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des-Formylflustrabromine (dFBr): A Structure-Activity Study on its Ability to Potentiate the Action of Acetylcholine at α4β2 Nicotinic Acetylcholine Receptors

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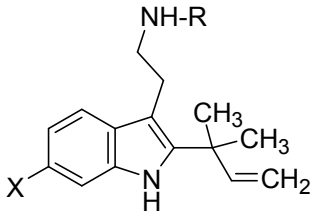
ABSTRACT

The naturally-occurring indole alkaloid *des*-formylflustrabromine (dFBr; **1**) is one of the first agents shown to act as a selective positive allosteric modulator (PAM) at $\alpha4\beta2$ nicotinic acetylcholine receptors (nAChRs). We previously deconstructed this agent to determine which of its structural features contribute to its actions and have identified an agent that might serve as the basis for a "*working pharmacophore*". Here, we elaborate the dFBr (**1**; EC_{50} = 0.2 μM) structure to identify how various structural modifications impact its actions. Electrophysiological studies with *Xenopus laevis* oocytes identified several compounds with dFBr-like potency and one, the 5-bromo analog of **1** (i.e., 5-bromo dFBr; **25**; EC_{50} = 0.4 μM), with more than twice the efficacy of **1** as a PAM at $\alpha4\beta2$ nAChRs.

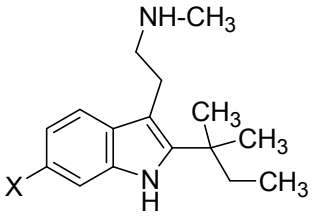
Keywords: Positive allosteric modulator, electrophysiology, nAChRs, α4β2 PAM

Introduction

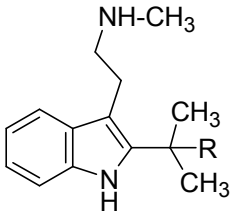
The $\alpha4\beta2$ subpopulation of nicotinic acetylcholine receptors (nAChRs) is implicated in drug and nicotine addiction, Parkinson's disease, depression, and pain.[1-6] However, there are very few, if any, agents that show substantial selectivity for this receptor subpopulation as agonists. Hence, there remains an unmet need for the development of $\alpha4\beta2$ nAChR-selective agents. The natural product *des*-formylflustrabromine (dFBr; **1**) has been identified as one of the first positive allosteric modulators (PAMs) of $\alpha4\beta2$ nAChRs with little to no action at other populations of nAChRs.[7,8] We "deconstructed" the dFBr (**1**) molecule in a stepwise manner to determine the contribution of its various structural features to $\alpha4\beta2$ PAM action.[9] Found was that: *i)* conversion of the secondary amine of dFBr to a primary amine (i.e., **2**) reduced potency and efficacy, *ii)* reduction of the unsaturated side chain of dFBr (i.e., **4**) doubled its potency, with retention of efficacy, *iii)* debromination of **1** and **4** (i.e., **3** and **5**, respectively) reduced potency by at least one order of magnitude, but did not detract from ACh-potentiating action, and that *iv)* a branched indole 2-position substituent was favored.[9] Removal of the 2-position substituent, and even the simple removal of both *gem* dimethyl groups (i.e., of **1** and **4**), resulted in loss of ACh-potentiating action. Although compound **6** was substantially less potent than dFBr (**1**), it was the structurally simplest compound shown to potentiate the action of ACh at $\alpha4\beta2$ nAChRs. In contrast, compound **7** failed to potentiate the actions of ACh.[9] Compound **6** would appear to represent the basis for a "*working pharmacophore*" and is the most abbreviated agent that retains dFBr-like PAM action.



1 R = CH_3, X = Br (dFBr)
2 R = H, X = Br
3 R = CH_3, X = H



4 X = Br
5 X = H



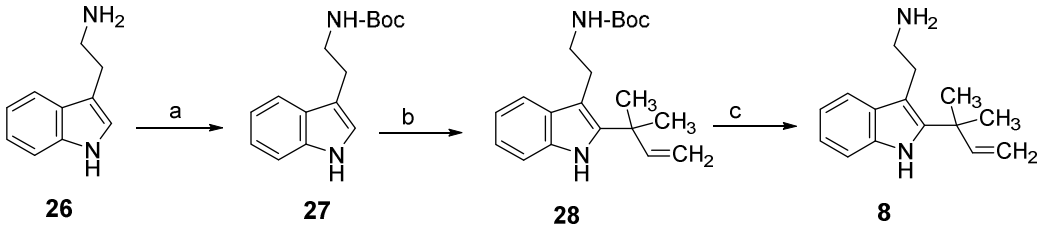
6 R = CH_3
7 R = H

The above "*deconstruction*" investigation introduced no new substituents to the dFBr molecule; that is, dFBr substituents were simply "deleted" in a systematic fashion.[9] In the present study, we "*elaborated*" (for the most part) the structure of dFBr by adding new substituents or by modifying various structural aspects of dFBr. An initial goal was to confirm that a primary amine analog of dFBr (more specifically, of **3**; i.e., **8**) retains action in the absence of the 6-bromo group (and it might be noted that a decrease in potency was expected based on our earlier studies). But, the major goal of the study was to further explore the structure-activity relationships of dFBr to identify what structural features are required to retain or enhance its PAM actions on ACh at $\alpha4\beta2$ nAChRs expressed in *Xenopus laevis* oocytes in an effort to identify those that might be ultimately exploited for the development of agents with enhanced potency and/or efficacy. At the time our work began, essentially nothing was known about the SAR of dFBr as an $\alpha4\beta2$ nAChRs PAM other than what we have previously published.[9]

Results

Synthesis. The compounds examined in this study were prepared as described below. For the purpose of this investigation, dFBr (**1**) was resynthesized as its hydrochloride salt as previously reported by us.[8] Compound **8**, the primary amine analog of **3**, was prepared in a similar manner (Scheme 1). That is, the primary amine of tryptamine (**26**) was protected by a Boc group (i.e., **27**), prenylated to **28** using prenyl 9-BBN,[10] and deprotected to afford **8**. This sequence also was used to prepare several other targets.

Scheme 1. Synthesis of compound **8**.[a]



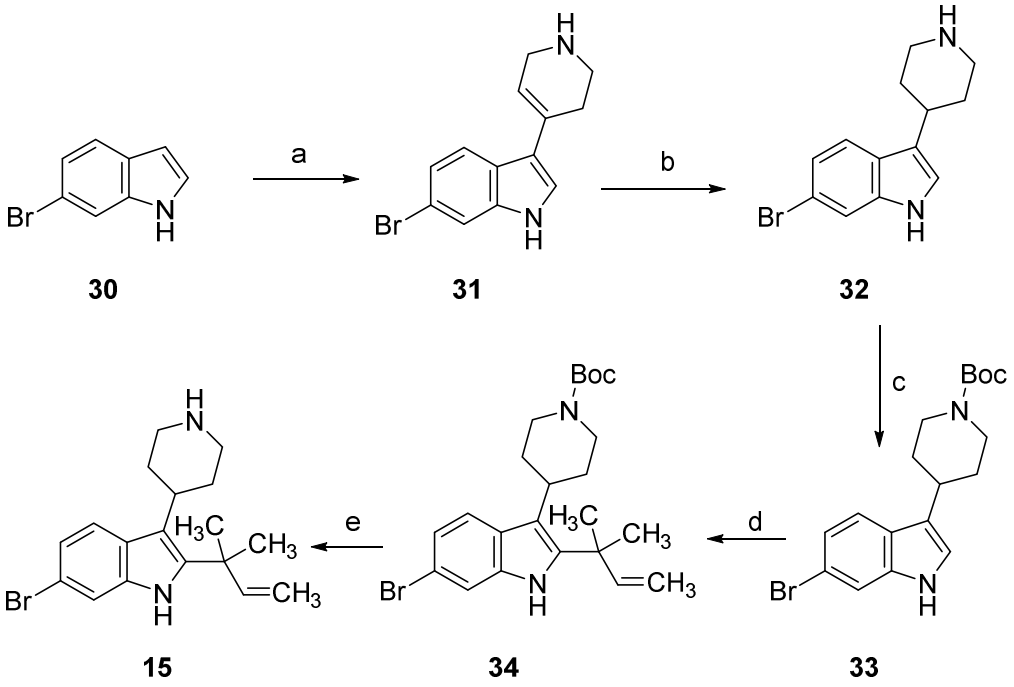
[a]Reagents: (a) Boc_2O, Et_3N, DMF, room temperature, 20 h; (b) *t*-butyl hypochlorite, THF, -78 ºC, 45 min; prenyl 9-BBN, 3 h, room temperature; (c) HCl, Et_2O, 0 ºC.

The free base of dFBr (**1**) was reductively alkylated using formaldehyde and $NaCNBH_3$ to tertiary amine **9**, which was subsequently methylated using MeI to give the N,N,N-trimethyl quaternary amine **10**. Alkylation of 5-bromo-2-(1,1-dimethylallyl)indole (**29**) with *N*,*N*-dimethylaminoethyl chloride furnished isotryptamine **11**. The free base of gramine **12** was prepared according to a literature procedure,[11] and converted to a water-soluble oxalate salt, and N-methylhomotryptamine (i.e., 3-(3-methylaminopropyl)-indole)[12] was Boc-protected, prenylated, and deprotected to afford **13** using the same

sequence of reactions shown in Scheme 1. Tetrahydro-β-carboline **14** was obtained by cyclization of 6-bromotryptamine with acetone under Pictet-Spengler conditions.

Compound **15** was obtained as shown in Scheme 2. Condensation of 6-bromoindole (**30**) with 4-piperidone afforded tetrahydropyridine **31**; catalytic reduction of **31** to piperidine **32** followed by amine protection gave **33**. Prenylation (as described in Scheme 1) with subsequent de-protection provided **15**.

Scheme 2. Synthesis of compound **15**.[a]

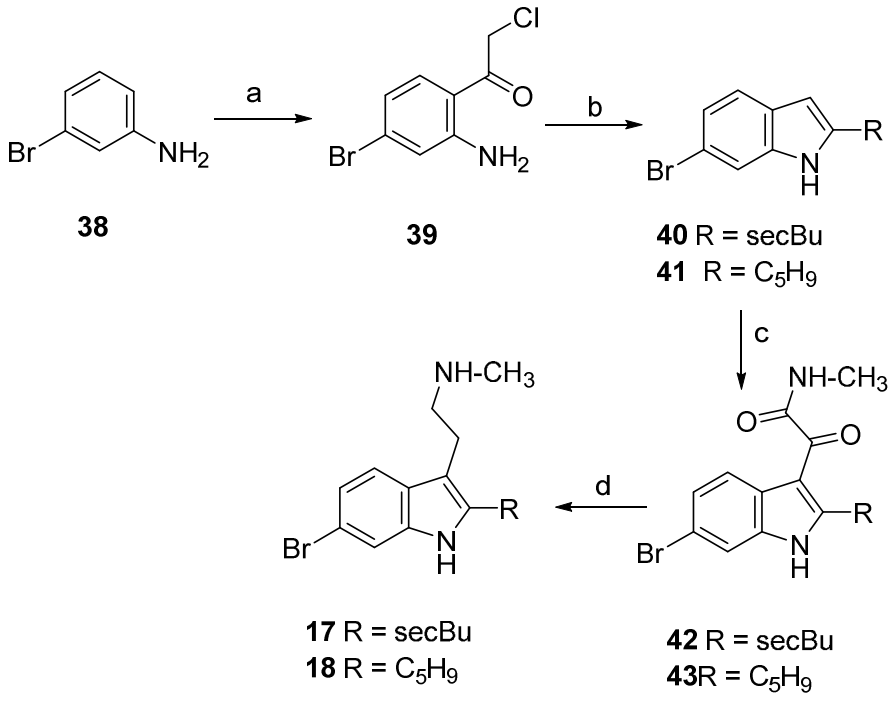


[a]Reagents: (a) 4-piperidone·HCl, KOH, CH_3OH; (b) H_2, PtO_2, AcOH; (c) Boc_2O, Et_3N, CH_2Cl_2; (d) *i t*-BuOCl, THF, *ii* prenyl 9-BBN, THF; (e) HCl, EtOAC.

Compound **16**, the α-methyl analog of **2**, was prepared in the same manner shown in Scheme 1 using 1-(6-bromo-1*H*-indol-3-yl)propan-2-amine (**35**) as starting material; **35**

was obtained by catalytic reduction of 3-[2-nitroprop-1-en-1-yl]-6-bromoindole which had been previously reported,[13] but not thoroughly characterized.

Compounds **17** and **18** were synthesized via a common route (Scheme 3). Acylation of 3-bromoaniline (**38**) with chloroacetyl chloride under Friedel-Crafts conditions afforded **39**, which was subsequently cyclized by treatment with the appropriate Grignard reagents to yield compounds **40** and **41**. The cyclization step involved a 1,2-aryl migration as described by Pei et al.;[14] although the latter investigators synthesized a variety of 2-substituted indoles, they never synthesized or utilized **39**. The remainder of the syntheses involved a Speeter-Anthony[15] sequence where an indole is acylated with oxalyl chloride, treated with an amine, and the resulting glyoxalyl amides reduced to the corresponding amines.



Scheme 3. Synthesis of **17** and **18.**[a]

[a]Reagents: (a) $AlCl_3$, BCl_3, $ClCH_2CN$, DCM, reflux, overnight; (b) THF, rt, 1.5h; (c) *i* $(COCl)_2$, Et_2O, 0 ºC, 1h, *ii* CH_3NH_2 (40% in H_2O), rt, overnight; (d) BH_3·DMS, THF, reflux, overnight.

Compounds **19** and **20** were prepared from their corresponding 2-substituted indoles using a Speeter-Anthony sequence. Likewise, this reaction scheme was also used in the preparation of **22**-**25**; however, for the latter four compounds, 2-unsubstituted indoles were used as starting materials and once the desired *N*-methyltryptamines were obtained, the amine was Boc-protected prior to prenylation, followed by deprotection. N_1-Methyl dFBr (**21**) was obtained by treatment of N-Boc-protected **1** with NaOH and MeI, followed by deprotection.

Electrophysiology. dFBr (**1**) was re-examined in *Xenopus* oocytes in the same manner we described earlier,[9] and its potency (EC_{50} = 0.2 μM) and efficacy (265%, relative to ACh control: 100%) were consistent with what we have reported.[8,9] Data obtained for the present compounds are detailed in Table 1, and "maximal effect" is defined as the I_{max} (calculated maximum current) for the potentiation dose-response curve. The examined compounds also are shown in Figure 1 and their action and potency are briefly summarized there for easy comparison (i.e., the figure provides their potency as EC_{50} values, and maximal potentiation, or it is indicated that they failed to potentiate the actions of ACh – as noted by "NP" – no potentiation).

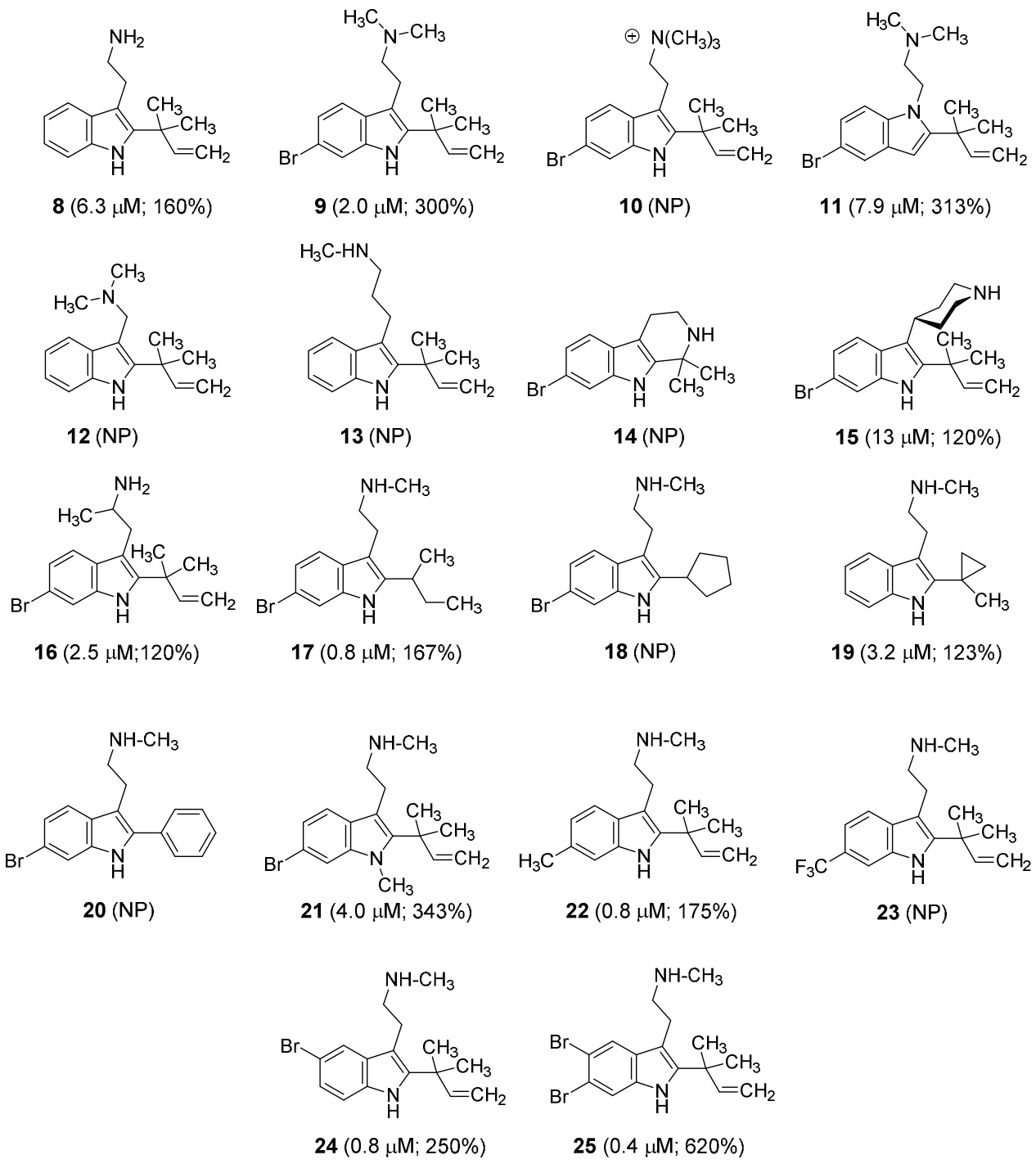


Figure 1. Compounds examined in the present investigation. Values in parenthesis are potencies for potentiation of ACh responses, followed by maximal potentiation effect; NP = no potentiation. More detailed data are provided in Table 1. As comparator, the EC_{50} value for dFBr (**1**) = 0.2 μM, with 265% potentiation.

Table 1. Potencies of dFBr (**1**) analogs to potentiate and/or inhibit the actions of 100 μM ACh at α4β2 nAChRs.[a]

	EC_{50}, μM (pEC_{50} ± SEM)	% change in I_{max} (95% CL)[b]	Maximal Effect[c]	IC_{50}, μM (pIC_{50} ±SEM)
1 (dFBr)	0.2 (6.7 ± 0.3)	350-510	265%	50 (4.3 ± 0.4)
8[d]	6.3 (5.2 ± 0.2)	250-310	160%	160 (3.8 ± 0.1)
9[d]	2.0 (5.7 ± 0.2)	360-610	300%	79 (4.1 ± 0.2)
10	NP[e]			2.5 (5.6 ± 0.1)
11	7.9 (5.1 ± 0.2)	240-540	313%	79 (4.1 ± 0.2)
12	NP[e]			5.0 (5.3 ± 0.1)
13	NP[e]			13 (4.9 ± 0.1)
14	NP[e]			5.0 (5.3 ± 0.1)
15	13 (4.9 ± 0.1)	280-290	120%	32 (4.5 ± 0.1)
16[d]	2.5 (5.6 ± 0.2)	220-250	120%	250 (3.6 ± 0.1)
17[d]	0.8 (6.1 ± 0.4)	300-350	167%	160 (3.8 ± 0.1)
18	NP[e]			25 (4.6 ± 0.1)
19[d]	3.2 (5.5 ± 0.3)	150-390	123%	32 (4.5 ± 0.2)
20	NP[e]			13 (4.9 ± 0.1)
21	4.0 (5.4 ± 0.2)	210-650	343%	32 (4.5 ± 0.2)
22[d]	0.8 (6.1 ± 0.2)	250-330	175%	100 (4.0 ± 0.1)
23	NP[e]			16 (4.8 ± 0.6)
24	0.8 (6.1 ± 0.3)	210-460	250%	32 (4.5 ± 0.2)
25	0.4 (6.4 ± 0.1)	710-870	620%	50 (4.3 ± 0.2)

[a]EC_{50}, I_{max}, and IC_{50} values were calculated using non linear regression to a bell-shaped dose response model. Hill slopes were fixed at 1 both for potentiation and inhibition. [b]I_{max} values represent the percent change of the ACh I_{max} after fitting the data to a bell-shaped dose response curve. [c]Maximal effect; no value is provided where there was no potentiation. [d]Maximum inhibition was set at 0 to enable estimation of IC_{50} values and it was necessary to fit the data using the bell-shaped dose response equation. [e]NP: No potentiation.

We had previously shown that N-*des*-methyl dFBr (**2**) was nearly equipotent with dFBr (**1**).[9] Consistent with this finding, the primary amine counterpart of *des*-bromo dFBr (**8**; EC_{50} = 6.3 μM, Table 1) was nearly equipotent with its primary amine counterpart (i.e., *des*-bromo dFBr; **3**, EC_{50} = 7.2 μM).[9] Nevertheless, **8** was substantially less potent than, and only about half as efficacious as, **1**. The N,N-dimethyl tertiary amine analog of dFBr, **9** (EC_{50} = 2.0 μM), retained dFBr-like action, was at least equi-efficacious, but was 10-fold less potent than dFBr (**1**) (Figure 2). In contrast, the N,N,N-trimethyl quaternary amine analog of dFBr (i.e., **10**) failed to potentiate the actions of ACh and only produced an inhibitory effect. The isotryptamine isostere of **9** (i.e., **11**; EC_{50} = 7.9 μM) retained activity but was 4-fold less potent than **9**.

Representative concentration-response curves (exemplified for ACh, 100 μM, in combination with compounds **1**, **9**, **15**, and **24**) showing enhancement of the ACh-induced response at low concentrations followed by inhibition at higher concentrations are shown in Figure 2.

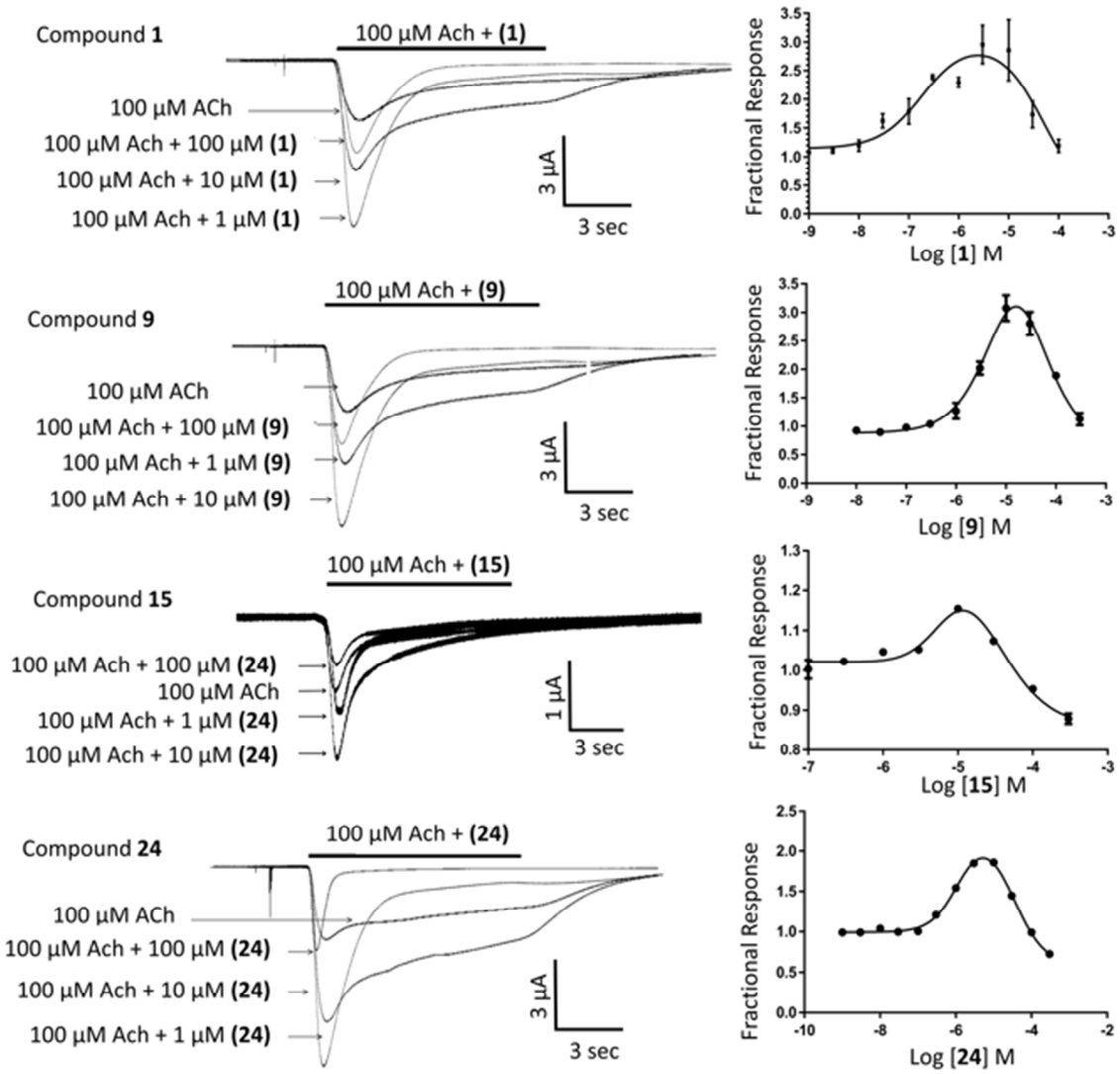


Figure 2. Typical responses and biphasic dose response curves for **1, 9, 15** and **24** showing potentiation and inhibition kinetics of analogs with different maximum observed responses. All potentiating compounds showed enhancement of ACh-induced responses at low concentrations followed by inhibition at higher concentrations. Inhibited responses typically displayed more rapid desensitization kinetics.

The side chain-shortened and side chain-elongated analogs **12** and **13** (Table 1), respectively, failed to potentiate the actions of ACh, as did the conformationally-constrained analog of **4** where the amine was tethered to the 2-position substituent in the form of a tetrahydro-β-carboline (i.e., **14**). Piperidine **15** (EC_{50} = 13 μM) was a very weak potentiating agent and only slightly (120%) enhanced the action of ACh. Compound **16** (EC_{50} = 2.5 μM), the racemic α-methyl analog of **2**, displayed substantially reduced potency and efficacy compared to dFBr.

Compound **17 (**EC_{50} = 0.8 μM), the racemic mono *des*-methyl counterpart of **4**, retained dFBr-like action, but only half its efficacy; it might be noted that the individual optical isomers of **17** were not examined. Other 2-modified compounds were either less potent/efficacious (i.e., **19**) or inactive (i.e., **18**, **20**). N_1-Methyl dFBr (**21**; EC_{50} = 4.0 μM) was 20-fold less potent than dFBr (**1**) but retained its efficacy (Table 1). Replacement of the 6-bromo substituent of dFBr (**1**) by a methyl group (i.e., **22**) decreased potency by 4-fold and halved efficacy, whereas replacement with a trifluoromethyl group (i.e., **23**) resulted in no potentiation. Moving the bromo substituent to the 5-position (i.e., **24**) reduced potency by 4-fold but had relatively little effect on efficacy. 5-Bromo dFBr (i.e., **25**), although only half as potent as **1**, displayed twice its efficacy.

Low concentrations of dFBr potentiated the actions of ACh whereas higher concentrations had an inhibitory effect;[8,9] for example, see Figure 2. In general, similar actions were observed for all compounds in the present investigation, although several

failed to enhance the action of ACh and simply displayed an inhibitory action (by a mechanism that has yet to be fully elucidated).

Discussion

We previously examined "*deconstructed*" analogs of dFBr (**1**) to understand which of (and to what extent) its various structural attributes impact its action as a PAM at $\alpha4\beta2$ ACh receptors.[9] A branched indole 2-position chain (although not chain unsaturation) was a critical feature for activity, the simple 2-*n*-propyl counterpart of dFBr (i.e., dFBr lacking both *gem*-dimethyl substituents) was inactive, but the 6-position bromo substituent, although not required, was found important for efficacy.[9] Compound **6**, although with 10-fold reduced potency, was demonstrated to be the minimal structure retaining dFBr-like action. Here, we extend these structure-activity findings.

Confirming a prior finding (i.e., with **2**),[9] a primary amine (i.e., **8**) is tolerated for dFBr-like action. Lacking a 6-bromo group, **8** was expected to be >10-fold less potent than **1**. This was found to be the case. Table 1 shows that a tertiary amine, the simple N,N-dimethylamine counterpart of **1**, also was tolerated, but that **9** was 10-fold less potent than **1**; however, a quaternary amine counterpart of dFBr, **10**, was inactive. The latter finding is compatible with the concept that dFBr-like compounds do not bind at the $\alpha4\beta2$ receptor orthosteric site (i.e., ACh, which is a quaternary amine, binds at the orthosteric site by definition). Whereas introduction of an α-methyl group also was tolerated (i.e., **16**), other changes to the indole 3-position substituent (e.g. chain shortening, chain extension, conformational restriction; i.e., **12**-**14**) resulted in loss of PAM action.

A branched 2-position substituent was identified earlier as being important for activity.[9] Here, it was found that removal of one of the methyl groups is tolerated (i.e., **17**); this confirms the earlier suggestion that only one of the *gem* dimethyl groups might be required,[9] and this might be related to the lipophilicity of the substituent. Nevertheless, **17** displayed only half the ACh potentiating effect of dFBr. Compound **6** showed potentiating action whereas its 2-isopropyl counterpart, **7**, was inactive.[9] Likewise, "extending" the isopropyl group to a bulkier cyclopentyl group (i.e., **18**) resulted in loss of action. At this time, given the results with **17**, we cannot explain this finding. However, like **6**, the cyclopropyl analog **19** was active but only weakly potentiated the action of ACh (i.e., to 123%). The 2-phenyl counterpart of dFBr (i.e., **20**), which also might be considered a "branched" substituent, was found to be inactive.

Because none of the above structural alterations resulted in enhanced potency or efficacy, attention was directed to the indole nucleus; the 2- and 3-position substituents of dFBr (**1**) were retained. Compound **11**, the isotryptamine counterpart of **9**, was about 40-fold less potent than than **1**, and 4-fold less potent than **9**, suggesting that the location or presence of the indolic N or NH substituent plays a role in PAM potency. Interestingly, the N_1-methyl counterpart of dFBr (i.e., **21**) displayed 20-fold reduced potency relative to dFBr suggesting that the indolic 1-position hydrogen atom might be involved in the potency of these compounds.

Because removal of the 6-bromo group of dFBr decreased its potency by at least 10-fold (reference 9 and the present study), an attempt was made to determine if this was related to the lipophilic or electronic character of the bromo substituent. Because the bromo group of dFBr (**1**) and the methyl group of **22** are electronically opposite, and because a bromo group is more lipophilic than a methyl group (π = 0.86 and 0.56, respectively[16]), it would seem that its contribution might be related to lipophilicity. The trifluoromethyl group of inactive **23** is equi-lipophilic (π = 0.88) with a bromo group; however, it is sterically larger than a bromo or methyl group[17,18] and might not be accommodated by the binding pocket. Indeed, several "size" indicies were examined and are consistent with this concept (see Table S1, Supporting Information).

Translocation of the 6-bromo group to the 5-position resulted in a compound (i.e., **24**) that was slightly less potent but equi-efficacious with dFBr. Thus, the position of the 6-bromo group, although perhaps optimal, would not appear to be critical for ACh-enhancing PAM action. The 5,6-dibromo indolic compound **25** was only slightly less potent than dFBr, but displayed twice its efficacy (620%). Additional 5-substituted and 5,6-disubstituted compounds remain to be examined.

Over the last few years attempts have been made, using site-directed mutagenesis, to localize sites on $\alpha4\beta2$ receptors where dFBr might act to elicit its allosteric effects; several potential sites have been identified.[19-21] In particular, Deba et al.[21] have proposed two distinct sites and, additionally, have modeled possible dFBr interactions. In one site, the protonated amine is proposed to form a hydrogen bond with the

carbonyl oxygen atom of a valine (V609) residue, and the 2-position alkyl group is involved in hydrophobic interactions with lysine (L317) and phenylalanine (F312) residues. This is consistent with our findings that the quaternary amine **10** is inactive, and that a 2-position (branched) alkyl substituent contributes to activity.[9] Furthermore, the enhanced potency of **4** over dFBr might be related to the greater lipophilicity of the ethyl group relative to its ethylene counterpart (π = 1.02 and 0.82 for CH_2CH_3 and $CH{=}CH_2$, respectively[16]). Docked in this site, the 6-bromo group of dFBr is within 3 Å of a phenylalanine (F316) residue, implicating a possible hydrophobic interaction. This is also in accord with the action of **22**. The second binding site involves, in addition to hydrophobic interactions between the (branched) 2-position alkyl group and a leucine moiety (L256), a hydrogen bond between the indole NH of dFBr and a cysteine (C259) residue.[21] The latter is consistent with the reduced potency of **21** (although it might be noted that **21** retained dFBr-like efficacy as a potentiating agent). Future homology modelling/docking studies can be specifically informed by results such as those provided here to validate and/or refine future models.

Thus far, the naturally-occurring **1** seems to be a nearly optimal structure (i.e., with regard to potency *and* efficacy) for dFBr-like compounds to potentiate the actions of ACh at α4β2 nAChRs. But, it might be noted that potency and efficacy do not co-vary. Apart from the saturated dFBr analog **4**, which was about twice as potent as, but equi-efficacious with, dFBr (**1**),[9] none of the compounds examined here displayed substantially enhanced potency ***and*** efficacy. Potential structural modifications of **1** resulting in retention of potency and efficacy, thus far, appear limited. It is evident that

manipulation of substituents on the indole nucleus (apart from the 2- and 3-positions) can favorably impact activity and result in compounds with sub-micromolar potency and even, as with **25**, enhanced efficacy. Future studies will focus primarily on the indole ring and substituents at the indole 4- – 7-positions. The ideal goal would be an agent that enhances the actions of ACh without acting as an antagonist of ACh at higher concentrations. This goal has yet to be achieved. But, the present findings provide insight as to how dFBr substituents impact the action of ACh, provide the most complete SAR study of dFBr (**1**) to date, and can inform future modeling/docking studies.

Experimental

Synthesis

Melting points (mp) were measured on a Thomas-Hoover melting point apparatus using glass capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer using tetramethylsilane (TMS) as an internal standard; peak positions are given in parts per million (δ). UHPLC-MS were recorded on a Perkin Elmer Flexar UHPLC with AxION 2 Time of Flight (TOF) mass spectrometer and the molecular weight of the compounds was within 0.05% of calculated values. Elemental analysis was performed on all target compounds by Atlantic Microlab Inc. (Norcross, GA) for the elements indicated and the results were typically within 0.4% of calculated values. Infrared spectra were obtained on a Thermo Nicolet iS10 FT-IR. Flash chromatography was performed on a CombiFlash Companion/TS instrument (Telodyne Isco Inc., Lincoln, NE) using packed silica gel (Silica Gel 230-400 mesh) columns (RediSep Rf Normal-phase Silica Flash Column, Teledyne Isco Inc., Lincoln, NE). All reactions were monitored by thin-layer chromatography (TLC) on silica gel GHLF plates (250 μ, 2.5 x 10 cm; Analtech Inc., Newark, DE).

2-(1,1-Dimethylallyl)tryptamine Hydrochloride (8). Gaseous HCl was bubbled into a solution of *tert*-butyl-2-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate **28** (0.4 g) in anhydrous Et_2O (20 mL) at 0 °C. The reaction mixture was allowed to stir for 24 h and the solvent was evaporated to yield a purple solid which was recrystallized from *i*-PrOH/Et_2O to yield 0.1 g (28%) of **8** as brown crystals: mp 231-232 °C; ^{1}H NMR (DMSO-d_6) δ 1.50 (s, 6H, CH_3), 2.86 (t, 2H, CH_2), 3.07 (t, 2H, CH_2), 5.06-5.11 (m, 2H,

CH_2), 6.12-6.18 (m, 1H, CH), 6.95-7.06 (m, 2H, Ar), 7.34 (d, *J* = 7.9 Hz, 1H, Ar), 7.51 (d, *J* = 7.8 Hz, 1H, Ar), 8.05 (br s, 3H, NH_3^+ aliphatic), 10.59 (br s, 1H, indolic NH). Anal. Calcd for ($C_{15}H_{20}N_2 \cdot HCl$) C, H, N.

6-Bromo-2-(1,1-dimethylallyl)-*N*,*N*-dimethyltryptamine Oxalate (9). Formaldehyde (0.09 mL, 1.25 mmol) and $NaBH_3CN$ (0.03 g, 0.5 mmol) were added to a stirred solution of **1** (free base, 0.08 g, 0.25 mmol) in CH_3CN (15 mL) under a N_2 atmosphere. The reaction mixture was allowed to stir for 1 h at room temperature, and then 2N HCl (8 mL) was added in a dropwise manner. After stirring for 2 h, the reaction mixture was diluted with Et_2O (10 mL), and the organic layer was separated and washed with H_2O (2 x 10 mL). The aqueous portions were combined, basified by addition of $NaHCO_3$ (pH 8), and extracted with CH_2Cl_2 (3 x 10 mL). The organic portions were combined, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (Aldrich silica gel 60) using DCM/MeOH (10:1) as eluent to afford the free base of **9** (0.06 g, 60% yield) as an oil: ^{1}H NMR ($CDCCl_3$): δ 1.53 (s, 6H, 2 x CH_3), 2.34 (s, 6 H, 2 x CH_3), 2.48-2.52(m, 2H, CH_2), 2.96-3.00 (m, 2H, CH_2), 5.14-5.18 (m, 2H, CH_2), 6.07-6.14 (m, 1H, CH), 7.15 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.36 (d, *J* = 1.6, 1H, Ar-H), 7.42 (s, 1H, 2 x Ar-H), 7.83 (b s,1H, NH). A solution of the free base (0.05 g, 0.15 mmol) and oxalic acid (0.01 g, 0.15 mmol) in $CHCl_3$ (3 mL) was allowed to stir for 0.5 h and the precipitate was collected by filtration. The solid product was recrystallized from *i*-PrOH to afford **9** (0.02 g, 36% yield) as a white solid: mp 172-173 ºC; ^{1}H NMR (DMSO-d_6) δ 1.58 (s, 6H, 2 x CH_3), 2.73 (s, 6H, 2 x N-CH_3), 4.39 (br.s, 2H, CH_2), 5.25-5.29 (m, 2H, CH_2), 6.31-6.38 (m,1H, CH), 7.25 (d, *J* = 8.44 Hz, 1H, ArH),

7.59 (d, *J* = 1.60 Hz, 1H, ArH), 7.70 (d, *J* = 8.44 Hz, 1H, ArH), 11.27 (s, oxalic acid). Anal. Calcd for ($C_{17}H_{23}BrN_2 \cdot C_2H_2O_4$) C, H, N.

6-Bromo-2-(1,1-dimethylallyl)-*N*,*N*,*N*-trimethyltryptamine Methiodide (10). A solution of 6-bromo-2-(1,1-dimethylallyl)-*N*,*N*-dimethyltryptamine (**9**) (0.05g, 0.2 mmol) in anhydrous *i*-PrOH (4 mL) was cooled to 0 °C (ice-bath). Iodomethane (0.06 g, 0.5 mmol) was added and the reaction mixture was allowed to stir for 22 h at room temperature under a N_2 atmosphere. The precipitate was collected by filtration and washed with anhydrous Et_2O (3 x 15 mL), air dried, and recrystallized from MeOH/*i*-PrOH to afford 0.02 g (30%) of **10** as yellow crystals: mp 208-210 °C; ^{1}H NMR (CD_3CN) δ 1.54 (s, 6H, $(CH_3)_2$), 3.15 (s, 9H, $N(CH_3)_3$), 3.22-3.38 (m, 4H CH_2), 5.25 (m, 2H, CH_2), 6.22 (m, 1H, CH), 7.22 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.46 (d, *J* = 8.5 Hz, 1H, Ar), 7.59 (d, *J* = 1.5 Hz, 1H, Ar), 9.35 (br s, 1H, indolic NH). Anal. Calcd for ($C_{18}H_{26}BrIN_2 \cdot C_3H_8O$) C, H, N.

5-Bromo-2-(1,1-dimethylallyl)-*N,N*-dimethylisotryptamine Hydrogen Oxalate (11). 3,5-Dibromo-1*H*-indole[22] (0.5 g, 1.8 mmol) was added in one portion to freshly prepared prenyl 9-BBN[10] (5.4 mmol) and Et_3N (0.9 mL, 6.3 mmol) in anhydrous THF (10 mL) at room temperature. The reaction mixture was allowed to stir at room temperature for 4 h and then quenched with a saturated solution of $NaHCO_3$ (20 mL). The organic layer was separated and the aqueous portion was extracted with Et_2O (2 x 20 mL). The combined organic portion was washed with H_2O (2 x 30 mL), brine (30 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure to yield a crude, dark-yellow oil. The residue was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to

30:1) to afford 0.3 g (78%) of 5-bromo-2-(1,1-dimethylallyl)indole (**29**) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 6H, CH_3), 5.10 (m, 2H, CH_2), 6.01 (m, 1H, CH), 6.24 (s, 1H, Ar), 7.20 (dd, 2H, Ar), 7.89 (br s, 1H, indolic NH).

In a dry, round-bottom flask NaH (60% oil dispersion) (0.06 g, 1.4 mmol) was allowed to stir with anhydrous toluene in an ice bath for 10 min. After removal of the toluene, a solution of **29** (0.15 g, 0.6 mmol) in anhydrous DMF (5 mL) was added and stirring was continued in an ice bath (0 °C) for another 20 min. Prepared in a separate beaker, a solution of *N,N*-dimethylaminoethyl chloride hydrochloride (0.2 g, 1.1 mmol), K*t*BuO (0.2 g, 1.4 mmol) and KI (0.08 g, 0.6 mmol) in cold anhydrous DMF (3 mL) was then added to the round-bottom flask and the reaction mixture was heated at reflux for 17 h. The reaction mixture was quenched with H_2O (20 mL) and extracted with EtOAc (2 x 25 mL) and CH_2Cl_2 (2 x 25 mL). The combined organic portions were washed with H_2O (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), and evaporated to dryness to yield 0.1 g (63%) of free base as a brown-colored oil. Saturated oxalic acid solution in anhydrous Et_2O (5 mL) was added to a solution of the free base (0.1 g) in anhydrous Et_2O (5 mL) at 0 °C and allowed to stir overnight. The precipitate formed was collected by filtration and recrystallized from MeOH/Et_2O to afford 0.04 g (32%) of **11** as white flakes: mp 185-188 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (s, 6H, $(CH_3)_2$), 2.69 (s, 6H $N(CH_3)_2$), 3.03 (t, 2H, CH_2), 4.42 (t, 2H, CH_2), 5.17 (m, 2H, CH_2), 6.09 (m, 1H, CH), 6.37 (s,1H, CH), 7.25 (d, 1H, *J* = 8.7 Hz, Ar), 7.43 (s, 1H, *J* = 8.7 Hz, Ar), 7.70 (d, 1H, Ar). Anal. Calcd for ($C_{17}H_{23}BrN_2 \cdot C_2H_2O_4$) C, H, N.

2-(1,1-Dimethylallyl)gramine Hydrogen Oxalate (12). Dimethylamine (40%, 0.1 mL, 0.8 mmol) and HCHO (37%, 0.7 mL, 0.8 mmol) were added to a solution of 2-(1,1-dimethylallyl)-1*H*-indole[11] (0.10 g, 0.5 mmol) in glacial HOAc (3 mL) maintained at 5 °C. When the vapors ceased, MeOH (4 mL) was added to make a clear solution and the reaction mixture was allowed to stir overnight at room temperature. The organic solvent was removed by evaporation under reduced pressure and the solution was basified with NaOH (3M, to pH 10). The aqueous portion was extracted with Et_2O (3 x 30 mL) and the combined organic portion was washed with H_2O (3 x 50 mL), brine (50 mL), dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield the gramine as a free base which was converted to an oxalate salt by addition of a saturated oxalic acid solution in anhydrous Et_2O (5 mL) to a solution of crude 2-(1,1-dimethylallyl)gramine in anhydrous Et_2O (5 mL) at 0 °C. The precipitate was collected by filtration, dried and recrystallized from *i*-PrOH to afford 0.04 g (22%) of **12** as white crystals: mp 155-158 °C; ^{1}H NMR (DMSO-d_6) δ 1.54 (s, 6H, $(CH_3)_2$), 2.72 (s, 6H, $N(CH_3)_2$), 4.39 (s, 2H, CH_2), 5.22 (m, 2H, CH_2), 6.33 (m, 1H, CH), 7.04-7.68 (m, 4H, Ar), 11.13 (br s, 1H, COOH). Anal. Calcd for ($C_{16}H_{22}N_2 \cdot C_2H_2O_4 \cdot 0.5\ H_2O$) C, H, N.

***N*-Methyl-3-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propanamine Hydrogen Oxalate (13).** Triethylamine (0.4 mL, 2.6 mmol) and di-*tert*-butyl dicarbonate (0.6 g, 2.6 mmol) were added to a CH_2Cl_2 (15 mL) solution of 3-(3-methylaminopropyl)indole[12] (0.5 g, 2.6 mmol) and allowed to stir at room temperature for 19 h. The reaction mixture was quenched with H_2O (50 mL) and extracted with CH_2Cl_2 (2 x 30 mL). The combined organic portion was washed with H_2O (3 x 50 mL), dried (Na_2SO_4) and evaporated

under reduced pressure to obtain a yellow oil. The crude product was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 25:1) to afford 0.4 g (58%) of *tert*-butyl-3-(1*H*-indol-3-yl)propyl-*N*-methylcarbamate as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, $(CH_3)_3$), 1.83-1.90 (m, 2H, CH_2), 2.68 (t, 2H, CH_2), 2.78 (s, 3H, CH_3), 3.24 (m, 2H, CH_2), 6.93 (s, 1H, Ar), 7.01-7.03 (dt, 1H, Ar), 7.09-7.13 (dt, 1H, Ar), 7.28 (d, *J* = 8.0 Hz, 1H, Ar), 7.52 (d, *J* = 7.8 Hz, 1H, Ar), 7.86 (br s, 1H, indolic NH).

tert-Butyl hypochlorite (0.2 g, 1.7 mmol) was added to a solution of the above carbamate (0.4 g, 1.4 mmol) and Et_3N (0.2 g, 1.7 mmol) in THF (15 mL) at -78 °C and allowed to stir for 45 min. Freshly prepared prenyl-9-BBN[10] (2.8 mmol) was added in a dropwise manner over 10 min while maintaining temperature at -78 °C. The reaction mixture was allowed to warm to room temperature and stirred for additional 2 h. Sodium hydroxide (3M, 5 mL) and H_2O_2 (30% v/v, 5 mL) were added in a dropwise manner and the solution was allowed to stir for 1 h and diluted with Et_2O (100 mL). The organic layer was washed with H_2O (3 x 60 mL), brine (80 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield a yellow oil which was subjected to purification by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 25:1) to afford 0.2 g (40%) of *tert*-butyl-3-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-*N*-methylcarbamate as a glassy solid: mp 116-118 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H, $(CH_3)_3$), 1.45 (m, 8H, CH_2, CH_3), 2.72 (t, 2H, CH_2), 2.81 (s, 3H, CH_3), 3.25 (m, 2H, CH_2), 5.05-5.10 (m, 2H, CH_2), 6.01-6.08 (m, 1H, CH), 6.97-7.06 m, 2H, Ar), 7.20 (d, *J* = 7.8 Hz, 1H, Ar), 7.41 (d, *J* = 7.6 Hz, 1H, Ar), 7.74 (br s, 1H, indolic NH).

Gaseous HCl was bubbled into a solution of *tert*-butyl-3-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-*N*-methylcarbamate (0.1 g, 0.3 mmol) in dry EtOAc (10 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature overnight. The solvent was evaporated under reduced pressure to yield a brown solid which was converted to the oxalate salt and recrystallized from *i*-PrOH to yield 0.03 g (28%) of **13** as a yellow solid: mp 135-138 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (s, 6H, $(CH_3)_2$), 1.83 (m, 2H, CH_2), 2.55 (s, 3H, CH_3), 2.76 (t, 2H, CH_2), 2.94 (t, 2H, CH_2), 5.09 (m, 2H, CH_2), 6.16 (m, 1H, CH), 6.95 (t, 1H, Ar), 7.21 (t, 1H, Ar), 7.32 (d, *J* = 8.0 Hz, 1H, Ar), 7.44 (d, *J* = 7.8 Hz, 1H, Ar), 10.47 (s, 1H, COOH). Anal. Calcd for ($C_{17}H_{24}N_2 \cdot 1.3C_2H_2O_4$) C, H, N: 62.95, 7.56, 8.09%. Found: 63.04, 7.18, 7.50%. UHPLC-MS; [M^+1] calculated for $C_{17}H_{24}N_2$ 257.15, found 257.15.

7-Bromo-1,1-dimethyl-1,2,3,4-tetrahydro-β-carboline oxalate (14). A mixture of 6-bromotryptamine[13] (2.0 g, 8.4 mmol), acetone (0.6 mL, 8.8 mmol) and 2N HCl (8.40 mL, 16.80 mmol) in 30 mL of H_2O was heated at reflux for 5 h. The reaction mixture was washed with Et_2O (30 mL), basified with 1N NaOH and extracted with CH_2Cl_2 (2 x 30 mL). The combined organic portion was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography (silica gel) using CH_2Cl_2/MeOH (8:2) to afford 0.40 g (17%) of the desired product as a yellow solid: mp 188-192 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 6H, CH_3 x 2), 1.48 (br s, 1H, NH), 2.61 (t, *J* = 5.76 Hz, 2H, CH_2), 3.13 (t, *J* = 5.76 Hz, 2H, CH_2), 7.11 (dd, *J* = 8.36, 1.68 Hz, 1H, ArH), 7.25 (d, *J* = 8.36 Hz, 1H, ArH), 7.38 (s, *J* = 1.68 Hz, 1H, ArH), 7.61 (br s, 1H, ArNH).

The free base (0.40 g, 1.43 mmol) was dissolved in Et_2O and an ethereal solution of oxalic acid (0.13 g, 1.43 mmol) was added to afford 0.37 g (70%) of **14** as a white powder following recrystallization from abs EtOH/anhydrous Et_2O: mp 249-250 °C (with dec.); ^{1}H NMR (DMSO-d_6) δ 1.53 (s, 6H, CH_3 x 2), 2.73 (t, *J* = 5.76 Hz, 2H, CH_2), 3.24 (t, *J* = 5.6 Hz, 2H, CH_2), 7.11 (dd, *J* = 8.36, 1.56 Hz, 1H, ArH), 7.37 (d, *J* = 8.36 Hz, 1H, ArH), 7.48 (s, *J* = 1.56 Hz, 1H, ArH), 11.86 (br s, 1H, ArNH). Anal. Calcd for $[(C_{13}H_{15}BrN_2)_2 \cdot C_2H_2O_4 \cdot H_2O)]$ C, H, N.

6-Bromo-2-(1,1-Dimethylallyl)-3-(piperidin-4-yl)-1*H*-indole Hydrochloride (15). Gaseous HCl was bubbled through a solution of **34** (0.10 g, 0.70 mmol) in anhydrous EtOAc (10 mL). The solid product was recrystallized from *i*-PrOH to afford **15** (0.04 g, 41% yield) as a white solid: mp > 300 ºC; ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 6H, 2xCH_3), 1.60-1.63 (m, 2H, CH_2), 2.21-2.30 (m, 2H, CH_2), 2.79-2.86 (m, 2H, CH_2), 3.09-3.21 (m, 1H, CH), 3.27-3.31 (m, 2H, CH_2), 5.05-5.10 (m, 2H, CH_2), 6.08-6.15 (dd, *J* = 10.5, *J* = 17.3 Hz, 1H, CH), 7.04-7.07 (dd, *J* = 1.8, J = 8.5 Hz, 1H, Ar-H), 7.51 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.71 (d, *J* = 8.5 Hz, 1H, Ar-H), 10.63 (s, 1H, $R_2NH_2^+Cl^-$). Anal. Calcd for $(C_{18}H_{23}BrN_2 \cdot HCl \cdot 2H_2O$) C, H, N.

1-(6-Bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propan-2-amine Hydrochloride (16). Gaseous HCl was bubbled into a solution of **37** (300 mg, 0.2 mmol) in anhydrous Et_2O (20 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 24 h and the solvent was evaporated to yield a white solid which was recrystallized from *i*-

PrOH/Et_2O to yield 60 mg (27%) of **16** as white crystals: mp 229-230 °C; ^{1}H NMR (DMSO-d_6) δ 1.09 (d, 3H, CH_3), 1.51 (d, 6H, $(CH_3)_2$), 2.95-3.14 (m, 2H, CH_2), 3.43 (m, 1H, CH), 5.08-5.15 (m, 2H, CH), 6.14-6.21 (m, 1H, CH), 7.14 (dd, 1H, Ar), 7.51 (m, 2H, Ar), 8.07 (br s, 3H, NH_3^+ aliphatic), 10.86 (br s, 1H, indolic NH). Anal. Calcd for ($C_{16}H_{21}BrN_2$·HCl) C, H, N.

***N*-Methyl-6-bromo-2-*sec*-butyltryptamine Oxalate** (**17**)**.** Oxalyl chloride (0.2 mL, 2.4 mmol) was added in a dropwise manner to a stirred solution of **40** (0.5 g, 1.98 mmol) in anhydrous Et_2O (20 mL) at 0 °C. The reaction mixture was allowed to stir for 30 min at 0 °C. The solvent was removed under reduced pressure and the residual yellow oil was washed with Et_2O (2 x 5 mL) to remove excess oxalyl chloride. A solution of 40% aq. $MeNH_2$ (25 mL), cooled to 0 °C, was slowly added to 6-bromo-3-yl-2-*sec*-butyl-glyoxyl chloride and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was concentrated in vacuo and the crude product, **42**, (0.5 g) was used in the next step.

A solution of **42** (0.50 g, 1.5 mmol) in anhydrous THF (12 mL) was heated to 60 °C. Then BH_3·DMS (0.42 mL, 4.5 mmol) was added and the reaction mixture was heated at reflux with stirring overnight. The reaction mixture was cooled in an ice bath, quenched with 2N HCl (10 mL), heated at reflux for 30 min, cooled and extracted with Et_2O (2 x 20 mL). The aqueous portion was treated with 10% NaOH to pH 14, and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic portion was washed with H_2O (2 x 15 mL), dried (Na_2SO_4), and the solvent removed under reduced pressure. The solution of crude

residue (0.23 g, 0.74 mmol) and oxalic acid (0.07 g, 0.74 mmol) in $CHCl_3$ (3 mL) was allowed to stir for 0.5 h and the precipitate was collected by filtration. The solid product was recrystallized from *i*-PrOH to afford **17** (0.12 g, 43% yield) as a white solid: mp 126-129 ºC; ^{1}H NMR (DMSO-d_6) δ 0.70 (t, *J* = 7.0 Hz, 3H, CH_3), 0.85-0.87 (d, *J* = 7 Hz, 3H, CH_3), 1.42-1.50 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.76-2.81 (m, 5H, 2 x CH_2, CH_3), 6.92 (d, *J*=4Hz, 1H, ArH), 7.24-7.28 (m, 2H, ArH,), 10.78 (s, 1H, oxalic acid). Anal. Calcd. For ($C_{15}H_{21}BrN_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

***N*-Methyl-6-bromo-2-cyclopentyltryptamine Hydrochloride (18).** A solution of **43** (0.40 g, 1.15 mmol) in anhydrous THF (12 mL) was heated to 60 °C. Then BH_3·DMS (0.33 mL, 3.44 mmol) was added and the reaction mixture was heated at reflux with stirring overnight. The reaction mixture was cooled in an ice bath, quenched with 2N HCl (10 mL), heated at reflux for 30 min, cooled and extracted with Et_2O (2 x 20 mL). The aqueous portion was treated with 10 % NaOH until pH 14, and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic portion was washed with H_2O (2 x 15 mL), dried (Na_2SO_4), and the solvent removed under reduced pressure. The crude product was collected by filtration through a small amount of silica gel using CH_2Cl_2/MeOH (9:1/1% TEA) to give 0.24 g of a yellow oil. The oil was dissolved in CH_2Cl_2 (20 mL) and washed with 10% HCl (10 mL); the organic portion was dried (Na_2SO_4) and the solvent was removed to provide 0.03 g (6%) of **18** as white crystals following recrystallization from absolute EtOH/Et_2O: mp 262-264 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 1.67-2.01 (m, 8H, CH_2), 2.58 (s, 4H, CH_2-CH_2-N), 2.98 (s, 3H, CH_3), 3.24 (m, 1H, CH_2), 7.10 (dd, *J*=8.4, 1.8 Hz, 1H, ArH), 7.43 (d, *J*=1.8 Hz, 1H, ArH,), 7.45 (d, *J*=8.4, 1H, ArH,), 8.61 (br

s, 2H, NH_2^+ ex with D_2O), 10.97 (br s, ArNH, ex with D_2O). Anal. Calcd for ($C_{16}H_{21}BrN_2 \cdot HCl$) C, H, N.

***N*-Methyl-2-(1-methylcyclopropyl)tryptamine Oxalate (19).** Oxalyl chloride (0.72 mL, 8.2 mmol) was added in a dropwise manner to a stirred solution 2-(1-methylcyclopropyl)-1*H*-indole[23] (0.7 g, 4.1 mmol) in anhydrous Et_2O (20 mL) at 0 °C. The reaction mixture was allowed to stir for 30 min at 0 °C. The solvent was removed under reduced pressure and the residual solid was washed with Et_2O (2 x 5 mL) to remove excess oxalyl chloride. A solution of 40% aq. $MeNH_2$ (25 mL), cooled to 0 °C, was slowly added to 2-(1-methylcyclopropyl-1H-indol-3-yl)-glyoxyl chloride and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was filtered and the solid was washed with H_2O (20 mL) to afford 0.28 g (26% over two steps) of the desired product as a pink solid: mp 260-265 °C (dec.); 1H NMR (DMSO-d_6) δ 0.76 (m, 2H), 0.93 (m, 2H), 1.44 (s, 3H), 2.78 (d, 3H, *J* = 4.6 Hz), 7.12-7.20 (m, 2H), 7.38-7.39 (m, 1H), 7.86-7.88 (m, 1H), 7.87 (d, 1H, *J* = 4.6 Hz), 12.12 (br s, 1H).

The above glyoxylamide (0.20 g, 0.8 mmol) was added in a portionwise manner to a suspension of $LiAlH_4$ (0.15 g, 4.0 mmol) in anhydrous THF (10 mL) under N_2 at 0 °C. The stirred reaction mixture was heated at reflux for 4 h. Then the reaction mixture was cooled to room temperature, and excess $LiAlH_4$ was decomposed by addition of 0.1 mL of H_2O, 0.1 mL of NaOH (15%) and 0.3 mL of H_2O. The mixture was filtered to remove the salts of Li and the filtrate was dried (Na_2SO_4) and the solvent was removed under reduced pressure to provide 0.03 g (17%) of the product as a white oil. The free base

(0.03 g, 0.13 mmol) was dissolved in Et_2O and oxalic acid (0.01g, 0.13 mmol) was added. The precipitate was recrystallized from absolute EtOH/anhydrous Et_2O to afford the product as a white powder: mp 133-135 °C; 1H NMR (DMSO-d_6) δ 0.76 (m, 2H), 0.86 (m, 2H), 1.38 (s, 3H), 2.64 (s, 3H), 6.96 (t, 1H, J = 14.7, 7.6 Hz), 7.03 (t, 1H, J = 14.7, 7.6 Hz), 7.26 (d, 1H, J = 7.6 Hz), 7.49 (d, 1H, J = 7.6 Hz). Anal. Calcd for ($C_{15}H_{20}N_2 \cdot C_2H_2O_4 \cdot 0.25\ H_2O$) C, H, N

***N*-Methyl-6-bromo-2-phenyltryptamine Hydrochloride (20).** Oxalyl chloride (0.27 mL, 3.1 mmol) was added in a dropwise manner to a stirred solution of 6-bromo-2-phenylindole[24] (0.42 g, 1.54 mmol) in anhydrous Et_2O (10 mL) at 0 °C. The reaction mixture was allowed to stir for 2 h at room temperature. The solvent was removed under reduced pressure and the residual yellow oil was washed with Et_2O (2 x 5 mL) to remove excess oxalyl chloride. A solution of 40% aq. $MeNH_2$ (25 mL), cooled to 0 °C, was slowly added to 6-bromo-3-yl-2-cyclopentylglyoxylchloride and the reaction mixture was allowed to stir overnight at room temperature (~1 mL of THF was added to aid the dissolution). The reaction mixture was concentrated in vacuo and the crude product was purified by column chromatography (silica gel) using hexanes/EtOAc 7:3 to give 0.37 g (67% over two steps) of the desired product as a yellow powder: mp 290 °C with decomposition; ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 3H, CH_3), 6.47(br s, 1H, NH), 7.33 (dd, *J* = 8.56, 1.72 Hz, 1H, ArH), 7.40-7.44 (m, 5H, Ph), 7.49 (d, *J* = 1.72 Hz, 1H, ArH), 7.99 (d, *J* = 8.56 Hz, 1H, ArH), 8.49 (br s, 1H, ArNH). The product was used without further characterization in the preparation of **20**.

A solution of 6-bromo-3-yl-2-phenyl-*N*-methylglyoxylamide (0.24 g, 0.67 mmol) in anhydrous THF (7 mL) was heated to 60 °C. Then BH_3-DMS (0.2 mL, 2.0 mmol) was added and the stirred reaction mixture was heated at reflux overnight. The reaction mixture was cooled in an ice bath, quenched with 2N HCl (10 mL), heated at reflux for 30 min, cooled and extracted with Et_2O (2 x 20 mL). The aqueous portion was treated with 10 % NaOH until pH 14, and extracted with EtOAc (2 x 20 mL). The combined organic portion was washed with H_2O (2 x 15 mL), dried (Na_2SO_4), and the solvent was removed to provide 0.05 g (2%) of the hydrochloride salt as a grey powder. The salt was recrystallized from absolute EtOH/anhydrous Et_2O to afford 0.03 g (1%) of the target product as a grey powder: mp 245-247 °C, with decomposition; ^{1}H NMR (DMSO-d_6) 2.53 (s, 3H, CH_3), 3.08 (s, 4H, CH_2-CH_2-N), 7.14 (dd, 1H, ArH), 7.38-7.40 (m, 1H, ArH), 7.46-7.49 (m, 3H, ArH), 7.57-7.59 (m, 3H, ArH), 8.54 (br s, 2H, NH_2^+ ex with D_2O), 11.47(br s, ArNH ex with D_2O). Anal. Calcd for ($C_{17}H_{17}BrN_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

***N*-Methyl-6-bromo-1-methyl-2-(1,1-dimethylallyl)tryptamine Hydrochloride (21).** In a dry, round-bottom flask NaH (60% oil dispersion) (0.04 g, 0.9 mmol) was allowed to stir with anhydrous toluene at 0 °C (ice bath) for 10 min. After removal of the toluene, a solution of *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate[25] (0.3 g, 0.7 mmol) in anhydrous DMF (5 mL) was added and stirring was continued at 0 °C (ice bath) for 30 min. Iodomethane (0.15 g, 1.1 mmol) was added to the cold solution and the reaction mixture was allowed to stir at room temperature for 1.5 h. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic portions were washed with H_2O (3 x 25 mL) and brine (40 mL), dried (Na_2SO_4) and evaporated to dryness under reduced pressure to yield a

crude, yellow-colored oil which was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to 10:1) to afford 0.2 g (79%) of *tert*-butyl-2-(6-bromo-1-methyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methylcarbamate as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, $(CH_3)_3$), 1.56 (s, 6H, $(CH_3)_2$), 1.97-2.11 (m, 2H, CH_2), 2.51 (br s, 5H, NCH_3, CH_2), 5.18-5.26 (m, 2H, CH_2), 6.28-6.35 (m, 1H, CH), 6.77 (d, 2H, $ArCH_2$), 7.13-7.18 (m. 7H, Ar), 7.72 (s, 1H, Ar). The product was used without further characterization in the preparation of **21**.

Gaseous HCl was bubbled into a 0 °C solution of *tert*-butyl-2-(6-bromo-1-methyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methylcarbamate (100 mg) in anhydrous EtOAc (10 mL). The reaction mixture was allowed to stir at room temperature for 7 h and the solvent was evaporated under reduced pressure to yield a white solid which was recrystallized from MeOH to yield 35 mg (41%) of **21** as white crystals: mp 251-252 °C; ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 6H, $(CH_3)_2$), 2.53 (s, 3H $NHCH_3$), 2.87 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 3.6 (s, 3H, NCH_3), 4. 88 (d, 1H CH_2), 5.05 (d, 1H, CH_2), 6.15 (m, 1H, CH), 7.12 (d. 1H, Ar), 7.54 (m, 2H, Ar), 8.78 (s, 2H, NH_2^+ aliphatic). Anal. Calcd for ($C_{17}H_{23}BrN_2 \cdot HCl$) C, H, N.

2-(1,1-Dimethylallyl)-3-[2-(*N*-methylamino)ethyl]-6-methylindole Hydrochloride (22). Gaseous HCl was bubbled through a solution of **46** (0.13 g, 0.36 mmol) in dry EtOAc (10 mL). The precipitate was collected by filtration and recrystallized from MeOH/Et_2O to afford 0.09 g (85%) of **22** as brown crystals: mp 236-237 ºC; ^{1}H NMR (DMSO-d_6) δ 1.50 (s, 6H, 2 x CH_3), 2.39 (s, 3H CH_3), 2.59 (s, NH), 2.87 - 3.00 (m, 2H,

CH_2), 3.05 - 3.16 (m, 2H, CH_2), 3.37 (s, 3H, CH_3), 5.08 (s, 1H, CH_2), 5.13 (d, *J* = 6 Hz, 1H, CH_2), 6.15 (dd, *J* = 6 Hz, *J* = 18 Hz, 1H, CH), 6.82 (d, *J* = 8.4 Hz, 1H, ArH), 7.13 (s, 1H, Ar), 7.45 (d, *J* = 8.4 Hz, 1H, Ar), 9.04 (br s, 1H, NH), 10.41 (s, 1H, NH_3^+). Anal. Calcd for ($C_{17}H_{24}N_2 \cdot HCl$) C, H, N.

2-(1,1-Dimethylallyl)-3-[2-(*N*-methylamino)ethyl]-6-trifluoromethylindole Hydrochloride (23). A solution of 6-trifluoromethyl-*N*-methyltryptamine (**47**) (0.12 g, 0.5 mmol) and di-*tert*-butyl dicarbonate (0.12 g, 0.55 mmol) in CH_2Cl_2 (7 mL) was allowed to stir at room temperature for 4 h, and then solvent was removed under reduced pressure. The oily residue (0.14 g, 81%) was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.40 (m, 9H, Boc), 2.66 - 3.23 (m, 5H, CH_2, CH_3), 3.56 (m, 2H, CH_2), 7.03 (s, 1H, CH), 7.30 (m, 1H, Ar-H), 7.56 (m, 2H, Ar-H), 8.34 (br s, 1H, NH).

Hypochlorous acid *tert*-butyl ester (0.06 mL, 0.5 mmol) was added in a dropwise manner to a solution of the above product (0.14 g, 0.41 mmol) and Et_3N (0.07 mL, 0.5 mmol) in dry THF (6.0 mL) at -78 ºC. The clear reaction solution was allowed to stir for 0.5 h before a freshly prepared prenyl 9-BBN[10] (0.16 g, 1.2 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. Addition of aqueous NaOH (3M, 0.5 mL) and 30% H_2O_2 (0.5 mL) was followed by allowing the reaction to stir for 1 h. The reaction mixture was diluted with Et_2O (30 mL), the organic layer was separated and washed with semisaturated solution of NaCl (3 x 20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column

chromatography (Aldrich silica gel 60) using hexanes/ EtOAc (7:1) as eluent to afford (0.07 g, 40 % yield) of an oil: ^{1}H NMR ($CDCCl_3$): δ 1.40 (s, 9H, Boc), 1.48 (s, 6H, 2x CH_3), 2.65-3.05 (m, 5H, CH_3, CH_2), 3.19-3.51 (m, 2H, CH_2), 4.94-5.30 (m, 2H, CH_2), 5.86-6.21 (m, 1H, CH), 7.22 (m, 1H, Ar), 7.50 (m, 2H, Ar), 8.52 (b s,1H, NH).

Gaseous HCl was bubbled through a solution of the oil (0.065 g, 0.16 mmol) in anhydrous EtOAc (10 mL). The solid product was recrystallized from MeOH/Et_2O to afford 0.035 g (0.04 g, 65% yield) of **23** as a white solid: decomp at 236 ºC; ^{1}H NMR (DMSO-d_6) δ 1.53 (s, 6H, 2x CH_3), 2.59 (s, 3H, CH_3), 2.93-2.98 (m, 2H, CH_2), 3.13-3.16 (m, 2H, CH_2), 5.11 (d, *J* = 6 Hz, 1H, CH_2), 5.13 (s, 1H, CH_2), 6.12-6.21 (dd, *J* = 6 Hz, *J* = 12 Hz, 1H, CH), 7.27 (d, *J* = 9 Hz, 1H Ar), 7.67 (s, 1H, Ar), 7.81 (d, *J* = 9 Hz, 1H, Ar), 9.03 (b s, 1H, NH), 11.16 (s, 1H, $R_2NH_2^+Cl^-$). Anal. Calcd for ($C_{17}H_{21}F_3N_2 \cdot HCl$) C, H, N.

2-(1,1-Dimethylallyl)-3-[2-(N-methylamino)ethyl]-5-bromoindole Hydrochloride (24). Hypochlorous acid *tert*-butyl ester (0.15 mL, 1.36 mmol) was added in a dropwise manner to a solution of [2-(5-bromo-1*H*-indol-3-yl)-ethyl]-methyl-carbamic acid *tert*-butyl ester[2026] (0.40 g, 1.13 mmol) and Et_3N (0.19 mL, 1.36 mmol) in dry THF (10.0 mL) at -78 ºC. The clear reaction solution was allowed to stir for 0.5 h before a freshly prepared prenyl 9-BBN[10] (0.43 g, 2.26 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. Addition of aqueous NaOH (3M, 0.5 mL) and 30% H_2O_2 (0.5 mL) was followed by allowing the reaction mixture to stir for 1 h. The reaction mixture was diluted with Et_2O (30.0 mL), the organic layer was separated and washed with a

semisaturated solution of NaCl (3 x 20.0 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (Aldrich silica gel 60) using hexanes/ EtOAc (7:1) as eluent to afford N-[2'-[2-(1", 1"-dimethyl-allyl)-5-bromo-1H-indol-3-yl]-ehyl]-N-methylcarbamic acid *tert*-butyl ester (0.3 g, 63 % yield) as an oil: ^{1}H NMR ($CDCCl_3$): δ 1.44-1.46 (m, 15 H, 2 x CH_3, Boc), 2.84-2.92 (m, 5H, CH_3, CH_2), 3.31 (m, 2H, CH_2), 5.07-5.11 (m, 2H, CH_2), 5.99-6.07 (m, 1H, CH), 7.06-7.13 (m, 2H, 2 x Ar-H), 7.60 (s, 1H, Ar-H), 7.90 (b.s.,1H, NH).

Gaseous HCl was bubbled through a solution of the ester (0.29 g, 0.70 mmol) in anhydrous EtOAc (10 mL). The solid product was recrystallized from *i*-PrOH to afford **24** (0.04 g, 16% yield) as a brown solid: mp 248-249 ºC; ^{1}H NMR (DMSO-d_6) δ 1.55 (s, 6H, 2 x CH_3), 2.64 (s, 3H, N-CH_3), 2.96-3.00 (m, 2H, CH_2), 3.11-3.14 (m, 2H, CH_2), 5.13 -5.17(m, 2H, CH_2), 6.15-6.22 (m, 1H, CH), 7.19 (dd, *J* = 1.84 Hz, *J* = 8.52 Hz, 1H Ar-H), 7.36 (d, *J* = 8.52 Hz, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 8.92 (b s, H, NH), 10.89 (s, 1H, $R_2NH_2^+Cl^-$). Anal. Calcd for ($C_{16}H_{21}BrN_2 \cdot HCl \cdot 0.5\ H_2O$) C, H, N.

2-(1,1-Dimethylallyl)-3-[2-(N-methylamino)ethyl]-5,6-dibromoindole Hydrochloride (25). Gaseous HCl was bubbled through a solution of *tert*-butyl-2-(5,6-dibromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**50**) (280 mg) in dry anhydrous EtOAc (10 mL) at 0 °C. The reaction mixture was allowed to stir for 24 h and the solvent was evaporated to yield a white solid which upon recrystallization from *i*-PrOH, yielded 60 mg (25%) of **25** as white crystals: mp 238-239 °C; ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 6H, CH_3), 2.62 (s, 3H NH(CH_3), 2.95 (t, 2H, CH_2), 3.09 (t, 2H, CH_2), 5.10-5.16 (m, 2H,

CH_2), 6.12-6.19 (m, 1H, CH), 7.71 (s, 1H, Ar), 8.03 (s, 1H, Ar), 8.81 (br s, 1H, NH^+ aliphatic), 10.98 (s, 1H, indolic NH). Anal. Calcd for ($C_{16}H_{20}Br_2N_2 \cdot HCl$) C, H, N.

***tert*-Butyl-2-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate (28).** *tert*-Butyl hypochlorite (0.5 g, 4.6 mmol) was added to a solution of *tert*-butyl-2-(1*H*-indol-3-yl)ethylcarbamate **27**[27] (1.0 g, 3.8 mmol) and Et_3N (0.5 g, 4.6 mmol) in anhydrous THF (25 mL) at -78 °C and the solution was allowed to stir for 45 min. Freshly prepared prenyl 9-BBN[10] (7.7 mmol) was added in a dropwise manner over 15 min while maintaining the temperature at -55 °C. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 3 h. Sodium hydroxide (3M, 10 mL) and H_2O_2 (30% v/v, 10 mL) were added in a dropwise manner and the reaction mixture was allowed to stir for 1 h at room temperature. The reaction was diluted with Et_2O (100 mL), the organic layer was separated and washed with H_2O (3 x 60 mL), brine (80 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to yield a crude residue which was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 5:1) to afford 0.5 g (36%) of **28** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, CH_3), 1.47 (s, 6H, CH_3), 2.96 (t, 2H, CH_2), 3.32 (m, 2H, CH_2), 4.53 (br s, 1H, NH aliphatic), 5.06-5.11 (m, 2H, CH_2), 6.01-6.08 (m, 1H, CH), 6.98-7.08 (m, 2H, Ar), 7.21 (d, *J* = 8.0 Hz, 1H, Ar), 7.48 (d, *J* = 7.8 Hz, 1H, Ar), 7.79 (br s, 1H, indolic NH). Compound **28** was used in the preparation of **8**.

6-Bromo-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (31). 4-Piperidone hydrochloride monohydrate (1.96 g, 12.75 mmol) was added in one portion to a solution

of 6-bromoindole (**30**) (1.0 g, 5.1 mmol) and KOH (1.5 g, 27.03 mmol) in methanol (25 mL) and the mixture was heated at reflux for 5 h. After cooling the reaction mixture, the potassium chloride precipitate was removed by filtration and the solution volume was reduced to 1/3 under reduced pressure. Addition of water (5 mL) was followed by filtration of the precipitate. After washing with dichloromethane **31** was obtained as yellow solid (1.20 g, 85%): mp 143-145 ºC; ^{1}H NMR (DMSO-d_6) δ 2.43 (m, 2H, CH_2), 2.99-3.02 (t, J = 5.7 Hz, 2H, CH_2), 3.46-3.48 (m, 2H, CH_2), 6.20 (m, 1H, CH), 7.18-7.21 (dd, J = 1.8 Hz, J = 8.5 Hz, 1H, Ar-H), 7.46 (d, J = 2.1 Hz, 1H, Ar-H), 7.61 (d, J = 1.8 Hz, 1H, Ar-H), 7.82 (d, J = 8.5 Hz, 1H, CH), 11.29 (s, 1H, NH).

6-Bromo-3-(piperidin-4-yl)-1*H*-indole (32). Starting material **31** (0.41 g, 1.48 mmol) was hydrogenated in a Parr apparatus (50 Psi) overnight with PtO_2 (0.03g) in HOAc (25 mL). Upon completion of the reaction the catalyst was removed by filtration, pH of the reaction mixture was adjusted to 8 (3N NaOH), and the solution was extracted with EtOAc (3 x 15 mL). The organic portion was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure to obtain **32** (0.28 g, 70%) as a light-yellow solid: mp 197-199 °C; ^{1} H NMR (CD_3CN-d_3) δ 1.61-1.72 (m, 2H), 1.92-2.01 (m, 2H), 2.75-2.82 (td, J = 12.4 Hz, J = 2.4 Hz, 2H), 2.91-2.98 (m, 1H, CH), 3.14-3.17 (m, 2H), 7.06 (s, 1H, CH), 7.15-7.18 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.57-7.59 (m, 1H, Ar-H), 9.16 (br s, 1H, NH).

***tert*-Butyl 4-(6-bromo-1*H*-indol-3-yl)piperidine-1-carboxalate (33).** A solution of **32** (0.20 g, 0.72 mmol), Et_3N (0.12 mL, 0.86 mmol) and di-*tert*-butyl dicarbonate (0.16 g,

0.72 mmol) in CH_2Cl_2 (8 mL) was allowed to stir at room temperature for 2 h, and then solvent was removed under reduced pressure. The oily residue (0.21 g, 76%) was used without further purification. ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, Boc), 1.54-1.60 (m, 2H), 1.90-1.96 (m, 2H), 2.77 – 2.86 (m, 2H), 4.14 (m, 1H, CH), 6.84 (d, *J* = 1.72 Hz, 1H, CH), 7.10-7.12 (dd, *J* = 8.5 Hz, *J* = 1.7 Hz, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 7.38 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.42 (d, *J* = 1.7 Hz, 1H, Ar-H), 8.29 (br s, 1H, NH).

***tert*-Butyl 4-[6-bromo-2-(1,1-dimethylallyl)-1*H*-indole-3-yl]piperidine-1-carboxylate (34).** Hypochlorous acid *tert*-butyl ester (0.08 mL, 0.66 mmol) was added in a dropwise manner to a solution of **33** (0.21 g, 0.55 mmol) and Et_3N (0.09 mL, 0.66 mmol) in dry THF (6.0 mL) at -78 ºC. The clear reaction solution was allowed to stir for 0.5 h before a freshly prepared prenyl 9-BBN[10] (0.21 g, 1.1 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. Addition of aqueous NaOH (3M, 0.5 mL) and 30% H_2O_2 (0.5 mL) was followed by allowing the reaction mixture to stir for1 h. The reaction mixture was diluted with Et_2O (30 mL); the organic layer was separated and washed with 25% aqueous solution of NaCl (3 x 20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (Aldrich silica gel 60) using hexanes/EtOAc (7:1) as eluent to afford **34** (0.10 g, 40% yield) as a white solid: mp 190-191 °C; ^{1} H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$): δ 1.44–1.47 (m, 15H, 2 x CH_3, Boc), 1.55-1.58 (m, 2H), 1.97-2.08 (m, 2H), 2.62-2.65 (m, 2H), 3.00-3.7 (m, 1H, CH), 4.17 (m, 2H), 5.04-5.09 (m, 2H, CH_2), 5.86-6.21 (dd, *J* = 17.6 Hz, *J* = 27.9 Hz, 1H, CH), 7.06 (dd, *J* = 8.6 Hz, *J* = 1.8 Hz, 1H, Ar-H),

7.37 (d, *J* = 1.8 Hz, 1H. Ar-H), 7.41 (d, *J* = 17.6 Hz, 1H, Ar-H), 7.78 (br s, 1H, NH). Compound **34** was used in the preparation of **15**.

1-(6-Bromo-1*H*-indol-3-yl)propan-2-amine (35). Sodium borohydride (2.8 g, 73 mmol) was added to a solution of anhydrous THF (40 mL) and $BF_3{\cdot}Et_2O$ (10 mL, 79 mmol) at 0 °C, and the resulting suspension was allowed to stir for 15 min while maintaining the temperature at 0 °C. 3-[(*E*)-2-Nitroprop-1-en-1-yl)-6-bromo-1*H*-indole[13] (3.7 g, 13 mmol) was then added, and the reaction mixture was allowed to stir at reflux for 4 h, cooled in an ice bath, and quenched by the careful addition of H_2O (2 mL). The mixture was acidified with HCl (2N, to pH 1) and heated at reflux for a further 2 h. After cooling, the acidic solution was extracted with Et_2O (2 x 25 mL). The aqueous portion was basified with NaOH (3M, to pH 10) and extracted with Et_2O (3 x 50 mL). The combined organic portion was washed with H_2O (3 x 100 mL), brine (50 mL), dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to yield 1.5 g (44%) of **35** as a white foam which was used in the preparation of **36** without purification.

***tert*-Butyl-1-(6-bromo-1*H*-indol-3-yl)propyl-2-carbamate (36).** Di-*tert*-butyl dicarbonate (1.3 g, 5.8 mmol) and Et_3N (0.6 g, 5.8 mmol) were added to a solution of **35** (1.5 g, 1.3 mmol) in anhydrous DMF (20 mL) and the reaction mixture was allowed to stir for 24 h. The reaction mixture was poured into H_2O with ice (100 mL) and extracted with EtOAc (3 x 40 mL). The combined organic portion was washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield the crude product. The crude residue was purified by column chromatography (silica gel;

hexanes/EtOAc; 100:1 to 3:1) to afford 1.5 g (75%) of **36** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.04 (d, 3H, CH_3), 1.36 (s, 9H, $(CH_3)_3$), 2.72-2.89 (m, 2H, CH_2), 3.92 (m, 1H, CH), 4.34 (s, 1H, NH aliphatic), 6.93 (s, *J* = 2.1 Hz, 1H, Ar), 7.14 (dd, *J* = 8.4, 1.6 Hz, 1H, Ar), 7.43 (m, 2H, Ar), 7.95 (s, 1H, indolic NH).

***tert*-Butyl-1-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-2-carbamate (37).** *tert*-Butyl hypochlorite (0.4 g, 3.3 mmol) was added to a solution of **36** (0.9 g, 2.5 mmol) and Et_3N (0.3 g, 3.3 mmol) in anhydrous THF (30 mL) at -78 °C and the solution was allowed to stir for 45 min. Freshly prepared prenyl-9-BBN[10] (5.1 mmol) was added in a dropwise manner over 20 min while maintaining the temperature at -55 °C. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 3 h. Aqueous NaOH (3M, 7 mL) and H_2O_2 (30% v/v, 7 mL) were added in a dropwise manner and stirring was continued for another 1 h. The reaction mixture was diluted with Et_2O (100 mL), the organic layer was separated and washed with H_2O (3 x 100 mL), brine (80 mL), dried (Na_2SO_4) and evaporated under reduced pressure to yield a crude residue which was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 100:8) to afford 0.5 g (45%) of **37** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.09 (d, 3H, CH_3), 1.36 (s, 9H, $(CH_3)_3$), 1.51 (d, 6H, $(CH_3)_2$), 2.95-3.14 (m, 2H, CH_2), 3.43 (m, 1H, CH), 4.34 (s, 1H, NH aliphatic), 5.08-5.15 (m, 2H, CH), 6.14-6.21 (m, 1H, CH), 7.14 (dd, 1H, Ar), 7.51 (m, 2H, Ar), 7.95 (s, 1H, indolic NH).

1-(2-Amino-4-bromophenyl)-2-chloroethanone (39). 3-Bromoaniline (**38**) (5.0 g, 29.1 mmol) and chloroacetonitrile (4.6 mL, 72.7 mmol) were added sequentially to a mixture

of $AlCl_3$ (4.64 g, 34.8 mmol) and a solution of 1.0 M BCl_3/CH_2Cl_2 (34.8 mL, 34.8 mmol) with an extra 30 mL of CH_2Cl_2 cooled in an ice bath. The cloudy reaction mixture was allowed to stir for 0.5 h at room temperature before being heated at reflux for 12 h. The reaction mixture was then cooled in an ice bath, quenched with 2N HCl (45 mL), heated at reflux for 20 min, cooled to room temperature and extracted with CH_2Cl_2 (2 x 50 mL). The combined organic portion was dried (Na_2SO_4) and the solvent was removed under reduced pressure. Recrystallization in hexane gave 3.1 g (43%) of the product as a yellow solid: mp 139-141 °C. ^{1}H NMR ($CDCl_3$) δ 4.54 (s, 2H, CH_2), 6.28 (br s, 2H, NH_2), 6.70 (dd, *J*=8.68, 1.84 Hz, 1H, ArH), 6.81 (d, *J*=1.84 Hz ,1H, ArH), 7.40 (d, *J*=8.68 Hz, 1H, ArH). Compound **39** was used in the preparation of compounds **40** and **41**.

6-Bromo-2-sec-butyl-1*H*-indole (40). A solution of 2.0 M cyclopentyl magnesium chloride in THF (5 mL, 10.0 mmol) was added in a dropwise manner to a solution of **39** (1.0 g, 4.0 mmol) in anhydrous THF (20 mL) at -10 °C. The reaction mixture was kept below 10 °C during the addition and allowed to stir in an ice bath for 15 min. After 15 min at room temperature, the reaction mixture was quenched with NH_4Cl (10 mL), extracted with MTBE (2 x 30 mL) and washed with brine (10 mL). The organic portion was combined, dried (Na_2SO_4) and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography (silica gel) using hexanes/EtOAc (9:1) to afford 0.64 g (38%) of the desired product as yellow solid: mp 37-38 °C; ^{1}H NMR ($CDCl_3$) δ 0.83 (t, *J*=3Hz, 3H, CH_3), 1.25 (d, *J*=3Hz, 2H, CH_2), 1.6-1.65 (m, 2H, CH_2), 2.72-2.75 (m, 1H, CH), 6.14 (s, 1H, ArH), 7.07 (d, J=8.0 Hz, 1H, ArH), 7.29 (s, 1H, ArH), 7.35 (s, 1H, ArH), 7.78 (br s, 1H, ArNH).

6-Bromo-2-cyclopentylindole (41). A solution of 2.0 M cyclopentyl magnesium chloride in THF (10 mL, 20.0 mmol) was added in a dropwise manner to a solution of **39** (2.0 g, 8.0 mmol) in anhydrous THF (20 mL) at -10 °C. The reaction mixture was kept below 10 °C during the addition and allowed to stir in an ice bath for 15 min. After 15 min at room temperature, the reaction mixture was quenched with a saturated solution of NH_4Cl (10 mL), extracted with MTBE (2 x 30 mL) and washed with brine (10 mL). The organic portion was combined, dried (Na_2SO_4), and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography (silica gel) using hexanes/EtOAc (9:1) to afford 0.64 g (24%) of the desired product as a white solid: mp 121-123 °C; 1H NMR ($CDCl_3$) δ 1.70 (m, 6H, CH_2), 2.04 (m, 2H, CH_2), 3.09 (m, 1H, CH), 6.14 (m, 1H, ArH), 7.08 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.29 (d, *J* = 8.4 Hz, 1H, ArH), 7.35 (d, *J* = 1.8 Hz, 1H, ArH), 7.78 (br s, 1H, ArNH).

6-Bromo-3-yl-2-cyclopentyl-*N*-methylglyoxylamide (43). Oxalyl chloride (0.7 mL, 7.56 mmol) was added in a dropwise manner to a stirred solution of **41** (1.0 g, 3.78 mmol) in anhydrous Et_2O (20 mL) at 0 °C. The reaction mixture was allowed to stir for 30 min at 0 °C.The solvent was removed under reduced pressure and the residual yellow oil was washed with Et_2O (2 x 5 mL) to remove excess oxalyl chloride. A solution of 40% aq. $MeNH_2$ (25 mL), cooled to 0 °C, was slowly added to 6-bromo-3-yl-2-cyclopentylglyoxyl chloride and the reaction mixture was allowed to stir overnight at room temperature (~1 mL of THF was added to aid the dissolution). The reaction mixture was concentrated in vacuo and the crude product was purified by column

chromatography (silica gel) using hexanes/EtOAc (7:3) to give 1.1 g (83% over two steps) of the desired product as a beige powder: mp 196 °C with decomposition; 1H NMR ($CDCl_3$) δ 1.70 (m, 6H, CH2), 2.04 (m, 2H, CH_2), 3.09 (t, 1H), 6.14 (m, 1H, ArH), 7.08 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 7.29 (d, *J* = 8.4 Hz, 1H, ArH), 7.35 (d, *J* = 1.8 Hz,1H, ArH), 7.78 (br s, 1H, ArNH).

6,*N*-Dimethyltryptamine (44). Oxalyl chloride (3.19 mL, 36.5 mmol) was added in a dropwise manner at 0 °C to a stirred solution of 6-methylindole (4.0 g, 30.4 mmol) in anhydrous Et_2O (25 mL). The reaction mixture was allowed to stir for 4 h, the precipitate was collected by filtration, thoroughly washed with cold Et_2O (20 mL), and added to an ice-cold 40% aqueous solution of $MeNH_2$ (30 mL). The reaction mixture was allowed to stir at room temperature overnight and concentrated under reduced pressure. The precipitate was collected by filtration and recrystallized from MeOH to afford 3.3 g (50%) of an off-white solid: mp 226-226 °C; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 2.76 (d, J = 6 Hz, CH_3), 7.09 (d, J = 9 Hz, 1H), 7.33 (s, 1H Ar), 8.65 (d, J = 9 Hz, 1H, Ar), 8.71 (s, 1H, Ar), 12.10 (b.s., 1H, NH).

A solution of the above glyoxylamide (0.5 g, 2.3 mmol) in dry THF (10 mL) was added in a dropwise manner at 0 °C to a stirred suspension of $LiAlH_4$ (0.87 g, 23 mmol) in dry THF (15 mL). The reaction mixture was heated under a N_2 atmosphere for 5 h, cooled to 0 °C and quenched with MeOH (1 mL), NaOH (15%, 1.5 mL), and H_2O (1 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated under reduced pressure to give an oily yellow residue. Purification by chromatography on a silica gel column

(Aldrich silica gel 60) using CH_2Cl_2/MeOH/NH_4OH (8:1:0.1) as eluent gave **44** (free base, 0.26 g, 60%) as a brown oil: ^{1}H NMR ($CDCCl_3$) δ 1.81 (b.s., NH), 2.47 (m, 6H, 2 x CH_3), 2.95 - 3.05 (m, 4H, 2 x CH_2), 6.77 - 7.13 (m, 2H, Ar), 7.19 (s, 1H, CH), 7.56 (d, J = 9 Hz, 1H, Ar), 8.06 (b.s., 1H, NH).

[2-(6-Methyl-1*H*-indol-3-yl)-ethyl]-methyl-carbamic acid *tert*-butyl ester (45). A solution of 6,*N*-dimethyltryptamine **(44)** (0.16 g, 0.85 mmol), and di-*tert*-butyl dicarbonate (0.2 g, 0.93 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 3 h, solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel column (Aldrich silica gel 60) using hexanes/EtOAc (5:1) as eluent to obtain **45** (0.16 g, 65%) as a white solid: mp 104-106 ºC; ^{1}H NMR ($CDCCl_3$) δ 1.39 (s, 9H, Boc), 2.46 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.89 - 3.06 (m, 4H, 2 x CH_2), 6.67 - 6.87 (m, 2H, Ar), 7.05 (s, 1H, Ar), 7.51 (d, J = 9 Hz, 1H, Ar), 8.03 (b.s., 1H, NH).

***N*-[2'-[2-(1,1-Dimethylallyl)-6-methyl-1*H*-indol-3-yl]-ehyl]-*N*-methylcarbamic acid *tert*-butyl ester (46).** Hypochlorous acid tert-butyl ester (0.07 mL, 0.66 mmol) was added in a dropwise manner at -78 ºC to a solution of **45** (0.16 g, 0.55 mmol) and Et_3N (0.12 mL, 1.1 mmol) in dry THF (7.0 mL). The clear reaction solution was stirred for 0.5 h before freshly prepared prenyl 9-BBN[10] (0.21 g, 1.1 mmol) solution in dry THF (5 mL) was added in a dropwise manner. After stirring for 30 min, the reaction mixture was warmed to room temperature, and stirring was continued for 1 h. The addition of 3M NaOH (0.5 mL) and 30% H_2O_2 (0.5 mL) was followed by continued stirring for 1 h. The reaction mixture was dilluted with Et_2O (30 mL); the organic layer was separated and

washed with a saturated solution of NaCl (3 x 50 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by chromatography on silica gel column (Aldrich silica gel 60) using hexanes/EtOAc (10:1) as eluent to obtain **46** (0.13 g, 65 %) as a yellow oil: ^{1}H NMR ($CDCCl_3$) δ 1.54 (b s, 9H, Boc), 1.86 - 1.96 (m, 6H, 2 x CH_3), 2.48 (s, 3H, CH_3), 2.80 - 3.10 (m, 5H, CH_2, CH_3), 3.44 (m, 2H, CH_2), 5.17 (s, 1H, vinylic H), 5.23 (d, *J* = 9 Hz, 1H, vinylic H), 6.00 - 6.38 (dd, *J* = 9 Hz, 17 Hz, 1H, vinylic H), 6.97 (m, 1H, Ar), 7.13 (s, 1H, Ar), 7.40-7.63 (m, 1H, Ar), 8.05 (b s, 1H, NH).

6-Trifluoromethyl-*N*-methyltryptamine (47). Oxalyl chloride (0.28 mL, 3.2 mmol) was added in a dropwise manner at 0 °C to a stirred solution of 6-trifluoromethylindole[2228] (0.50 g, 2.7 mmol) in dry Et_2O (10 mL). The reaction mixture was allowed to stir for 4 h; the precipitate was collected by filtration, thoroughly washed with cold Et_2O (20 mL) and added to an ice-cold 40% aqueous solution of $MeNH_2$ (15 mL). The reaction mixture was allowed to stir at room temperature overnight. The precipitate was collected by filtration and recrystallized from MeOH to afford the gloxylamide (0.53 g, 73%) as an off-white solid: mp 281-283 °C; ^{1}H NMR (DMSO-d_6) δ 2.79 (d, *J* = 6 Hz, 3H, CH_3), 7.60 (d, *J* = 9 Hz, 1H, Ar-H), 7.91 (s, 1H, CH), 8.43 (d, *J* = 9 Hz, 1H, Ar-H), 8.79 (m, 1H, Ar-H), 8.99 (s, 1H, NH), 12.51 (s, 1H, NH). Borane dimethylsulfide complex (2.0 M in THF, 1.1 mL) was added in a dropwise manner at 60 °C to a stirred solution of the glyoxylamide (0.2 g, 0.7 mmol) in dry THF (10 mL). The reaction mixture was heated at reflux under a N_2 atmosphere for 5 h, cooled to 0 °C, and quenched with aqueous HCl (3 M, 1.5 mL). The reaction mixture was heated at reflux for 0.5 h, cooled to room temperature and

diluted with H_2O (10 mL). After extracting the solution with CH_2Cl_2 (20 mL), the aqueous portion was basified (3N NaOH) and again extracted with CH_2Cl_2 (20 mL). The organic portion was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give **47** (0.12 g, 67%) as an off-white solid: mp 135-137 °C; ^{1}H NMR (DMSO-d_6) δ 2.51 (m, 3H, CH_3), 2.92-2.95 (m, 4H, 2xCH_2), 7.12 (dd, J = 1.2 Hz, J = 6 Hz, 1H, Ar-H), 7.23 (s, 1H, CH), 7.52-7.54 (m, 2H, Ar-H).

***N*-Methyl-5,6-dibromotryptamine (48).** A solution of 5,6-dibromo-1*H*-indole[2329] (1.8 g, 6.6 mmol) in anhydrous Et_2O (20 mL) in a 2-neck flask was chilled to -5 °C and N_2 was bubbled in for 5 min. Oxalyl chloride (1.2 mL, 13.1 mmol) was added in a dropwise manner and the reaction mixture was heated at reflux for 5 h. The reaction mixture was filtered and the precipitate was washed with cold Et_2O (2 x 10 mL) and air dried. The solid was added to cold $MeNH_2$ (40% in H_2O, 25 mL) and the solution was allowed to stir at room temperature overnight. The reaction mixture was diluted with H_2O (100 mL) and the residue was collected by filtration and air dried to yield 1.8 g (76%) of the glyoxylamide as a buff-colored solid: mp 263-264 °C (decomp); ^{1}H NMR (DMSOd_6) δ 2.75 (s, 3H, CH_3), 7.14 (s, 1H, Ar), 7.63 (s, 1H, Ar), 7.84 (s, 1H, Ar), 8.09 (br s, 1H, indolic NH). The glyoxylamide was used without further purification in the next step.

Borane dimethylsulfide (10.1M in THF, 1.4 mL, 14 mmol) was added in a dropwise manner at 60 °C to a stirred solution of *N*-methyl-5,6-dibromo-1*H*-indol-3-glyoxylamide (1.7 g, 5 mmol) in dry THF (35 mL). The reaction mixture was allowed to stir at reflux for 7 h, cooled to 0 °C, quenched with H_2O (4 mL), acidified with HCl (2N, to pH 1), and

heated at reflux for 1 h. The THF was evaporated under reduced pressure and the remaining aqueous portion was diluted with H_2O (100 mL), basified with NaOH (3M, to pH 9) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic portions were washed with H_2O (2 x 50 mL), dried (Na_2SO_4), and solvent was evaporated under reduced pressure to yield 1.5 g (96%) of **48** as a white solid: mp 107-111 °C; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, $NHCH_3$), 2.71 (t, 2H, CH_2), 2.76 (t, 2H, CH_2), 7.25 (s, 1H, Ar), 7.73 (s, 1H, Ar), 7.91 (s, 1H, Ar), 11.09 (br s, 1H, indolic NH). The material was used without further purification for synthesis of compound **49**.

***tert*-Butyl-2-(5,6-dibromo-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (49).** Triethylamine (0.5 g, 4.5 mmol) and di-*tert*-butyl dicarbonate (1.0 g, 4.5 mmol) were added to a solution of *N*-methyl-5,6-dibromotryptamine (**48**) (1.5 g, 4.5 mmol) in DMF (20 mL) and the solution was allowed to stir at room temperature for 24 h. The reaction mixture was quenched with H_2O (40 mL) and extracted with EtOAc (3 x 40 mL). The combined organic portion was washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 3:1) to afford 1.2 g (60%) of **49** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, CH_3), 2.84 (s, 3H, $NHCH_3$), 2.91 (t, 2H, CH_2), 3.48 (t, 2H, CH_2), 7.01 (s, 1H, Ar), 7.66 (s, 1H, Ar), 7.87 (s, 1H, Ar), 8.07 (br s, 1H, indolic NH).

***tert*-Butyl-2-(5,6-dibromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (50).** *tert*-Butyl hypochlorite (0.3 g, 3.0 mmol) was added to a

solution of **49** (1.0 g, 2.3 mmol) and Et_3N (0.3 g, 3 mmol) in THF (25 mL) at -78 ^oC and the solution was allowed to stir for 90 min. Freshly prepared prenyl 9-BBN[10] (4.6 mmol) was added in a dropwise manner over 20 min while maintaining the temperature at -55 ^oC. The reaction mixture was allowed to warm to room temperature and stirring continued for an additional 6 h. Sodium hydroxide (3M, 10 mL) and H_2O_2 (30%, 10 mL) were added in a dropwise manner, and reaction mixture was stirred for an additional 2 h and then diluted with Et_2O (100 mL). The organic portion was washed with H_2O (3 x 60 mL), brine (80 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 10:1) to afford 0.4 g (33%) of the product as a white foam: mp 55-57 °C; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, CH_3), 2.71-2.90 (m, 5H, CH_2, CH_3), 3.29 (m, 2H, CH_2), 5.07-5.12 (m, 2H, CH_2), 5.99-6.06 (m, 1H, CH), 7.49 (s, 1H, Ar), 7.72 (s, 1H, Ar), 7.82 (br s, 1H, indolic NH).

Electrophysiology.

Two electrode voltage clamp (TEVC) electrophysiology was used to evaluate dFBr and dFBr analogs. cDNA for α4 and β2 receptors was synthesized by GeneArt Inc. (Burlingame, CA) from sequences obtained from the National Center for Biotechnology Information (sequences NM_000744.5 and NM_000748.2 respectively) and inserted into a pcDNA3.1 expression vector. High yield capped mRNA transcripts were obtained using the mMessage mMachine transcription kit (Ambion, Austin, TX) and injected by microinjection into collagenase treated Xenopus oocytes using a ratio of α4 to β2 of 1:1.

Current recordings were made using an automated TEVC recording system incorporating a Gilson auto sampler injection system and a Warner Instruments OC-725C oocyte voltage clamp amplifier as previously described.[30] Data collection was performed using Axon instruments pClamp software.

Current and Voltage electrodes (1-4 MΩ) were filled with a 3M KCl solution. Oocytes were held in a vertical flow chamber of 200 µl volume and clamped at a holding potential of -60 mV. Oocytes were continuously perfused with a modified ND-96 buffer incorporating phosphate rather than HEPES buffer due to the previous finding that HEPES modulates the high sensitivity isoform of the α4β2 receptor (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 mM phosphate, pH 7.4).[31] Test compounds were dissolved in identical Phosphate-ND-96 buffer and injected into the chamber at a rate of 20 ml/min using the auto sampler injection system.

Oocytes were first exposed to increasing concentrations of each analog alone to determine if any were capable of activating receptors. Since no analogs elicited responses when applied alone, oocytes were co-perfused for a duration of 10 s with each dFBr analog and 100 µM ACh to identify inhibition or potentiation of ACh-induced responses. Responses for dFBr analogs were evaluated at concentrations ranging from 0.01µM to 300 µM and the peak amplitudes used to create dose response curves. In order to permit comparison of responses from different oocytes, individual responses to drug application were normalized to control responses elicited using 100 µM ACh in the absence of dFBr or its analogs. Oocytes were washed with HEPES-ND96 for 7 min

prior to reapplication of another drug concentration. Oocytes were periodically exposed to 100 μM ACh to assure responses returned to normal following washout of the analog/ACh combination. All data were collected from at least four replicate experiments using four oocytes obtained from at least two different frogs.

Currents were recorded by either application of ACh alone or by co-application of test compounds with a fixed 100 μM concentration of ACh. To compare responses from different oocytes, individual responses to drug application were normalized to the control responses elicited by 100 μM ACh. Data were collected from at least four replicate experiments using oocytes obtained from at least two different frogs.

$EC_{50,}$ I_{max} and IC_{50} values were determined from normalized, pooled data.

Concentration-response profiles were determined using nonlinear curve fitting and GraphPad Prism software (GraphPad Software, La Jolla, CA) using standard built-in algorithms. All analogs that potentiated ACh-induced responses showed biphasic (bell shaped) dose response curves. EC_{50} and (where possible) IC_{50} values were determined for these curves using a biphasic dose response equation that simultaneously fit both the potentiation and inhibition curves and enabled determination of the I_{max} for potentiation.[31] The percent change in I_{max} calculated using this equation (Table 1) represents the calculated change in Imax due to the addition of the potentiator. A maximum observed effect was also determined from the peak of the dose response curve. Typically the maximum effect is substantially less than the I_{max} change due to the

contribution of the inhibition phase of the curve. Dose-response curves where the IC_{50}/EC_{50} ratio was less than 10 were particularly challenging to fit using the biphasic equation and thus required constraint of some variables. For consistency, the initial plateau for all fits was typically fixed at 1 (i.e., dFBr alone) and the Hill slopes were constrained to 1 for both the potentiation and inhibition phases. Some curves required an additional constraint of plateau 2 (maximum inhibition) to be constrained to 0 in order to determine an IC_{50} value as indicated in Table 1. For compounds that showed only inhibition of ACh-induced responses, IC_{50} values were determined using a standard inhibition dose-response equation.

References

1. Rollema, H.; Coe, J. W.; Chambers, L. K.; Hurst, R. S.; Stahl, S. M.; and Williams, K. E. (2007) Rationale, pharmacology and clinical efficacy of partial agonists of α4β2 nACh receptors for smoking cessation. *Trends Pharmacol. Sci. 28*, 316–325
2. Quik, M.; and Wonnacott, S. (2011) α6β2* and α4β2* nicotinic acetylcholine receptors as drug targets for Parkinson's disease. *Pharmacol. Rev*. 63, 938–966.
3. Yu, L. F.; Zhang, H. K.; Caldarone, B. J.; Eaton, J. B.; Lukas, R. J.; and Kozikowski, A. P. (2014) Recent developments in novel antidepressants targeting $\alpha 4\beta 2$-nicotinic acetylcholine receptors. *J. Med. Chem*. *57*, 8204–8223.
4. Nirogi, R.; Goura, V.; Abraham, R.; and Jayarajan, P. (2013) α4β2* neuronal nicotinic receptor ligands (agonist, partial agonist and positive allosteric modulators) as therapeutic prospects for pain. *Eur. J. Pharmacol*. *712*, 22–29.
5. Zhu, C. Z.; Chin, C. L.; Rustay, N. R.; Zhong, C.; Mikusa, J.; Chandran, P. et al. (2011) Potentiation of analgesic efficacy but not side effects: co-administration of an α4β2 neuronal nicotinic acetylcholine receptor agonist and its positive allosteric modulator in experimental models of pain in rats. *Biochem. Pharmacol*. *82*, 967–976.
6. Nirogi, R.; Jabaris, S. L.; Jayarajan, P.; Abraham, R.; Shanmuganathan, D.; Rasheed, M. A. et al. (2011) Antinociceptive activity of α4β2* neuronal nicotinic receptor agonist A-366833 in experimental models of neuropathic and inflammatory pain. *Eur. J. Pharmacol*. *668*, 155–162.

7. Sala, F.; Mulet, J.; Reddy, K. P.; Bernal, J. A.; Wikman, P.; Valor, L. M.; Peters, L.; König, G. M.; Criado, M.; and Sala, S. (2005) Potentiation of human α4β2 neuronal nicotinic receptors by a *Flustra foliacea* metabolite. *Neurosci. Lett. 373*, 144–149.

8. Kim, J.-S.; Padnya, A.; Weltzin, M.; Edmonds, B. W.; Schulte, M. K.; and Glennon, R. A. (2007) Synthesis of desformylflustrabromine and its evaluation as an α4β2 and α7 nACh receptor modulator. *Bioorg. Med. Chem. Lett*. *17*, 4855–4860.

9. German, N.; Kim, J.-S.; Jain, A.; Dukat, M.; Pandya, A.; Ylong, M.; Weltzin, M.; Schulte, M. K.; and Glennon, R. A. (2011) Deconstruction of the α4β2 nicotinic acetylcholine receptor positive allosteric modulator *des*formylflustrabromine. *J. Med. Chem*. *54*, 7259–7267.

10. Kramer, G. W.; and Brown, H. C. (1977) The preparation and some unusual chemistry of B-allyl derivatives of 9-borabicyclo[3.3.1]nonane. *J. Organomet. Chem. 132*, 9–27.

11. Kuttruff, C.A.; Zipse H.; and Trauner, D. (2011) Concise total syntheses of variecolortides A and B through an unusual hetero-Diels-Alder reaction. *Angew. Chem. Int. Ed. 50*, 1402–1405.

12. Celik, L.; Sinning, S.; Severinsen, K.; Hansen, G.G.; Moller, M.S.; Bols, M.; Wibarg, O.; and Schiott, B. (2008) Binding of serotonin to the human serotonin transporter: Molecular modeling and experimental validation. *J. Am. Chem. Soc. 130*, 3853–3865.

13. Schumacher, R.W.; and Davidson, B.S. (1999) Synthesis of didemnolines A-D, N9-substitued β-carbolines alkaloids from the marine Ascidian *Didemnum* sp. *Tetrahedron 55*, 935–942.

14. Pei , T.; Tellers, D. M.; Streckfuss, E.C.; Chen, C.; and Davies, I. W. (2009) [1,2]-Aryl migration in the synthesis of substituted indoles: scope, mechanism, and high throughput experimentation. *Tetrahedron 65*, 3285–3291.

15. Speeter, M. E.; and Anthony, W. C. (1954) The action of oxalyl chloride on indoles: a new approach to tryptamines. *J. Am. Chem. Soc*. *76*, 6208–6210.

16. a) Hansch,C., Leo, A., Unger, S. H., Kim, H., Nikaitani, D., and Lien, E. J. (1973) Aromatic substituent constants for structure-activity correlations. *J. Med. Chem. 16*, 1207–1216. b) Hansch, C., and Elkinns, D. (1971) Partition coefficients and their uses. *Chem. Rev. 71*, 525–616.

17. MacPhee, J. A., Panaye, A., and Dubois, J.-E. (1978). A critical examination of the taft steric parameter - E_s. Definition of a revised, broader and homogeneous scale. *Tetrahedron 34*, 3553–3562.

18. Charton, M. (1975). Steric effects. I. Esterification and acid-catalyzed hydrolysis of esters. *J. Am. Chem. Soc., 97*, 1552–1556.

19. Weltzin, M. W., and Schulte, M. K. (2015) Desformylflustrabromine modulates α4β2 neuronal nicotinic acetylcholine receptor high- and low-sensitivity isoforms at allosteric clefts containing the β2 subunit. *J. Pharmacol. Exp. Ther. 354*, 184–194.

20. Alcaino, C., Musgaard, M., Minguez, T., and Mazzaferro, S. (2017) Role of the Cys loop and transmembrane domain in the allosteric modulation of 42 nicotinic acetylcholine receptors. J. Biol. Chem. *292*, 551–562.

21. Deba, F., Ali, H. I., Tairu, A., Ramos, K., Ali, J., and Hamouda, A. K. (2018) LY2087101 and dFBr share transmembrane binding sites in the (α4)3(β2)2 nicotinic acetylcholine receptor. *Sci. Rep. 8*, 1249.

22. Hugon, B.; Anizon, F.; Bailly, C.; Golsteyn, R.M.; Pierre, A.; Leonce, S.; Hickman, J.; Pfeiffer, B.; and Prudhomme, M. (2007) Synthesis and biological activities of isogranulatimide analogues. *Bioorg. Med. Chem. 15*, 5965–5980.

23. Pirrung M.C.; Deng L.; Li Z.; and Park K. (2002) Synthesis of 2,5-dihydroxy-3-(indol-3-yl)benzoquinones by acid-catalized condensation of indoles with 2,5-dichlorobenzoquinone. *J.Org. Chem. 67*, 8374–8388.

24. Okuma, K.; Seto, J.; Sakaguchi, K.; Ozaki, S.; Nagahora, N.; and Shioji, K. (2009) Palladium-free zinc-mediated hydroamination of alkynes: efficient synthesis of indoles from 2-akynylaniline derivatives. *Tetrahedron Lett. 50*, 2943–2945.

25. Lindel, T.; Brauchle, L.; Golz, G.; and Bohrer, P. (2007) Total synthesis of flustramine C via dimethylallyl rearrangement. *Org. Lett. 9*, 283–286.

26. Burm, B.E.A.; Meijler, M.M.; Korver, J.; Wanner, M.J.; and Koomen, G.-J. (1998) Synthesis of the bromination marine alkaloids (±)-arborescidine A, B and C. *Tetrahedron 54*, 6135–6146.

27. Fuente, M.C.; and Dominquez, D. (2011) Normal electron demand Diels-Alder cycloaddition of indoles to 2,3-dimethyl-1,3-butadiene. *Tetrahedron 67*, 3997–4001.

28. Walkington, A.; Gray, M.; Hossner, F.; Kitteringham, J.; and Voyle, M. (2003) A simple two-step synthesis of indoles. *Synth*. *Commun*. *33*, 2229–2233.

29. Parsons, T.B.; Ghellamallah, C.; Male, L.; Spencer, N.; and Grainger, R.S. (2011) Regioselective dibromination of methyl indole-3-carboxylate and application in the synthesis of 5,6-dibromoindoles. *Org. Biomol. Chem. 9*, 5021–5023.

30. Joshi, P.; Suryanarayanan, A.; and Schulte, M. K. (2004) A vertical flow chamber for Xenopus oocyte electrophysiology and automated drug screening. *J. Neurosci. Methods 132*, 69–79.

31. Weltzin, M. M.; Huang, Y.; and Schulte, M. K. (2014) Allosteric modulation of $\alpha4\beta2$ nicotinic acetylcholine receptors by HEPES. *Eur. J. Pharmacol*. *732*, 159–168.

SUPPORTING INFORMATION

Physicochemical properties of 6-position substituents and the total volume of selected compounds.

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Author Contributions

The project was proposed by RAG and MKS. NG, AJ, and RF-P conducted the synthesis under the direct supervision of MD. YH and YM conducted the electrophysiological studies under the direct supervision of MKS. MD and RAG prepared the first draft of the manuscript. All co-authors had an opportunity to contribute and make necessary corrections to the manuscript prior to submission.

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Notes

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