93%) of a pale-yellow oil: ¹H NMR (CDCl₃) δ 1.01 (t, *J* = 7.5 Hz, 3H, CH₃), 1.64–1.78 (m, 2H, CH₂), 2.47 (s, 3H, N-CH₃), 2.75 (t, *J* = 7.5 Hz, 2H, CH₂), 2.83–3.00 (m, 4H, 2CH₂), 7.10 (dt, *J* = 1.5, 6.9 Hz, 1H, ArH), 7.15 (dt, *J* = 1.5, 6.9 Hz, 1H, ArH), 7.30 (m, 1H, ArH), 7.58 (dd, *J* = 1.5, 6.9 Hz, 1H, ArH), 8.00 (br s, 1H, NH). The free base in Et₂O (5 mL) was treated with an ethereal solution of oxalic acid to afford 0.07 g (69%) of **10**: mp 177–179 °C after recrystallization from absolute EtOH/anhydrous Et₂O; ¹H NMR (DMSO-*d*₆) δ 0.92 (t, *J* = 7.2 Hz, 3H, CH₃), 1.56–1.74 (m, 2H, CH₂), 2.60 (s, 3H, N-CH₃), 2.67 (t, *J* = 7.5 Hz, 2H, CH₂), 2.88–3.06 (m, 4H, 2CH₂), 6.91–7.04 (m, 2H, 2ArH), 7.25 (br d, *J* = 7.8 Hz, 1H, ArH), 7.48 (br d, *J* = 7.8 Hz, 1H, ArH), 10.85 (br s, 1H, NH). Anal. Calcd for (C₁₄H₂₀N₂·C₂H₂O₄) C, H, N.

N-Methyl-2-(*tert*-butyl)tryptamine Hydrogen Oxalate (11)

Oxalyl chloride (0.2 mL, 2.308 mmol) was added in a dropwise manner at –5 °C to a stirred solution of 2-*tert*-butyl-1H-indole¹⁴ (0.02 g, 1.154 mmol) in anhydrous Et₂O (10 mL). The reaction mixture was allowed to stir for 6 h at 0 °C; after evaporation of solvent under reduced pressure, methylamine (40% aqueous solution, 5 mL) was added at room temperature, and the reaction mixture was allowed to stir overnight. The precipitate was collected by filtration and recrystallized from MeOH to afford 0.17 g (56%) of the corresponding glyoxylamide as brown solid: mp 190–191 °C; ¹H NMR (CDCl₃) δ 1.46 (s, 9H, 3CH₃), 3.1 (s, 3H, N-CH₃), 6.77 (s, 1H, NH), 7.07–7.45 (m, 4H, ArH), 7.76 (br s, 1H, NH).

A solution of the glyoxylamide (0.17 g, 0.65 mmol) in dioxane (5 mL) was added to a stirred suspension of LiAlH₄ (0.25 g, 6.5 mmol) in dioxane (10 mL) at 60 °C, and then heated at reflux overnight. The reaction mixture was cooled to room temperature and diluted with THF (10 mL), MeOH (3 mL) and NaOH (3N, 3 mL). The mixture was filtered and the filtrate was heated at reflux with THF (15 mL). The combined organic portion was evaporated to dryness under reduced pressure, and the residue was purified by column chromatography using CH₂Cl₂/MeOH (10:1) as eluent to afford the desired amine. The oxalate salt was prepared to give 0.03 g (56%) of **11** as white flakes after recrystallization from 2-PrOH: mp 194–195 °C; ¹H NMR (DMSO-*d*₆) δ 1.43 (s, 9H, 3CH₃), 2.64 (s, 3H, N-CH₃), 2.94 (d, 2H, CH₂), 3.15 (d, 2H, CH₂), 6.96–7.51 (m, 4H, ArH), 10.55 (s, 1H, R₂NH₂⁺COO[–]). Anal. Calcd for (C₁₈H₃₀N₂O⁺(C₂H₂O₄)[–]·2-PrOH) C, H, N.

N-Methyl-2-isopropyltryptamine Hydrochloride (12)

A solution of **26** (0.17 g, 0.61 mmol) in dry THF (5 mL) was added to a stirred suspension of LiAlH₄ (0.14 g, 3.7 mmol) in anhydrous THF (10 mL) at 0 °C, and the reaction mixture was heated at reflux for 2 h, cooled in an ice bath and quenched with MeOH (1 mL), NaOH (15%, 1.5 mL) and H₂O (1 mL). The organic portion was separated, dried (Na₂SO₄), and solvent was evaporated under reduced pressure to yield an oily residue. Purification by column chromatography using CH₂Cl₂/MeOH (9:1) → CH₂Cl₂/MeOH/Et₃N (9:1:0.1)

afforded 0.07 g of the amine as a light-yellow oil: ^1H NMR (DMSO- d_6) δ 1.28 (d, J = 9 Hz, 6H, 2CH₃), 2.51 (s, 3H, CH₃), 2.60–2.70 (m, 2H, CH₂), 2.74–2.81 (m, 2H, CH₂), 3.22 (m, 1H, CH), 6.90–7.00 (m, 2H, ArH), 7.25 (d, J = 9 Hz, 1H, ArH), 7.48 (d, J = 9 Hz, 1H, ArH).

Gaseous HCl was bubbled through a solution of the amine in dry EtOAc (10 mL) to obtain 0.04 g (50%) of **12** as white crystals after recrystallization from MeOH/anhydrous Et₂O: mp 212–213 °C; ^1H NMR (DMSO- d_6) δ 1.30 (d, J = 6.9 Hz, 6H, 2 $\times$ CH₃), 2.59 (s, 3H, CH₃), 2.92–3.05 (m, 4H, 2CH₂), 3.24 (m, 1H, CH), 6.95–7.08 (m, 2H, ArH), 7.29 (d, J = 7.5 Hz, 1H, ArH), 7.53 (d, J = 7.5 Hz, 1H, ArH), 9.00 (s, 1H, NH), 10.87 (br s, 1H, NH₃⁺). Anal. Calcd for (C₁₄H₂₀N₂·HCl) C, H, N.

***N*-[2-(2-Allyl-6-bromo-1*H*-indol-3-yl)ethyl]-*N*-methylcarbamic acid *tert*-butyl ester (**15**)**

tert-Butyl hypochlorite (0.58 mL, 5.10 mmol) was added in a dropwise manner to a stirred solution of **14**⁸ (1.50 g, 4.25 mmol) and Et₃N (0.71 mL, 5.10 mmol) in THF (25 mL) at –78 °C under a N₂ atmosphere. After 1.5 h at –78 °C, allyltributyltin (2.61 mL, 8.49 mmol) was added followed by addition of BF₃·Et₂O (1.1 mL, 8.49 mmol). The reaction mixture was allowed to stir for 0.5 h at the same temperature, and then quenched by addition of saturated aqueous solution of NaHCO₃ (10 mL). Ethyl acetate (100 mL) and H₂O (100 mL) were added, and the aqueous portion was extracted with EtOAc (3 $\times$ 50 mL). The combined organic portion was dried (Na₂SO₄) and solvent was removed under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc; 7:1) to give 0.74 g (44%) of a white solid: mp 138–140 °C. The product was immediately used in the synthesis of **4**.

2-*sec*-Butyltryptamine (22**)**

A solution of 2-*sec*-butylindole (**21**)¹⁴ (0.35 g, 2 mmol) in CH₂Cl₂ (2 mL) was added to a stirred solution of 1-dimethylamino-2-nitroethylene (0.23 g, 2 mmol) in TFA (1.2 mL) at 0 °C. The reaction mixture was allowed to stir for 2 h, poured into ice-cold H₂O (50 mL) and extracted with EtOAc (2 $\times$ 20 mL). The combined organic portion was washed with brine (20 mL), dried (Na₂SO₄) and solvent was removed under reduced pressure. The residue was recrystallized from EtOAc to yield the corresponding nitroalkene (0.27 g, 75% based on recovered starting material) as orange crystals: mp 141 °C; ^1H NMR (CDCl₃) δ 0.94 (t, J = 7.5 Hz, 3H, CH₃), 1.43 (d, J = 7.0 Hz, 3H, CH₃), 1.72–1.84 (m, 2H, CH₂), 3.28 (m, 1H, CH), 7.30–7.34 (m, 2H, ArH), 7.42–7.48 (m, 1H, CH), 7.72–7.78 (m, 1H, CH), 7.87 (d, J = 13 Hz, 1H, ArH), 8.40 (d, J = 13 Hz, 1H, ArH), 8.74 (br s, 1H, NH).

A solution of the alkene (0.27 g, 1.1 mmol) in dry THF (5 mL) was added in a dropwise manner at 0 °C to a stirred suspension of LiAlH₄ (0.26 g, 6.9 mmol) in anhydrous THF (10 mL). The reaction mixture was heated overnight at reflux under a N₂ atmosphere, cooled to 0 °C, quenched with MeOH (1 mL), H₂O (1.5 mL), NaOH (1 mL) and diluted with CH₂Cl₂ (30 mL). After acid (2N HCl, 30 mL)/base (3N NaOH, 30 mL) extraction, the organic portion was separated, dried (Na₂SO₄) and solvent was removed under reduced pressure to give 0.14 g (62%) of **22** as a light-brown oil: ^1H NMR (CDCl₃) δ 0.91 (t, J = 7.5 Hz, 3H, CH₃), 1.33 (d, J = 6.0 Hz, 3H, CH₃), 1.62–1.79 (m, 2H, CH₂), 2.84–2.97 (m, 2H, CH₂), 2.98–3.12 (m, 3H, CH, CH₂), 7.03–7.25 (m, 2H, ArH), 7.33 (d, J = 8.0 Hz, 1H, ArH), 7.82 (br s, 1H, NH).

2-Isopropyl-1*H*-indole (23**)**

A solution of *n*BuLi (1.6 M in hexane; 14 mL, 22.4 mmol) was added to a stirred solution of *N*-(*o*-tolyl)isobutyramide¹⁵ (2.0 g, 11 mmol) in anhydrous THF (20 mL) at 0 °C. After stirring for 1 h, the reaction mixture was allowed to warm gradually to room temperature. After an additional 8 h, EtOAc (15 mL) and then saturated aqueous NH₄Cl (5 mL) were added. The organic portion was separated, dried (Na₂SO₄) and solvent was removed under

reduced pressure. The crude oily residue was purified by column chromatography using hexanes/EtOAc (20:1) as eluent to afford 0.70 g (66 % based on recovered material) of **23** as an off-white solid: mp 75–76 °C (lit.¹⁶ 73–74 °C); ¹H NMR (CDCl₃) δ 1.41 (d, *J* = 6 Hz, 6H, 2 × CH₃), 3.09–3.16 (m, 1H, CH), 6.31 (s, 1H, CH), 7.10–7.16 (m, 2H, ArH), 7.35 (d, *J* = 9 Hz, 1H, ArH), 7.60 (d, *J* = 9 Hz, 1H, ArH), 7.85 (br s, 1H, NH).

2-Isopropyltryptamine (25)

A solution of **23** (0.30 g, 1.9 mmol) in CH₂Cl₂ (2 mL) was added to a stirred solution of 1-dimethylamino-2-nitroethylene (0.22 g, 1.9 mmol) in TFA (1.2 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 2 h, poured into icecold water (20 mL) and extracted with EtOAc (20 mL). The organic portion was washed with brine (15 mL), dried (Na₂SO₄) and the solvent was removed under reduced pressure. The crude product was purified recrystallized from EtOAc to afford 0.18 g (60%) of **24** as orange crystals: mp 159 °C; ¹H NMR (CDCl₃) δ 1.44 (d, *J* = 6.0 Hz, 6H, 2 × CH₃), 3.51–3.62 (m, 1H, CH), 7.28–7.35 (m, 2H, ArH), 7.42–7.46 (m, 1H, CH), 7.72–7.75 (m, 1H, CH), 7.84 (d, *J* = 13 Hz, 1H, ArH), 8.42 (d, *J* = 13 Hz, 1H, ArH), 8.74 (br s, 1H, NH).

A solution of **24** (0.26 g, 1.1 mmol) in anhydrous THF (5 mL) was added in a dropwise manner to a stirred suspension of LiAlH₄ (0.26 g, 6.9 mmol) in THF (10 mL) at 0 °C. The reaction mixture was heated at reflux overnight, cooled in an ice bath, and quenched with MeOH (1 mL), NaOH (15%, 1.5 mL) and H₂O (1 mL). After acid (3N HCl, 10 mL)/base (3N NaOH, 15 mL) extraction, the organic portion was dried (Na₂SO₄) and solvent was removed under reduced pressure to afford 0.14 g (61%) of **25** as a light-yellow oil: ¹H NMR (DMSO-*d*₆) δ 1.28 (d, *J* = 7.5 Hz, 6H, 2CH₃), 1.73 (br s, 2H, NH₂), 2.73–2.84 (m, 4H, 2 × CH₂), 3.21 (m, 1H, CH), 6.89–7.01 (m, 2H, ArH), 7.27 (d, *J* = 7.5 Hz, 1H, ArH), 7.44 (d, *J* = 7.5 Hz, 1H, ArH), 8.78 (br s, 1H, NH). Compound **25** was used immediately in the synthesis of **26**.

Ethyl [2-(2-isopropyl-1*H*-indol-3-yl)ethyl]carbamate (26)

Ethyl chloroformate (0.07 mL, 0.70 mmol) was added in a dropwise manner at 0 °C to a stirred solution of **25** (0.14 g, 0.7 mmol) in CHCl₃ (5 mL), followed by addition of a 4N aqueous solution of NaOH (0.20 mL, 0.70 mmol). The reaction mixture was allowed to stir for 2 h at room temperature and diluted with CHCl₃ (15 mL). The organic portion was dried (Na₂SO₄), and solvent was evaporated under reduced pressure to afford 0.17 g (88%) of **26** as an oily residue which was used in the preparation of **12** without further purification.

Electrophysiology

The cDNA sequences for human α4 (NCBI Reference Sequence: NM_000744.5), β2 (NCBI Reference Sequence: NM_000748.2) and α7 (NCBI Reference Sequence: NM_000746.3) nACh receptor subunits were used to synthesize a full length cDNA for each subunit. cDNA synthesis was conducted by GeneArt Inc. (Burlingame, CA). The synthetic β2 cDNA was inserted into the pcDNA3.1/Zeo(+) expression vector and the α4 cDNA was inserted into the pcDNA3.1/hygromycin expression (vectors procured from Invitrogen, Carlsbad, CA).

Ovarian lobes were surgically removed from *Xenopus laevis* frogs and washed twice in Ca²⁺-free Barth's buffer (82.5 mM NaCl/2.5 mM KCl/1mM MgCl₂/5 mM HEPES, pH 7.4) then gently shaken with 1.5 mg/mL collagenase (Sigma type II, Sigma-Aldrich) for 1 h at 20–25 °C. Stage IV oocytes were selected for microinjection. Synthetic cRNA transcripts for human α7 and α4β2 were prepared using the mMACHINE™ High Yield Capped RNA Transcription Kit (Ambion, TX). Oocytes were injected with a total of 50 nL cRNA at a concentration of 0.2 ng/nL in appropriate subunit ratios then incubated at 19 °C for 24 to 72 h prior to their use in voltage clamp experiments. Recordings were made using

an automated two-electrode voltage-clamp system incorporating an OC-725C oocyte clamp amplifier (Warner Instruments, CT) coupled to a computerized data acquisition (Datapac 2000, RUN technologies) and autoinjection system (Gilson). Recording and current electrodes with resistance 1–4 M Ω were filled with 3M KCl. Details of the chambers and methodology employed for electrophysiological recordings have been described earlier.¹⁷ Oocytes were held in a vertical flow chamber of 200- μ L volume and perfused with ND-96 recording buffer (96 mM NaCl/2 mM KCl/1.8 mM CaCl₂/1 mM MgCl₂/5 mM HEPES; pH 7.4) at a rate of 20 mL/min. Test compounds were dissolved in ND-96 buffer and injected into the chamber at a rate of 20 mL/min using the Gilson auto-sampler injection system. Compounds were co-applied with the EC₇₅ concentration (100 μ M) of ACh. Data Analysis: Concentration/response curves were fit by non-linear curve fitting and GraphPad Prism Software (San Diego, CA) using standard built-in algorithms. For IC₅₀ determinations, data were fit to a single site competition model. For the potentiation/inhibition curves obtained for α 4 β 2 modulation, the data were fit to a bell shaped dose-response equation as previously described.⁹

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Abbreviations

ACh	acetylcholine
dFBr	<i>des</i> -formylflustrabromine
nACh	nicotinic acetylcholine
TFA	trifluoroacetic acid

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Figure 1.

Potentiation of ACh-evoked responses by representative deconstructed analogs *des*-formylflustrabromine (**1**). Responses were elicited by application of 100 μ M ACh to *Xenopus* oocytes expressing $\alpha 4\beta 2$ nicotinic receptors. Test compounds **6–8** were co-applied with ACh at the concentrations indicated. Each series of traces (A–C for compounds **6**, **7**, and **8**, respectively) as shown here were elicited from a single oocyte for each compound. Concentration/response curves for these compounds are shown in Figure 2.

Figure 2.

Concentration/response curves for representative deconstructed analogs of *des*-formylflustrabromine (**1**). Deconstructed compounds **6** (A), **7** (B), and **8** (C) were co-applied with 100 μ M ACh (i.e., a concentration of ACh that produced the maximal effect or Functional Response = 1) on human $\alpha 4\beta 2$ receptors. Peak amplitudes were normalized to peak currents obtained from 100 μ M ACh in the absence of test compound. Curves were fit to a hormetic model. A: Compound **6**, the half maximal potentiation (EC_{50}) is 0.10 ± 0.04 μ M; maximum potentiation was 290% of the controlled trace (100 μ M ACh). B: Compound **7**, $EC_{50} = 6.77 \pm 0.35$ μ M (250% of the control trace). C: Compound **8**, $EC_{50} = 1.22 \pm 0.66$ μ M (260% of the control trace).

Scheme 1.

Synthesis of target compounds **4–6** and **10**. The synthesis of **1** has been previously reported⁸ and is shown here only for comparison of methods. Reagents: (a) (1) CO_2Cl_2 , Et_2O ; (2) MeNH_2 ; (3) Dimethylethylamine-alane, THF; (b) $(\text{Boc})_2\text{O}$, Et_3N , DMF; (c) (1) *t*-BuOCl, THF; (2) allyltributyltin, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, THF; (d) NaBH_4 , CoCl_2 , EtOH ; (e) TFA, CH_2Cl_2 ; (f) (1) *t*-BuOCl, THF; (2) prenyl 9-BBN, THF; (g) H_2 10% Pd/C.

Scheme 2.

Synthesis of target compounds **7** and **8**. Reagents: (a) (1) *t*-BuOCl, THF; (2) prenyl 9-BBN, THF; (b) 10% Pd/C, H₂, 50 psi; (c) TFA, CH₂Cl₂.

Scheme 3.

Synthesis of compound **12**. Reagents: (a) 1-Dimethylamino-2-nitroethylene, TFA; (b) LiAlH_4 , THF; (c) $\text{Cl-COOC}_2\text{H}_5$, CHCl_3 . The exact same method was employed to synthesize **9** from 2-*sec*-butylindole (**21**).

Table 1

Effect of deconstructed analogs of *des*-formylflustrabromine (1) on the action (potentiation or antagonism) of the functional activity of ACh at $\alpha 4\beta 2$ nACh receptors expressed in *Xenopus* oocytes as measured using two-electrode voltage clamp methods.

1,3-12 (R = -CH₃)
2 (R = -H)

	R ₁	R ₂	R ₃	Agonist pEC ₅₀ (±S.E.M.)	Antagonist pIC ₅₀ (±S.E.M.)
1	-Br	-CH ₃	-CH=CH ₂	6.48 (0.28)	^c
2	-Br	-CH ₃	-CH=CH ₂	6.29 (0.24)	^c
3^a	-Br	-	-	Np ^b	4.40 (0.06)
4	-Br	-H	-CH ₂ CH ₃	Np ^b	4.14 (0.10)
5	-Br	-H	-CH=CH ₂	Np ^b	5.45 (0.08)
6	-Br	-CH ₃	-CH ₂ CH ₃	6.86 (0.16)	^c
7	-H	-CH ₃	-CH=CH ₂	5.14 (3.45)	^c
8	-H	-CH ₃	-CH ₂ CH ₃	5.52 (1.03)	^c
9	-H	-CH ₃	-CH ₂ CH ₃	Np ^b	4.78 (0.55)

1,3-12 (R = -CH₃)
2 (R = -H)

	R ₁	R ₂	R ₃	Agonist pEC ₅₀ (±S.E.M.)	Antagonist pIC ₅₀ (±S.E.M.)
10	-H	-H	-CH ₂ CH ₃	NP ^b	4.73 (0.06)
11	-H	-CH ₃	-CH ₃	5.40 (1.50)	- ^c
12	-H	-CH ₃	-CH ₃	NP ^b	- ^d

^a *N*-Methyl-6-bromotryptamine.

^b NP = No potentiation of the action of ACh.

^c could pIC₅₀ not be reliably determined.

^d Not determined.

Table 2

Potency of examined analogs of *des*-formylflustrabromine (1), relative to 1, to inhibit the effect of ACh on human $\alpha 7$ nACh receptors expressed in *Xenopus* oocytes as measured using two-electrode voltage clamp methods.

	pIC ₅₀ ($\pm$ SEM)
1	5.73 (0.05)
2	5.24 (0.14)
3	4.96 (0.32)
4	4.68 (0.10)
5	4.53 (0.14)
6	4.59 (0.24)
7	5.11 (0.14)
8	4.69 (0.20)
9	5.01 (0.10)
10	4.35 (0.19)
11	5.32 (0.11)
12	ND ^a

^aND = Not determined