2324 *J. Med. Chem.* **2010,** *53,* 2324–2328
DOI: 10.1021/jm901827x

Journal of
Medicinal
Chemistry
Brief Article

Toward Biophysical Probes for the 5-HT$_3$ Receptor: Structure−Activity Relationship Study of Granisetron Derivatives

Sanjeev Kumar V. Vernekar,[†] Hasan Y. Hallaq,[†] Guy Clarkson,[†] Andrew J. Thompson,[‡] Linda Silvestri,[‡] Sarah C. R. Lummis,[‡] and Martin Lochner*[,†]

[†]*Department of Chemistry, University of Warwick, Coventry CV4 7AL, U.K., and* [‡]*Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U.K.*

Received December 10, 2009

This report describes the synthesis and biological characterization of novel granisetron derivatives that are antagonists of the human serotonin (5-HT$_3$A) receptor. Some of these substituted granisetron derivatives showed low nanomolar binding affinity and allowed the identification of positions on the granisetron core that might be used as attachment points for biophysical tags. A BODIPY fluorophore was appended to one such position and specifically bound to 5-HT$_3$A receptors in mammalian cells.

Introduction

The serotonin (5-HT[a]) type 3 receptor (5-HT$_3$R) is a ligand-gated ion channel that is responsible for rapid transmission of nerve impulses at synapses of the central and peripheral nervous system.[1] It is a member of the Cys-loop family, which also includes nicotinic acetylcholine (nACh), γ-aminobutyric acid (GABA$_A$), and glycine receptors. These proteins consist of five pseudosymmetrically arranged subunits. Each subunit comprises a large extracellular N-terminal domain that is responsible for agonist binding, four transmembrane domains (M1−M4) that surround a central ion conducting pore, and a large intracellular loop between M3 and M4 that influences channel conductance and mediates the actions of intracellular messengers.

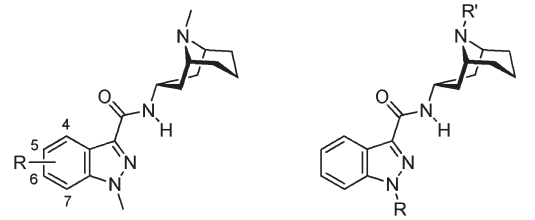
The 5-HT$_3$R is an attractive therapeutic target. Its antagonists are used to control chemotherapy-induced, radiotherapy-induced, and postoperative nausea and vomiting and for treatment of irritable bowel syndrome.[2] There is some evidence that 5-HT$_3$R antagonists might also be useful for treatment of psychiatric and neurological disorders such as anxiety, drug dependence, depression, bulimia nervosa, schizophrenia, and cognitive dysfunction.[3] Another potentially interesting widespread application is their capacity to reduce pain in certain conditions including rheumatoid arthritis, fibromyalgia, and migraine.[4]

Structural insight of Cys-loop receptors has been gained from high resolution structures of homologous acetylcholine binding proteins (AChBPs),[5] engineered nAChR subunits,[6] bacterial receptors,[7] and the cryoelectron microscopy data of the nAChR.[8] This has allowed the construction of 5-HT$_3$R homology models.[9−12] However, such models may be too inaccurate to be useful for rational structure-based drug design and often require labor-intensive validation by experimental methods (i.e., receptor mutagenesis). Furthermore, despite structural information and homology modeling, the exact mechanism that couples agonist binding to channel opening in Cys-loop receptors is still poorly understood and cannot be elucidated by static high resolution structures alone.

Using high-affinity biophysical probes to investigate the structure and function of ligand-gated ion channels is a complementary strategy to traditional biological approaches such as radioligand binding and electrophysiology. In addition, such molecular probes, for example, utilizing fluorescent ligands as an alternative to radioligand binding or immunolabeling, can be used for assays that target specific ligand-gated ion channels.

The aim of the present study was to identify tethering positions for potentially bulky biophysical tags on the high-affinity 5-HT$_3$R antagonist granisetron[13] (**1**, Chart 1). Because of the lack of granisetron structure−activity data in the public domain, we substituted every synthetically possible position with differently sized functional groups on the granisetron core, which yielded 14 different granisetron derivatives (Chart 1).

Chart 1. Reference and Title Compounds



1, R = H, granisetron

2a–c, R = 4-OH, 4-OMe, 4-OBn
3a–c, R = 5-OH, 5-OMe, 5-OBn
4a–c, R = 6-OH, 6-OMe, 6-OBn
5a–c, R = 7-OH, 7-OMe, 7-OBn

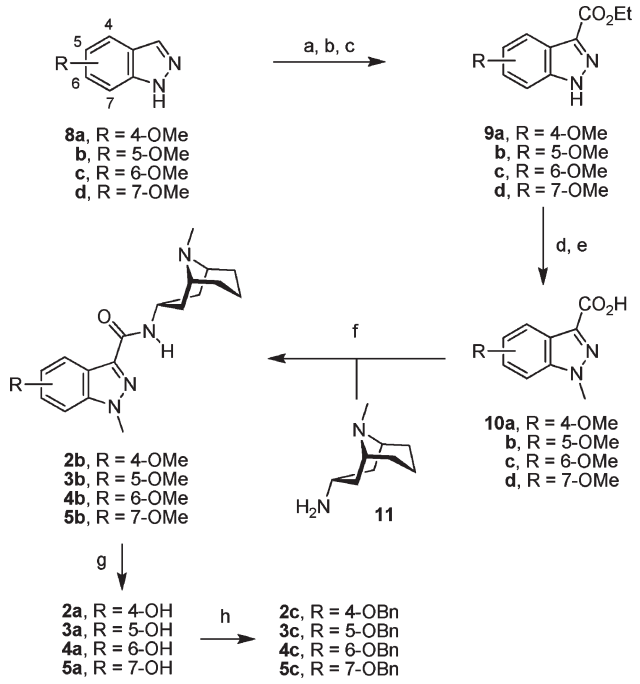
6, R = Me, R' = Bn
7, R = $(CH_2)_3NH_2$, R' = Me

*To whom correspondence should be addressed. Current address: Department of Chemistry and Biochemistry, University of Berne, Switzerland. Phone: +41-31-631-3311. Fax: +41-31-631-8057. E-mail: martin.lochner@dcb.unibe.ch.

[a]Abbreviations: 5-HT, serotonin; 5-HT$_3$R, 5-HT$_3$ receptor; AChBP, acetylcholine binding protein; nAChR, nicotinic acetylcholine receptor; Boc, *tert*-butoxycarbonyl; CD_3OD, tetradeuteriomethanol; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMF, *N*,*N*-dimethylformamide; GABA, γ-aminobutyric acid; HOBt, 1-hydroxybenzotriazole; SEM, 2-(trimethylsilyl)ethoxymethyl; THF, tetrahydrofuran.

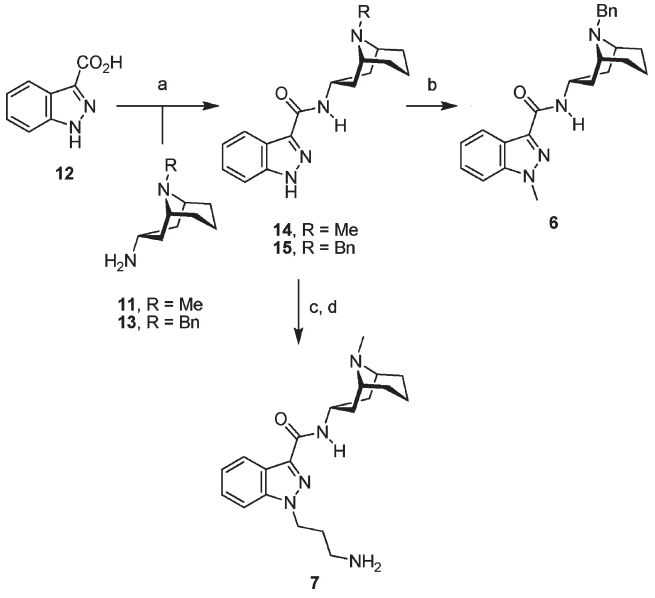
 Published on Web 02/10/2010

Scheme 1. Synthesis of Compounds **2**–**5**[a]



[a] Reagents and conditions: (a) $^{c}Hex_2NMe$, SEM-Cl, THF, room temp, 65–88%; (b) $^{n}BuLi$, THF, −78 °C, $CNCO_2Et$, 66–75%; (c) 2 M HCl, EtOH, room temp, 76–98%; (d) KO^{t}Bu, THF, 0 °C, MeI, room temp, 78–95%; (e) 2 M NaOH, MeOH, room temp, 97–99%; (f) DCC, HOBt, **11**, 4:1 CH_2Cl_2/DMF, room temp, 85–94%; (g) $[Me_3NH][Al_2Cl_7]$, CH_2Cl_2, reflux, or BBr_3, CH_2Cl_2, room temp, or NaSEt, DMF, 110 °C, 84–93%; (h) BnBr, Na_2CO_3, acetone, room temp, 63–94%.

Scheme 2. Synthesis of Compounds **6** and **7**[a]



[a] Reagents and conditions: (a) DCC, HOBt, **11** or **13**, 4:1 CH_2Cl_2/DMF, room temp, 81–97%. (b) For **15**, R = Bn: KO^{t}Bu, THF, 0 °C; MeI, room temp, 90%. (c) For **14**, R = Me: KO^{t}Bu, 5:1 THF/DMF, 0 °C, $Br(CH_2)_3NHBoc$, room temp, 78%. (d) 1.2 M HCl in MeOH, room temp, 98%.

Results and Discussion

Chemistry. The synthesis of **2**–**5** is shown in Scheme 1, and it was accomplished starting from indazoles **8a**–**d** which were prepared according to literature methods.[14,15] A SEM protecting group was selectively attached to N-2 of the indazoles which was followed by a SEM-directed C-3 lithiation and subsequent reaction with an ester group donor.[16] Protecting group cleavage, N-1 methylation, and subsequent ester hydrolysis furnished indazole carboxylic acids **10a**–**d** which were coupled with bicyclic amine **11**[17] to yield amides **2b**–**5b**.

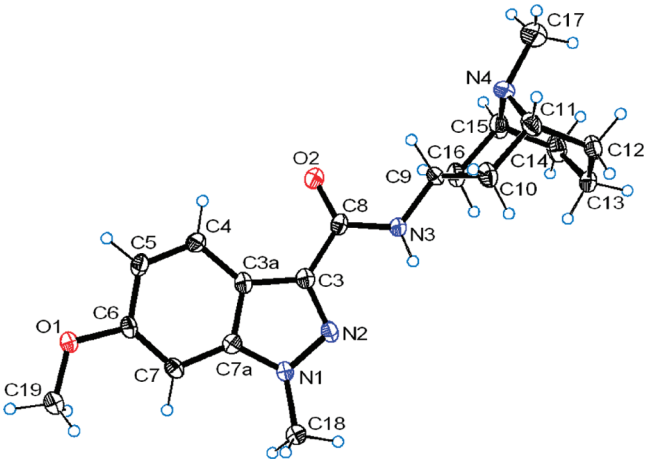


Figure 1. Crystal structure of **4b**. Thermal ellipsoids are shown at 50% probability.

The aromatic methoxy ethers were cleaved using either an excess of BBr_3 in CH_2Cl_2 or NaSEt in hot DMF. However, no product was obtained when these methods were used for the 7-methoxy derivative **5b**. Instead, when the latter compound was treated with a chloroaluminate ionic liquid[18] in refluxing CH_2Cl_2, the desired cleavage product **5a** was successfully produced in good yield (86%). Finally, hydroxyindazoles **2a**–**5a** were alkylated under basic conditions to give benzyl ethers **2c**–**5c**.

The synthesis of **6** and **7** started from commercially available 1*H*-indazole-3-carboxylic acid (**12**, Scheme 2). Coupling with bicyclic amines **11** and **13** afforded amides **14** and **15**. The amine **13** was synthesized from the corresponding bicyclic ketone, 9-benzyl-9-azabicyclo[3.3.1]nonan-3-one,[19] using an oxime formation and reduction sequence as described for **11** in ref 17. Amide **15** was methylated at N-1 to yield granisetron derivative **6**. Alkylation of amide **14** with 3-(Boc-amino)propyl bromide followed by deprotection under acidic conditions furnished **7**.

The granatane (9-methyl-9-azabicyclo[3.3.1]nonane) moiety of the synthesized granisetron derivatives **2**–**7** adopts a boat–chair conformation in solution, as confirmed by ^{1}H NMR. In CD_3OD, the 3-proton of the azabicycle appears as a triplet of triplets and has a coupling constant of 11.5 Hz with the 2- and 4-*endo* protons and a coupling constant of 6.8 Hz with the 2- and 4-*exo* protons (e.g., for **5a**). A coupling constant of 11.0 Hz between the 2-*exo* and the bridgehead 1-proton was observed that is indicative of an eclipsed arrangement. Thus, the obtained ^{1}H NMR data are in agreement with a 3-*exo* proton and a boat–chair conformation of the granatane.[13,20]

This particular structural aspect of granisetron derivatives **2**–**7** was further confirmed by the crystal structures of **4b** and **5a** (Figures 1 and 2). In both structures the indazole ring is coplanar with the amide bond and there appears to be flexibility around the bond between the amide nitrogen and the granatane. Intriguingly, the methyl group of the

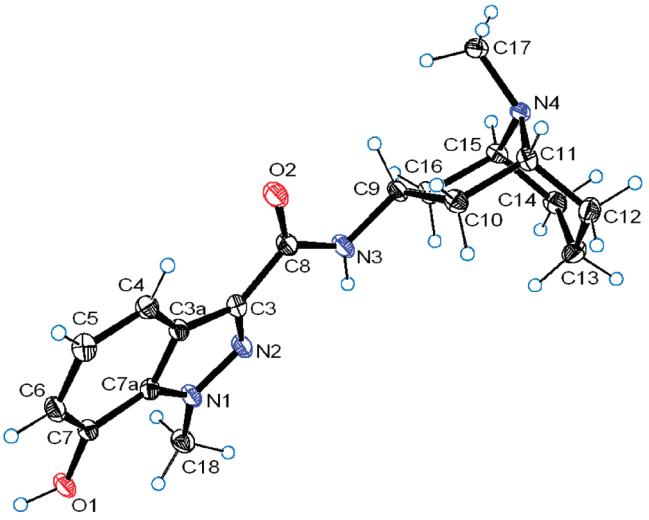
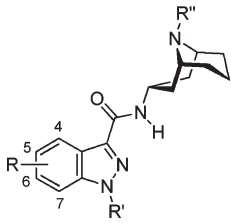


Figure 2. Crystal structure of **5a**. Thermal ellipsoids are shown at 50% probability.

Table 1. Binding Affinities of Granisetron Derivatives **2**−**7** for the Human 5-HT$_3$A Receptor[a]



compd	R	R′	R″	K_i (nM) mean ± SEM	*n*
1	H	Me	Me	1.45 ± 0.13[b]	3
2a	4-OH	Me	Me	0.17 ± 0.09	5
2b	4-OMe	Me	Me	26.3 ± 7.4	4
2c	4-OBn	Me	Me	375 ± 61	4
3a	5-OH	Me	Me	7.3 ± 1.5	3
3b	5-OMe	Me	Me	5306 ± 209	4
3c	5-OBn	Me	Me	3029 ± 956	4
4a	6-OH	Me	Me	279 ± 27	4
4b	6-OMe	Me	Me	237 ± 51	3
4c	6-OBn	Me	Me	749 ± 183	4
5a	7-OH	Me	Me	0.67 ± 0.28	3
5b	7-OMe	Me	Me	71.1 ± 8.0	6
5c	7-OBn	Me	Me	0.23 ± 0.03	5
6	H	Me	Bn	59.3 ± 0.8	3
7	H	$(CH_2)_3NH_2$	Me	1.89 ± 0.33	3

[a] K_i determined from competition binding with [^{3}H]granisetron using HEK293 cell membranes stably expressing h5-HT$_3$AR. [b] Reference 23.

granatane is in an axial position with respect to the chair in **4b** whereas it is equatorial in **5a**. To the best of our knowledge, this is the first time crystal structures of granisetron derivatives have been reported.

Biological Data. The affinities of granisetron derivatives **2**−**7** were assessed by competition with radiolabeled [^{3}H]granisetron, an established 5-HT$_3$AR competitive antagonist. All the compounds, except 5-substituted derivatives **3b** and **3c**, exhibited affinities in the nanomolar range (Table 1).

4-Hydroxy compound **2a** displayed an 8.5-fold increase in affinity for 5-HT$_3$AR relative to **1**, consistent with an H-bond here. Size is important at this location; as bulk was

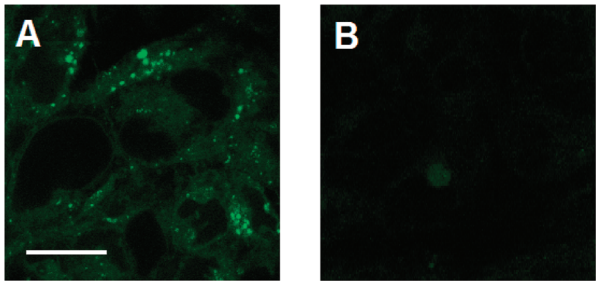
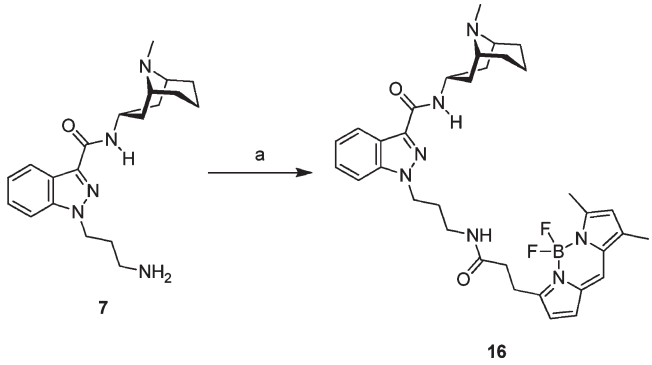


Figure 3. Labeling of HEK293 cells stably transfected with h5-HT$_3$AR with **16**. Cells were incubated in 10 nM **16** for 10 min with (B) or without (A) 10 μM quipazine, washed, mounted, and then observed in a confocal microscope.[31] (A) Fluorescence is observed in all cells, with clusters of receptors clearly present in many cells. Unlabeled areas are nuclei. (B) Quipazine (a competitive 5-HT$_3$R ligand) has displaced **16** from its binding sites, leaving only weak autofluorescence. Scale bar represents 20 μm.

Scheme 3. Synthesis of Fluorescent Probe **16**[a]



[a] Reagents and conditions: (a) ^{i}Pr$_2$EtN, BODIPY FL SE, DMF, room temp, 98%.

increased (**2b** and **2c**), the affinity decreased ~200-fold. However, at the 5-position size is more critical; substitution with large groups here resulted in a >1000-fold decrease in affinity (**3a**−**c**). This is consistent with previous studies that showed that the presence of a 5-chloro substituent resulted in a marked reduction in potency relative to **1**.[13] Substitution of the 6-position of the indazole ring was also poorly tolerated, and all the compounds in this series showed >100-fold decreases in affinities. 7-Hydroxy compound **5a** exhibited a similar affinity for the 5-HT$_3$AR when compared to **1**. The data for substitutions at the 7-position show no clear pattern. Compound **5a**, the major granisetron metabolite in humans,[21] is interestingly as potent as granisetron.[22] Affinity was decreased when the larger methoxy group was present at the 7-position but increased for the larger benzyl group, indicating that the π-system may interact with hydrophobic residues in the binding site. However, a benzyl group was less well tolerated at the bicycle 9-position: **6** exhibited a K_i of 59 nM. The aminopropyl substituent at the indazole N1-position (**7**) did not significantly alter the binding affinity.

Thus, the data show that the N1- and 7-positions of indazole and the 9-position of the granatane of granisetron are the most tolerant regarding substitution. This is in agreement with established pharmacophore models,[24,25] and modifications of the regions we have identified have previously been well tolerated in structurally similar ligands, in particular the position occupied by groups located at a position similar to that of N1 of granisetron.[26−29] Given the

tolerance of the N1-position to addition of the aminopropyl linker, we consider this location the most appropriate to attach large biophysical tags.

To confirm the validity of this hypothesis, we attached a BODIPY FL fluorophore at this position (Scheme 3) to create fluorescent analogue **16** (BFL-GR). K_i data show that **16** binds with high affinity to 5-HT_3AR (K_i = 2.80 ± 0.72 nM, n = 3) and can be visualized in HEK293 cells expressing 5-HT_3AR (Figure 3). Similar images have previously been obtained using fluorescein, rhodamine 6G, and cyanine Cy5 dyes attached via a linker at a similar position of ondansetron, another high-affinity 5-HT_3AR antagonist.[30]

Conclusion

In summary, several novel derivatives of granisetron (**1**) have been discovered. Some derivatives have high affinities for the human 5-HT_3AR, and most notably derivatives **2a**, **5a**, **5c**, and **7** equal or exceed the affinity measured for **1**. The data of our systematic structure−activity study show that substitution at three positions of granisetron are well tolerated and that large functional biophysical tags can be attached at the N1-position.

Experimental Section

General. Chemicals and solvents were either purchased from commercial suppliers or purified by standard techniques. All experiments involving air-sensitive reagents were performed under an inert atmosphere in oven-dried glassware. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F254 plates, and compounds were visualized by irradiating with UV light, by exposing to I_2 vapors, and/or by staining with cerium molybdate stain (Hanessian's stain) followed by heating. Flash chromatography was carried out using Matrex silica gel 60 unless otherwise stated. Infrared spectra were recorded neat on a Nicolet AVATAR 320 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300, DPX-400, and DRX-500. The chemical shifts are reported in δ (ppm), and the residual signal of the solvent was used as the internal standard. High resolution mass spectra were obtained using electrospray ionization mass (MS-ESI) technique on a Bruker MicroTOF instrument. Purity was determined by elemental analysis and/or HPLC; purity of key target compounds was ≥95%.

1-(3-Aminopropyl)-*N*-[(3-*endo*)-9-methyl-9-azabicyclo-[3.3.1]-non-3-yl]-1*H*-indazole-3-carboxamide Dihydrochloride (7·2HCl). Compound **14** (0.3 g, 1.0 mmol) was dissolved in anhydrous DMF/THF (1:5, 10 mL), cooled to 0 °C, and stirred for 5 min. Then a solution of KO^{t}Bu (0.135 g, 1.1 mmol) in anhydrous THF (2 mL) was added dropwise at 0 °C and stirred for 15 min, followed by the addition of a solution of *tert*-butyl *N*-(3-bromopropyl)carbamate (0.27 g, 1.2 mmol) in anhydrous THF (2 mL). The mixture was warmed to room temperature and stirred for 10 h. The progress of the reaction was monitored by TLC. The solvents were removed under vacuo, and the residue was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated to give the crude product. The crude product was further purified by crystallization (CH_2Cl_2/Et_2O) to afford the carbamate (0.36 g, 0.79 mmol, 78%) as a white solid: mp 156−158 °C; R_f = 0.23 (CH_2Cl_2/MeOH, 7:3); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.14−1.17 (m, 3H), 1.45 (s, 9H), 1.54−1.65 (m, 3H), 2.02−2.21 (m, 4H), 2.44−2.52 (m, 2H), 2.55 (s, 3H), 3.11−3.14 (m, 4H), 4.54 (t, J = 7.7 Hz, 2H), 4.60 (tt, J = 6.6 Hz, J = 11.5 Hz, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 8.25 (d, J = 8.2 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 15.1, 25.7, 28.8, 33.3, 34.8, 38.8, 40.7, 41.6, 47.7, 52.8, 110.9, 123.2, 123.6, 124.0, 128.0, 137.5, 142.5, 166.8; IR (neat) 3352, 3349, 2928, 1684, 1641, 1533, 1245, 1172, 1027, 748 cm^{-1}; HRMS-ESI(+) m/z calcd for $C_{25}H_{38}N_5O_3$ 456.2975 $[M + H]^+$, found 456.2990 $[M + H]^+$. Anal. Calcd for $C_{25}H_{37}N_5O_3$: C 65.91%, H 8.19%, N 15.37%. Found C 65.52%, H 8.27%, N 15.04%.

To a solution of carbamate (0.16 g, 0.35 mmol) in MeOH (8 mL) was added dropwise a solution of 1.2 M HCl in MeOH (10 mL) and stirred for 12 h at room temperature. The progress of the reaction was monitored by TLC, and the solvents were removed in vacuo and 3 times coevaporated with toluene to get the crude product. The crude product was further purified by crystallization (CH_2Cl_2/Et_2O) to afford **7**·2HCl (0.15 g, 0.34 mmol, 98%) as a white solid: mp 296−298 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.58−1.71 (m, 3H), 1.90−2.10 (m, 3H), 2.21−2.39 (m, 4H), 2.55−2.71 (m, 2H), 2.96−3.01 (m, 5H), 3.74 (d, J = 10.2 Hz, 2H), 4.61−4.70 (m, 3H), 7.30 (t, J = 7.5 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 8.22 (d, J = 8.2 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 15.2, 26.7, 31.1, 34.6, 40.9, 41.0, 41.9, 49.7, 57.8, 113.4, 125.6, 126.6, 127.1, 131.0, 137.5, 144.9, 166.8; IR (neat) 2884, 2749, 2676, 1630, 1553, 1208, 745 cm^{-1}; HRMS-ESI(+) m/z calcd for $C_{20}H_{30}N_5O$ 356.2450 $[M - 2HCl + H]^+$, found 356.2445 $[M - 2HCl + H]^+$. Anal. Calcd for $C_{20}H_{31}Cl_2N_5O$: C 56.07%, H 7.29%, N 16.35%, Cl 16.55%. Found C 55.21%, H 7.35%, N 16.00%, Cl 16.31%.

BFL-GR (16). To a solution of amine hydrochloride **7**·2HCl (9 mg, 0.021 mmol) in anhydrous DMF (0.5 mL) was added ^{i}Pr$_2$EtN (5.4 mg, 0.042 mmol) and stirred for 10 min. Then a solution of BODIPY FL SE (5 mg, 0.012 mmol) in DMF (1 mL) was added to the mixture and stirred at room temperature for 3.5 h. The progress of the reaction was monitored by TLC. The DMF was removed in vacuo and the crude product was purified by flash column chromatography (CH_2Cl_2 and then CH_2Cl_2/MeOH/Et_3N, 96:3:1) to afford **16** (7.9 mg, 0.012 mmol, 98%) as an orange solid: mp 190−192 °C (dec); R_f = 0.42 (CH_2Cl_2/MeOH, 7:3); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.07−1.18 (m, 3H), 1.40−1.59 (m, 4H), 1.88−2.05 (m, 6H), 2.15 (s, 3H), 2.32−2.40 (m, 4H), 2.48−2.52 (m, 5H), 3.08−3.16 (m, 4H), 4.37 (t, J = 6.8 Hz, 2H), 4.47 (tt, J = 6.7 Hz, J = 11.8 Hz, 1H), 6.10 (s, 1H), 6.23 (d, J = 4.0 Hz, 1H), 6.86 (d, J = 4.0 Hz, 1H), 7.17 (t, J = 7.7 Hz, 1H), 7.26 (s, 1H), 7.33 (t, J = 7.4 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 8.10 (d, J = 8.2 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.2, 14.6, 15.3, 25.6, 33.0, 36.2, 37.8, 40.4, 41.1, 47.6, 53.2, 110.9, 117.7, 123.0, 123.8, 125.8, 128.1, 129.6, 138.3, 139.6, 140.5, 145.2, 149.3, 164.5, 174.8; IR (neat) 2921, 1605, 1488, 1245, 1132, 1056, 974, 734 cm^{-1}; HRMS-ESI(+) m/z calcd for $C_{34}H_{43}BF_2N_7O_2$ 630.3539 $[M + H]^+$, found 630.3526 $[M + H]^+$; UV/vis/Fluo (MeOH) $\lambda_{max\,abs}$ = 506 nm, $\lambda_{max\,emiss}$ = 514 nm.

Acknowledgment. The authors thank the EPSRC (EP/E042139/1) and the Wellcome Trust (081925/Z/07/Z, A.J.T. and S.C.R.L.) for financial support. S.C.R.L. is a Wellcome Trust Senior Research Fellow in Basic Biomedical Science.

Supporting Information Available: Synthesis details and spectral data for compounds other than **7** and **16**, HPLC purity assessment for all target compounds, CIF files of **4b** and **5a**, experimental details for competition binding, and K_i determination. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reeves, D. C.; Lummis, S. C. R. The molecular basis of the structure and function of the 5-HT_3 receptor: a model ligand-gated ion channel. *Mol. Membr. Biol.* **2002**, *19*, 11–26.
(2) Thompson, A. J.; Lummis, S. C. R. The 5-HT_3 receptor as a therapeutic target. *Expert Opin. Ther. Targets* **2007**, *11*, 527–540.
(3) Greenshaw, A. J.; Silverstone, P. H. The non-antiemetic uses of serotonin 5-HT_3 receptor antagonists. Clinical pharmacology and therapeutic applications. *Drugs* **1997**, *53*, 20–39.

(4) Färber, L.; Drechsler, S.; Ladenburger, S.; Gschaidmeier, H.; Fischer, W. The neuronal 5-HT_3 receptor network after 20 years of research. Evolving concepts in management of pain and inflammation. *Eur. J. Pharmacol.* **2007**, *560*, 1–8.
(5) Brejc, K.; van Dijk, W. J.; Klaassen, R. V.; Schuurmans, M.; van der Oost, J.; Smit, A. B.; Sixma, T. K. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* **2001**, *411*, 269–276.
(6) Dellisanti, C. D.; Yao, Y.; Stroud, J. C.; Wang, Z.-Z.; Chen, L. Crystal structure of the extracellular domain of nAChR α1 bound to α-bungarotoxin at 1.94 Å resolution. *Nat. Neurosci.* **2007**, *10*, 953–962.
(7) Hilf, R. J. C.; Dutzler, R. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature* **2008**, *452*, 375–379.
(8) Unwin, N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J. Mol. Biol.* **2005**, *346*, 967–989.
(9) Reeves, D. C.; Sayed, M. F. R.; Chau, P.-L.; Price, K. L.; Lummis, S. C. R. Prediction of 5-HT_3 receptor agonist-binding residues using homology modeling. *Biophys. J.* **2003**, *84*, 2338–2344.
(10) Thompson, A. J.; Price, K. L.; Reeves, D. C.; Ling Chan, S.; Chau, P.-L.; Lummis, S. C. R. Locating an antagonist in the 5-HT_3 receptor binding site using modeling and radioligand binding. *J. Biol. Chem.* **2005**, *280*, 20476–20482.
(11) Joshi, P. R.; Suryanarayanan, A.; Hazai, E.; Schulte, M. K.; Maksay, G.; Bikadi, Z. Interactions of granisetron with an agonist-free 5-HT_{3A} receptor model. *Biochemistry* **2006**, *45*, 1099–1105.
(12) Yan, D.; White, M. M. Spatial orientation of the antagonist granisetron in the ligand-binding site of the 5-HT_3 receptor. *Mol. Pharmacol.* **2005**, *68*, 365–371.
(13) Bermudez, J.; Fake, C. S.; Joiner, G. F.; Joiner, K. A.; King, F. D.; Miner, W. D.; Sanger, G. J. 5-Hydroxytryptamine (5-HT_3) receptor antagonists. 1. Indazole and indolizine-3-carboxylic acid derivatives. *J. Med. Chem.* **1990**, *33*, 1924–1929.
(14) Schumann, P.; Collot, V.; Hommet, Y.; Gsell, W.; Dauphin, F.; Sopkova, J.; MacKenzi, E. T.; Duval, D.; Boulouard, M.; Rault, S. Inhibition of neuronal nitric oxide synthase by 7-methoxyindazole and related substituted indazoles. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1153–1156.
(15) Shimada, I.; Maeno, K.; Kazuta, K.-i.; Kubota, H.; Kimizuka, T.; Kimura, Y.; Hatanaka, K.-i.; Naitou, Y.; Wanibuchi, F.; Sakamoto, S.; Tsukamoto, S.-i. Synthesis and structure−activity relationships of a series of substituted 2-(1*H*-furo[2,3-*g*]indazol-1-yl)ethylamine derivatives as 5-HT_{2C} receptor agonists. *Bioorg. Med. Chem.* **2008**, *16*, 1966–1982.
(16) Luo, G.; Chen, L.; Dubowchik, G. Regioselective protection at N-2 and derivatisation at C-3 of indazoles. *J. Org. Chem.* **2006**, *71*, 5392–5395.
(17) Donatsch, P.; Engel, G.; Hügi, B.; Richardson, B. P.; Stadler, P. A.; Breuleux, G. Method of Inducing a Serotonin M Receptor Antagonist Effect with *N*-Quinuclidinyl-benzamides. US 5,017,582, **1991**.
(18) Kemperman, G. J.; Roeters, T. A.; Hilberink, P. W. Cleavage of aromatic methyl ethers by chloroaluminate ionic liquid reagents. *Eur. J. Org. Chem.* **2003**, 1681–1686.
(19) Dold, O.; Stach, K.; Schaumann, W. 3-Phenylnorgranat-2-enes. US 3,509,161, **1970**.
(20) Fernandez, M. J.; Huertas, R.; Galvez, E. Synthesis and structural and conformational study of some amines derived from the norgranatane system. *J. Mol. Struct.* **1991**, *246*, 359–366.
(21) Clarke, S. E.; Austin, N. E.; Bloomer, J. C.; Haddock, R. E.; Higham, F. C.; Hollis, F. J.; Nash, M.; Shardlow, P. C.; Tasker, T. C. Metabolism and disposition of ^{14}C-granisetron in rat, dog and man after intravenous and oral dosing. *Xenobiotica* **1994**, *24*, 1119–1131.
(22) Marr, H. E.; Haddock, R. E.; Ramsay, T. W. Preparation of 9-Azabicyclo[3.3.1]nonane Derivatives as 5-HT_3 Receptor Antagonists. WO 9,101,316, **1991**.
(23) Hope, A. G.; Peters, J. A.; Brown, A. M.; Lambert, J. J.; Blackburn, T. P. Characterization of a human 5-hydroxytryptamine3 receptor type A (h5-HT3R-AS) subunit stably expressed in HEK 293 cells. *Br. J. Pharmacol.* **1996**, *118*, 1237–1245.
(24) Parihar, H. S.; Suryanarayanan, A.; Ma, C.; Joshi, P.; Venkataraman, P.; Schulte, M. K.; Kirschbaum, K. S. 5-HT_3R binding of lerisetron: an interdisciplinary approach to drug−receptor interactions. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2133–2136.
(25) Schmidt, A. W.; Peroutka, S. J. Three-dimensional steric molecular modeling of the 5-hydroxytryptamine$_3$ receptor pharmacophore. *Mol. Pharmacol.* **1989**, *36*, 505–511.
(26) Cappelli, A.; Gallelli, A.; Manini, M.; Anzini, M.; Mennuni, L.; Makovec, F.; Menziani, M. C.; Alcaro, S.; Ortuso, F.; Vomero, S. Further studies on the interaction of the 5-hydroxytryptamine$_3$ (5-HT_3) receptor with arylpiperazine ligands. Development of a new 5-HT_3 receptor ligand showing potent acetylcholinesterase inhibitory properties. *J. Med. Chem.* **2005**, *48*, 3564–3575.
(27) Cappelli, A.; Anzini, M.; Vomero, S.; Mennuni, L.; Makovec, F.; Doucet, E.; Hamon, M.; Menziani, M. C.; De Benedetti, P. G.; Giorgi, G.; Ghelardini, C.; Collina, S. Novel potent 5-HT_3 receptor ligands based on the pyrrolidone structure: synthesis, biological evaluation, and computational rationalization of the ligand−receptor interaction modalities. *Bioorg. Med. Chem.* **2002**, *10*, 779–801.
(28) Clark, R. D.; Miller, A. B.; Berger, J.; Repke, D. B.; Weinhardt, K. K.; Kowalczyk, B. A.; Eglen, R. M.; Bonhaus, D. W.; Lee, C.-H.; Michel, A. D.; Smith, W. L.; Wong, E. H. F. 2-(Quinuclidin-3-yl)pyrido[4,3-*b*]indol-1-ones and isoquinolin-1-ones. Potent conformationally restricted 5-HT_3 receptor antagonists. *J. Med. Chem.* **1993**, *36*, 2645–2657.
(29) Tairi, A.-P.; Hovius, R.; Pick, H.; Blasey, H.; Bernard, A.; Surprenant, A.; Lundström, K.; Vogel, H. Ligand binding to the serotonin 5HT_3 receptor studied with a novel fluorescent ligand. *Biochemistry* **1998**, *37*, 15850–15864.
(30) Wohland, T.; Friedrich, K.; Hovius, R.; Vogel, H. Study of ligand−receptor interactions by fluorescence correlation spectroscopy with different fluorophores: evidence that the homopentameric 5-hydroxytryptamine type 3$_{As}$ receptor binds only one ligand. *Biochemistry* **1999**, *38*, 8671–8681.
(31) Spier, A. D.; Wotherspoon, G.; Nayak, S. V.; Nichols, R. A.; Priestley, J. V.; Lummis, S. C. R. Antibodies against the extracellular domain of the 5-HT_3 receptor label both native and recombinant receptors. *Mol. Brain Res.* **1999**, *71*, 369 (Erratum).