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Synthesis of *N*-substituted 9-azabicyclo[3.3.1]nonan-3α-yl carbamate analogs as σ_2 receptor ligands

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Abstract—A series of *N*-substituted 9-azabicyclo[3.3.1]nonan-3α-yl phenylcarbamate analogs was prepared and their affinities for sigma (σ_1 and σ_2) receptors were measured in vitro. The results of their structure–activity relationship study identified two new compounds, *N*-(9-(4-aminobutyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*′-(2-methoxy-5-methylphenyl)carbamate and *N*-(9-(6-aminohexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*′-(2-methoxy-5-methylphenyl)carbamate, having a high affinity and selectivity for σ_2 versus σ_1 receptors. These compounds were also used in the preparation of biotinylated and fluorescent probes of the σ_2 receptor.

1. Introduction

Sigma receptors represent a class of proteins that were initially thought to be a subtype of the opiate receptors.[1] The development of sigma selective ligands, such as (+)-pentazocine, DTG (1,3-di-*o*-tolylguanidine), and (+)-3-PPP (3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine), allowed sigma binding sites to be distinguished as a separate receptor system.[2] It is now widely accepted that there are at least two classes of sigma binding sites, denoted sigma-1 (σ_1) and sigma-2 (σ_2). These receptors are distinguishable functionally, pharmacologically, and by molecular size. The σ_1 receptor has a molecular weight of ~25 kDa, whereas the σ_2 receptor has a molecular weight of ~21.5 kDa. The σ_1 receptor gene has been cloned from guinea pig liver, human placental choriocarcinoma, rat brain, and mouse kidney, and displays a 30% sequence homology with the enzyme, yeast sterol isomerase.[3–5] The σ_2 receptor gene has not been cloned, although a number of studies have presented evidence linking the σ_2 receptor to potassium channels and intracellular calcium release in NCB-20 cells.[2,6] Radioligand binding studies using [^{3}H](+)-pentazocine and [^{3}H]DTG have revealed that both σ_1 and σ_2 receptors have a widespread distribution in the central nervous system and in a variety of tissues and organs[2,6] However, since [^{3}H]DTG possesses a similar affinity for σ_1 and σ_2 receptors, in vitro binding studies using this radiotracer to measure σ_2 receptor density require the use of the 100 nM (+)-pentazocine in the binding assay in order to mask σ_1 receptors.

Previous studies have reported that σ_2 receptors overexpressed in a wide variety of human and murine tumor cells grown in cell culture.[7–9] Furthermore, it has shown that the density of σ_2 receptors is 10-fold higher in proliferative versus quiescent mouse mammary adenocarcinoma cells in vitro[9,10] and in vivo.[11] Based on these data, we have proposed that the σ_2 receptor may serve as a receptor-based biomarker of the proliferative status of solid tumors. Additional studies have shown that σ_2 receptor ligands can induce apoptosis in tumor cells, and raise the possibility that σ_2 selective ligands may be useful as anticancer or chemosensitizing agent.[12,13] A potential role of the σ_2 receptor in regulating cellular proliferation and apoptosis has led to a renewed interest in identifying the biological function of this receptor.

A number of structurally diverse compounds have been shown to possess a high affinity to sigma receptors. Most of these compounds display either a high selectivity for the σ_1 receptor or bind with equal affinity to both σ_1 and σ_2 receptors. Until recently, only a few σ_2 selective ligands have been identified. For example, the phenyl morphan CD-184,[14] the trishomocubane analog ANSTO-20,[15] the potent 5-HT$_3$ and 5-HT$_4$ ligand, BIMU-1,[16] have been shown to possess a moderate affinity and selectivity for σ_2 versus σ_1 receptors (Fig. 1).

Keywords: σ_2 Receptors; Fluorescent probe; Two-photon microscopy.
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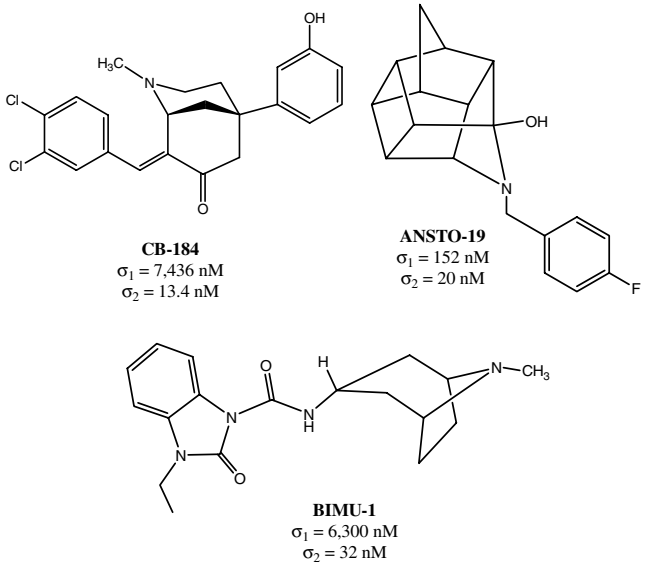
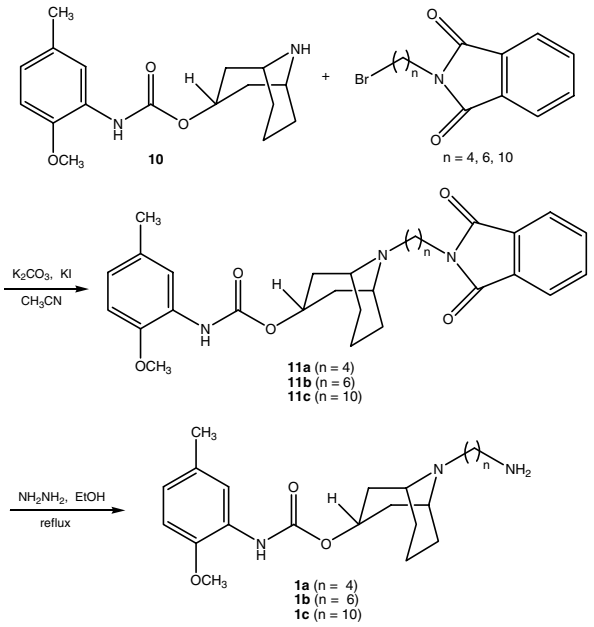
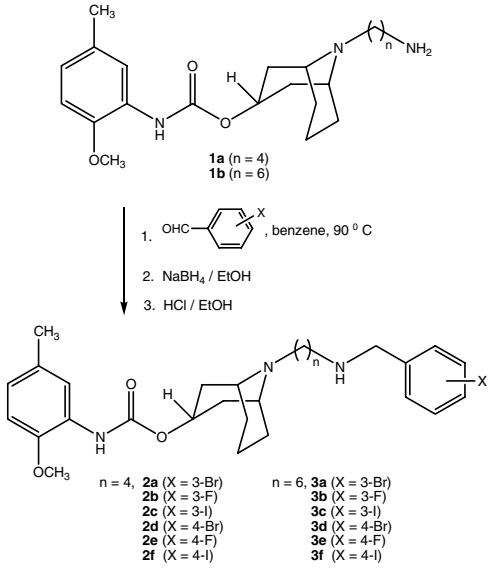


Figure 1. Structures of the σ_2 selective compounds.



Scheme 1.



Scheme 2.

We previously reported the synthesis and in vitro binding of a series of *N*-substituted-9-azabicyclo[3.3.1]nonan-3α-yl carbamate analogs having a modest affinity and selectivity for σ_2 versus σ_1 receptors.[17,18] The goal of the current study was to prepare radiolabeled, fluorescent, and biotinylated probes of the σ_2 receptor using the *N*-substituted-9-azabicyclo[3.3.1]nonan-3α-yl carbamate analogs as the lead structures. This strategy involved the incorporation of an aminoalkyl substituent in which the length of the spacer group separating the primary amino group and the bridgehead nitrogen atom varied by four, six, and ten methylene units. The primary amino group served as the point of attachment of the corresponding radiolabeled, fluorescent, and biotinylated probe, whereas the methylene spacer group separated the probe from the σ_2 receptor recognition fragment, the 9-azabicyclo[3.3.1]nonan-3α-yl carbamate moiety. This study developed two biotinylated probes that will be useful in the identification and purification of σ_2 receptors utilizing Biotin-Avidin coupling techniques, and one fluorescent probe that has been useful in sub-cellular visualization of σ_2 receptors using two-photon microscopy.

2. Chemistry

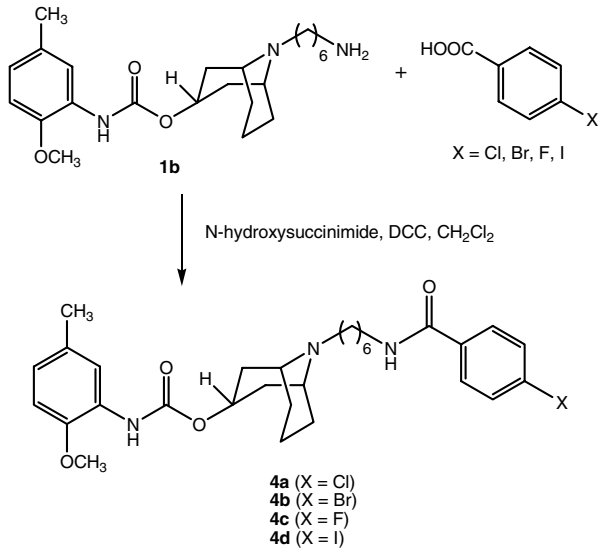
The synthesis of the target compounds is outlined in Schemes 1–6. The reaction between the secondary amine **10**[17,18] and *N*-(ω-bromoalkyl)phthalimides gave the intermediates **11a–c**. Treatment with anhydrous hydrazine gave the primary amines **1a–c** (Scheme 1). Reductive amination of compounds **1a**,**b** with halobenzaldehydes and sodium borohydride afforded the *N*-halobenzyl derivatives (**2a–f** and **3a–f**) (Scheme 2). Only amine **1b** was reacted with 4-halobenzoic acids to give the 4-halobenzoyl derivatives (**4a–d**) (Scheme 3).

Compounds **1b** and **1c** were condensed with (+)-biotin *N*-hydroxysuccinimide ester to give **5** and **6** (Scheme 4) in moderate yield (75% and 78%). Similarly, **1b** and **1c** were also condensed with (+)-biotinamidocaproate *N*-hydroxysuccinimidyl ester to give **7** and **8** (Scheme 5) in high yield (88% and 95%).

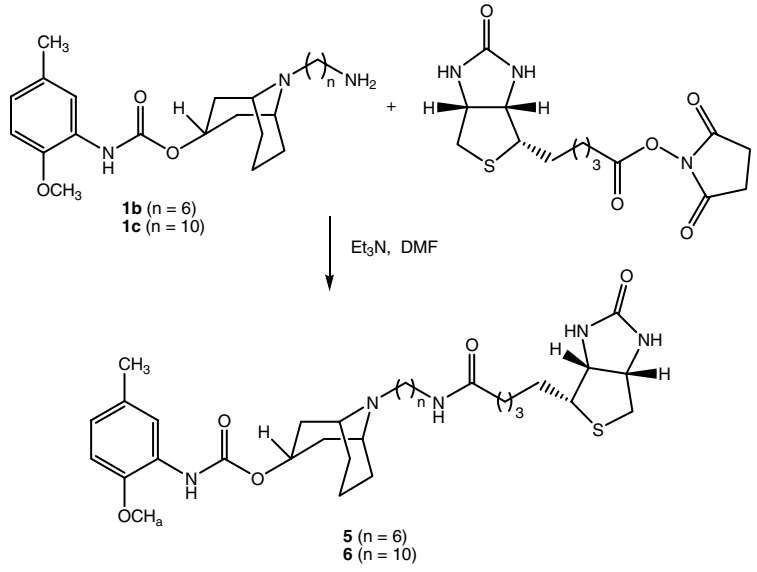
In an effort to gain some insight into the sub-cellular localization of σ_2 receptors in tumor cells, the fluorescent analog, **9**, was prepared by reacting **1b** with dansyl chloride as outlined in Scheme 6.[19]

3. Radioligand binding studies at sigma receptors

In vitro binding studies were conducted in order to determine the affinity of the target compounds at σ_1 and σ_2



Scheme 3.



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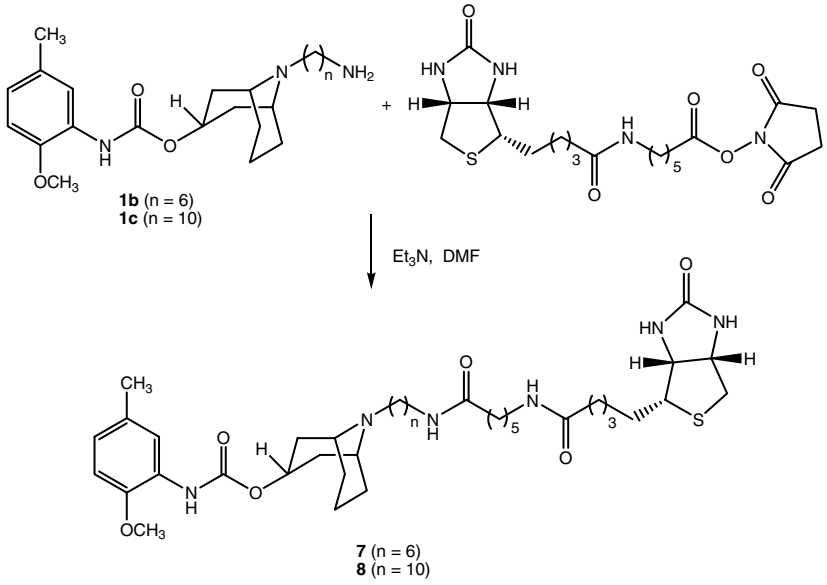
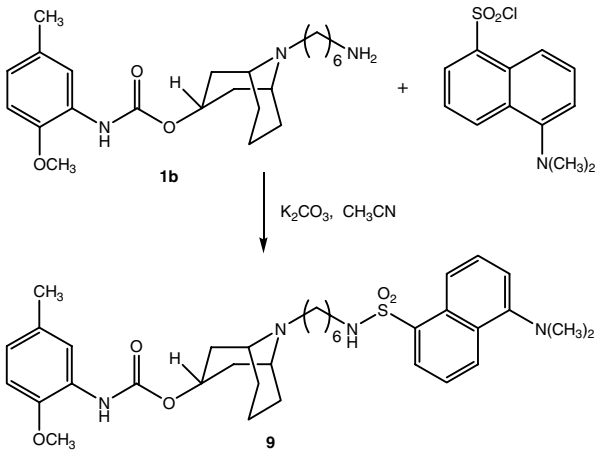


Scheme 5.

receptors. The σ_1 binding studies were conducted using the σ_1-selective radioligand, [^{3}H](+)-pentazocine in guinea pig brain membranes; σ_2 sites were assayed in rat liver membranes with [^{3}H]DTG in the presence of (+)-pentazocine (1 μM) to mask σ_1 sites or the σ_2 selective ligand [^{3}H]RHM-1 alone.[6,21]

The results of the in vitro binding studies are shown in Table 1. For compounds **1a**–**c**, the extension of the methylene linker separating the amino group and the bridgehead nitrogen atom (n = 4, 6, and 10) had some affect on the binding affinity for sigma receptors. Compounds **1a** and **1b** which have a linker group of 4 and 6 methylene units, respectively, between the amino group and the bridgehead nitrogen atom, both had moderate affinity and selectivity for σ_2 versus σ_1 receptors. Compound **1c** with the 10 methylene spacer group had high affinity at σ_2 receptor but low selectivity for σ_2 versus σ_1 receptor relative to **1a** and **1b**.



Scheme 6.

Table 1. In vitro binding data

Compound	K_i[a] (nM)		
	σ_1[b]	σ_2[c]	σ_1/σ_2 Ratio[d]
1a	2490 ± 271	12.94 ± 0.46	193
1b	1418 ± 439	5.19 ± 0.80	273
1c	134.3 ± 11.9	7.07 ± 1.27	19
2a	66.29 ± 5.30	3.06 ± 0.41	21.66
2b	0.30 ± 0.05	4.13 ± 0.33	0.07
2c	63.87 ± 5.61	1.21 ± 0.14	52.77
2d	1.70 ± 0.28	4.07 ± 0.61	0.42
2e	22.15 ± 1.58	29.07 ± 3.48	0.76
2f	0.34 ± 0.03	4.00 ± 0.54	0.08
3a	112.40 ± 4.80	8.94 ± 0.61	12.57
3b	0.46 ± 0.05	4.85 ± 0.49	0.09
3c	0.56 ± 0.06	32.68 ± 5.93	0.02
3d	43.16 ± 2.88	9.93 ± 1.61	4.35
3e	1.00 ± 0.26	70.88 ± 4.38	0.01
3f	0.65 ± 0.09	35.93 ± 1.50	0.02
4a	1397 ± 64	3.56 ± 0.08	392
4b	2041 ± 98	719 ± 84	2.84
4c	1243 ± 212	497 ± 103	2.50
4d	1412 ± 318	1,009 ± 110	1.40
5	3402 ± 538	114 ± 28	29.87
6	10,234 ± 2895	69.70 ± 7.67	147
7	10,365 ± 2313	1351 ± 78	7.67
8	3819 ± 518	733 ± 45	5.21
9	12,644 ± 3754	148 ± 8.85	85.43
Haloperidol	1.45 ± 0.33	24.20 ± 3.00	0.06

[a] Mean ± SEM, K_i values were determined by at least three experiments.
[b] K_i for inhibiting the binding of [^{3}H](+)-pentazocine to guinea pig brain homogenates.
[c] K_i for inhibiting the binding of [^{3}H]DTG or [^{3}H]RHM-1 to rat liver homogenates.
[d] K_i for σ_1/K_i for σ_2.

Attachment of the substituted benzyl groups to the amino side chain of compounds **1a** and **1b** resulted in compounds (**2a**–**f** and **3a**–**f**) having a dramatic increase in affinity at σ_1 receptors. The affinity at σ_2 receptors either slightly increased (**2a**–**d** and **2f** vs **1a**) or decreased relative to the primary amine (**3a**–**f** vs **1b**). On the contrary, attachment of the substituted benzoyl groups to the amino side chain of compound **1b** resulted in a dramatic reduction in affinity at σ_2 receptors (compounds **4a**–**d**). An exception to this was compound **4a**, which had a high affinity and selectivity for σ_2 receptors versus σ_1 receptors.

Coupling of compounds **1b** and **1c** to two different biotin activated esters showed that the biotinylated derivatives having a short chain (compounds **5** and **6**) displayed a higher affinity and selectivity for σ_2 versus σ_1 receptor than the biotin analogs having the additional amidocaproate spacer group between the biotin and bridgehead nitrogen atom (compounds **7** and **8**).

4. Fluorescent σ_2 ligands

Fluorescent σ_2 ligands can be used to study the subcellular localization of σ_2 receptors in cells growing under cell culture conditions, or in brain tissue sections, using two-photon microscopy. In vitro binding studies demonstrated that the fluorescent analog, **9**, prepared as outlined in Scheme 6, had a moderate affinity and selectivity for σ_2 versus σ_1 receptors.

Excitation and emission spectra demonstrated that **9** exhibited the maximum excitation wavelength at 333 nm, and the maximum emission wavelength with a range of 480–520 nm (Fig. 2). In order to use two-photon microscopy to study the sub-cellular localization of

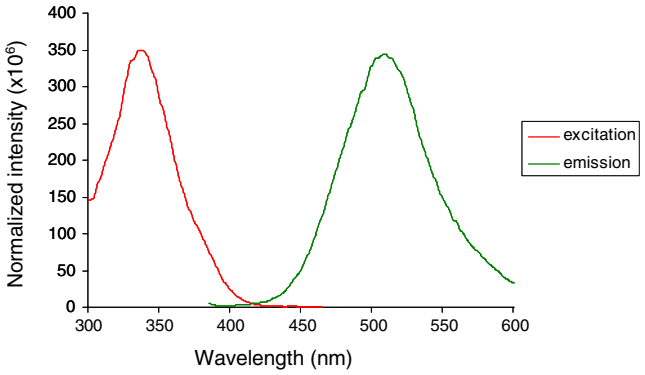


Figure 2. Excitation and emission spectra of **9**.

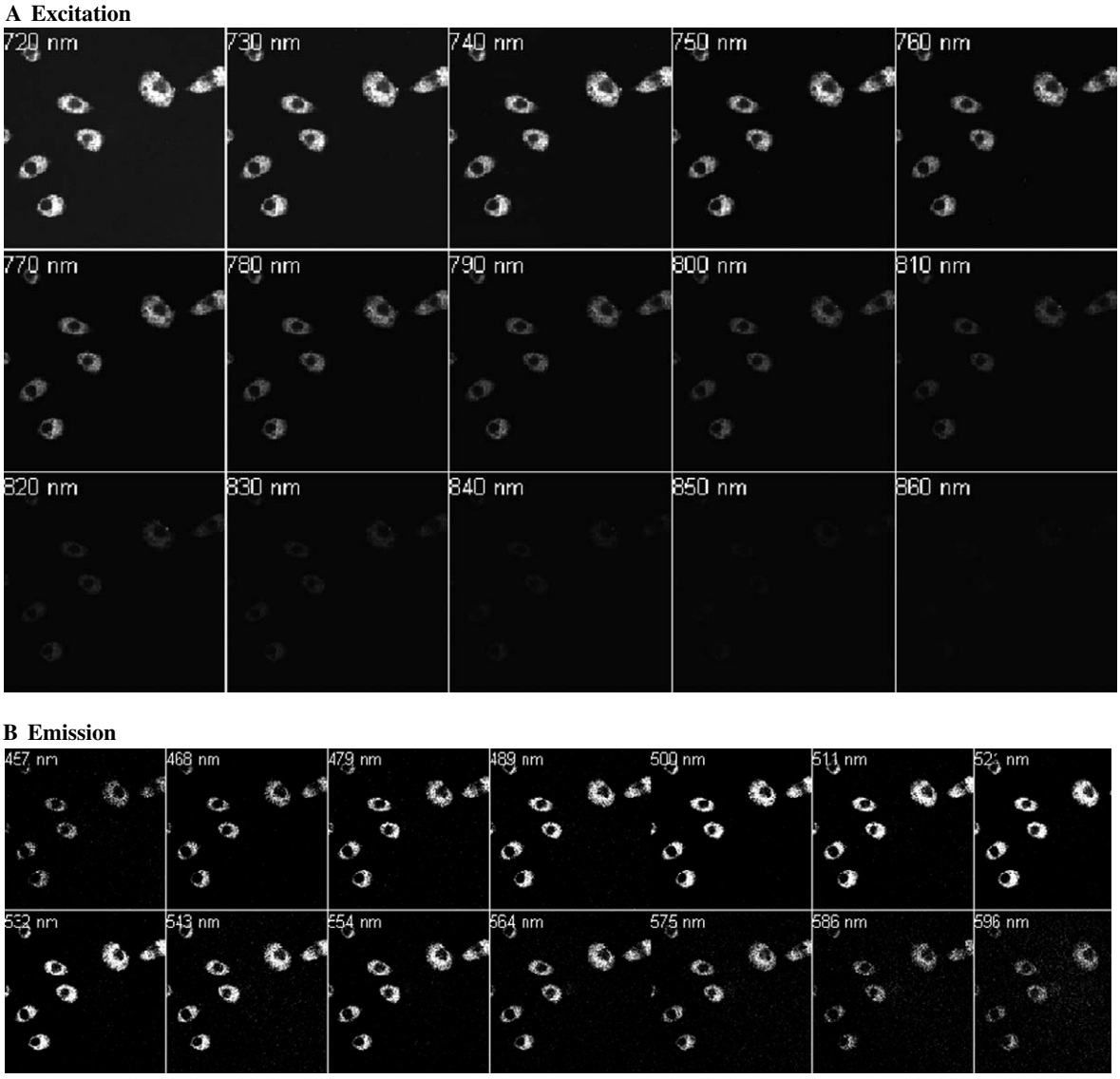


Figure 3. Excitation spectra (A) and emission spectra (B) of **9** in EMT6 cells by two-photon microscopy.

σ_2 receptors, EMT6 cells were incubated with **9** at a concentration of 200 nM. The maximum excitation wavelength and maximum emission wavelength were examined by two-photon microscopy. Compound **9** was found to localize in the cytoplasm of EMT6 cells. The maximum excitation wavelength for two-photon microscopy was found to be 720 nm, whereas the maximum emission wavelength ranged from 480 to 540 nm. The results of the two-photon sub-cellular localization study are shown in Figure 3.

5. Discussion

The current study is a continuation of our effort to develop ligands having a high affinity and selectivity for σ_2 versus σ_1 receptors. In earlier studies, we prepared a number of structural analogs of the mixed serotonin 5-HT_3/5-HT_4 ligand, BIMU-1, having a high affinity for σ_2 versus σ_1 receptors and a low (or negligible) affinity at serotonin 5-HT_3 and 5-HT_4 receptors.[17,18] The results of these initial studies revealed that extension of the length of the methylene spacer group between the bridgehead nitrogen atom and the benzene ring had little effect on binding to σ receptors. The goal of the present study was to further explore the structure–activity relationships of the groups attached to the bridgehead nitrogen atom of the granatane ring system in order to prepare probes of the σ_2 receptor that could be used in imaging studies and in the purification of the σ_2 receptor protein from tissue.

The first strategy involved extending the length of the methylene spacer group between the bridgehead nitrogen atom and the free amino group. The results of this study (Table 1) revealed that compounds **1a** and **1b**, having either a 4 or 6 methylene spacer group between the bridgehead nitrogen and the free amino group, had a high affinity for σ_2 versus σ_1 receptors. Extension of the spacer group to 10 methylene units resulted in an increase in affinity for σ_1 receptors and a reduction in the σ_1:σ_2 selectivity ratio (Table 1). As a next step, we prepared a series

of analogs having substituted benzyl groups attached to the primary amino group of **1a** and **1b**. In this study, the aromatic ring of the benzyl group was substituted with the halogen atoms F, Br, and I in the 3- or 4-position since the corresponding radiolabeled versions (i.e., ^{18}F-, ^{76}Br-, and ^{125}I-labeled analogs) could be used in imaging studies to assess the σ_2 receptor status of solid tumors. An unexpected finding was the dramatic increase in σ_1 receptor affinity and reduction in σ_1:σ_2 selectivity ratio when making this substitution on the amino group of **1a** and **1b**. This result was in stark contrast to that obtained when the amino group of **1b** was substituted with a benzoyl group. This substitution led to a large reduction in σ_2 receptor affinity and no change in σ_1 receptor affinity (Table 1). An exception to this observation was the 5-chlorobenzoyl analog, **4a**, which maintained a high affinity and selectivity for σ_2 versus σ_1 receptors.

A second goal of the current study was to prepare molecular probes that could be used in the purification of σ_2 receptors from tissue sources and in two-photon microscopy studies aimed at visualizing the sub-cellular localization of σ_2 receptor. In this regard, two potentially useful compounds were identified, the biotinylated analog, **6**, and the dansyl analog, **9**. Both compounds have a moderate affinity for σ_2 receptors and moderate σ_1:σ_2 selectivity ratio. We are currently using compounds **6** and **9** in studies aimed at identifying the biological function of σ_2 receptors.

6. Conclusion

The results of the current study provided additional information on the structure–activity relationships of 9-azabicyclo[3.3.1]nonan-3α-yl carbamate analogs with respect to binding at σ_1 and σ_2 receptors. The length of the methylene spacer group separating the primary amino group and the bridgehead nitrogen atom had effect on the binding affinity for σ_2 receptors and σ_1:σ_2 selectivity ratio. From this study, the six methylene linker group gave a compound (**1b**) having the highest affinity and selectivity for σ_2 versus σ_1 receptors. The substituted benzyl or benzoyl groups attached to the amino side chain (**2a–f**, **3a–f**, and **4a–d**) did not enhance the selectivity for σ_2 versus σ_1 receptors. The attachment of a shorter chain biotin moiety to either **1b** or **1c** gave compounds (**5** and **6**) with moderate affinity and selectivity for σ_2 receptors. Finally, the dansyl derivative, **9**, had a moderate affinity and selectivity for σ_2 receptors and may be a useful probe for two-photon microscopy studies of this receptor in cells growing under cell culture conditions and in tissue slices.

7. Experimental

7.1. Chemical analysis

^{1}H NMR spectra were recorded on a Varian 300 MHz NMR spectrometer. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane (TMS). The following abbreviations are used for multiplicity of NMR signals: br s = broad singlet, d = doublet, m = multiplet, q = quintet, s = singlet, t = triplet. Melting points were determined with an electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, and were within ±0.4% of the calculated values. Mass spectrometry was provided by the Washington University Mass Spectrometry Resource, an NIH Research Resource (Grant No. P41RR0954). All reactions were carried out under an inert atmosphere of nitrogen.

The general procedure for conversion to an HCl salt was the addition of excess ethereal HCl solution to a solution of the compound in dry ethanol. The solvent was evaporated and the resulting salt was triturated with anhydrous ether and dried in vacuo.

The general procedure for conversion to an oxalate salt was the addition of a stoichiometric amount of a solution of oxalic acid in ethyl acetate to a solution of the compound in ethyl acetate. The solvent was evaporated and the resulting salt was triturated with anhydrous ether and dried in vacuo.

Elemental analyses

Compound	% C		% H		% N	
	Calcd	Found	Calcd	Found	Calcd	Found
1a	52.06	51.93	8.11	7.89	8.67	8.41
1b	53.90	54.12	8.46	8.46	8.20	8.10
1c	59.88	59.92	8.93	8.83	7.76	7.57
2a	53.73	53.23	6.64	6.20	6.22	6.01
2b	60.90	60.63	7.15	6.83	7.10	6.97
2c	50.20	49.88	6.18	5.83	5.86	5.51
2d	53.73	53.08	6.64	6.12	6.22	5.99
2e	59.10	59.02	7.27	7.04	6.89	6.53
2f	52.18	52.01	5.98	5.88	6.08	5.90
3a	56.47	56.79	6.81	6.70	6.17	6.06
3b	62.02	61.82	7.48	7.23	6.78	6.61
3c	52.82	52.52	6.37	6.23	5.77	5.58
3d	55.73	55.98	6.87	6.58	6.09	5.92
3e	58.61	58.61	7.69	7.38	6.41	6.25
3f	52.18	52.09	6.43	6.17	5.70	5.60
4a	57.52	57.48	6.94	6.81	6.29	6.07
4b	55.33	55.32	6.38	6.16	6.05	5.82
4c	59.80	59.79	7.06	6.67	6.54	6.17
4d	51.20	51.55	6.04	5.78	5.60	5.46
9	59.30	59.05	7.06	6.86	7.48	7.18

7.2. General procedure for the synthesis of compounds 1a–c

A mixture of secondary amine **10** (1.42 g, 4.68 mmol), *N*-(ω-bromoalkyl)phthalimides (1 equiv), KI (1 equiv), and K_2CO_3 (5 equiv) in acetonitrile was stirred at reflux overnight. After filtration, volatile components were evaporated in vacuo. The resulting residue was purified by silica gel column chromatography (2% CH_3OH in

CH_2Cl_2) to give the intermediate products (**11a**–**c**), which were then refluxed with anhydrous hydrazine (1.2 equiv) in ethanol (30 mL) for 2 h. The solvent was evaporated and an aqueous solution of 10% NaOH (25 mL) was added. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated to give the target compounds. The products were converted to the corresponding hydrochloride salts for elemental analysis.

7.2.1. *N*-(9-(4-Aminobutyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate hydrochloride (1a). Obtained in 70% yield from *N*-(4-bromobutyl)phthalimide to give an off-white powder, mp 130–131 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.95 (br s, 1H), 7.14 (s, 1H), 6.72–6.79 (m, 2H), 5.13 (q, J = 6.8 Hz, 1H), 3.84 (s, 3H), 3.05–3.10 (m, 2H), 2.68–2.72 (m, 2H), 2.57–2.61 (m, 2H), 2.39–2.49 (m, 2H), 2.29 (s, 3H), 2.09–2.19 (m, 1H), 1.83–1.91 (m, 2H), 1.19–1.54 (m, 11H); Anal. ($C_{21}H_{33}N_2O_3\cdot 2HCl\cdot 2H_2O$) C, H, N.

7.2.2. *N*-(9-(6-Aminohexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate hydrochloride (1b). Obtained in 74% yield from *N*-(6-bromohexyl)phthalimide to give a white powder, mp 119–120 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.95 (br s, 1H), 7.14 (s, 1H), 6.73–6.80 (m, 2H), 5.14 (q, J = 6.8 Hz, 1H), 3.85 (s, 3H), 3.05–3.10 (m, 2H), 2.66–2.71 (m, 2H), 2.55–2.60 (m, 2H), 2.40–2.50 (m, 2H), 2.30 (s, 3H), 2.08–2.24 (m, 1H), 1.82–1.94 (m, 2H), 1.20–1.55 (m, 14H); Anal. ($C_{23}H_{37}N_2O_3\cdot 2HCl\cdot 2H_2O$) C, H, N.

7.2.3. *N*-(9-(10-Aminodecyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate hydrochloride (1c). Obtained in 70% yield from *N*-(10-bromodecyl)phthalimide to give a white powder, mp 114–115 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.96 (br s, 1H), 7.14 (s, 1H), 6.72–6.78 (m, 2H), 5.14 (q, J = 6.6 Hz, 1H), 3.85 (s, 3H), 3.04–3.09 (m, 2H), 2.65–2.70 (m, 2H), 2.53–2.58 (m, 2H), 2.39–2.49 (m, 2H), 2.30 (s, 3H), 2.09–2.20 (m, 1H), 1.81–1.93 (m, 2H), 1.19–1.53 (m, 23H); Anal. ($C_{27}H_{45}N_2O_3\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

7.3. General procedure for the synthesis of compounds 2a–f and 3a–f

Primary amines **1a** or **1b** (200 mg) and 3-halo- or 4-halobenzaldehydes (1.3 equiv) in benzene (6 mL) were heated at 90 °C for 2 h. After evaporation, the resulting residue was treated with sodium borohydride (4 equiv) in ethanol (10 mL) at ambient temperature overnight. The reaction mixture was quenched with 10% HCl solution and concentrated in vacuo. The residue was dissolved in water (8 mL), the pH was adjusted to 10 by dropwise addition of an aqueous solution of 10% NaOH, and the product was extracted with CH_2Cl_2 (2× 25 mL). The organic layer was dried over Na_2SO_4 and evaporated to give the products. The oxalate salts were made for elemental analysis.

7.3.1. *N*-(9-(4-(3′-Bromobenzylamino)butyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (2a). Obtained in quantitative yield from **1a** and 3-bromobenzaldehyde to give an off-white powder, mp 189–190 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.95 (br s, 1H), 7.13–7.50 (m, 5H), 6.72–6.80 (m, 2H), 5.12 (q, J = 6.8 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 2H), 3.04–3.08 (m, 2H), 2.57–2.64 (m, 4H), 2.38–2.49 (m, 2H), 2.29 (s, 3H), 2.09–2.24 (m, 1H), 1.81–1.91 (m, 2H), 1.47–1.56 (m, 8H), 1.19–1.25 (m, 2H); Anal. ($C_{28}H_{38}BrN_3O_3\cdot C_2H_2O_4\cdot 2H_2O$) C, H, N.

7.3.2. *N*-(9-(4-(3′-Fluorobenzylamino)butyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (2b). Obtained in 55% yield from **1a** and 3-fluorobenzaldehyde to give a white powder, mp 185–186 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.95 (br s, 1H), 6.72–7.31 (m, 7H), 5.12 (q, J = 6.7 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 2H), 3.04–3.08 (m, 2H), 2.57–2.65 (m, 4H), 2.38–2.48 (m, 2H), 2.29 (s, 3H), 2.08–2.22 (m, 1H), 1.80–1.94 (m, 2H), 1.47–1.68 (m, 8H), 1.18–1.24 (m, 2H); Anal. ($C_{28}H_{38}FN_3O_3\cdot C_2H_2O_4\cdot H_2O$) C, H, N.

7.3.3. *N*-(9-(4-(3′-Iodobenzylamino)butyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (2c). Obtained in 12% yield from **1a** and 3-iodobenzaldehyde to give a white powder, mp 200–201 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.94 (br s, 1H), 7.70 (s, 1H), 7.55–7.60 (m, 1H), 7.30–7.34 (m, 1H), 7.03–7.13 (m, 2H), 6.72–6.80 (m, 2H), 5.12 (q, J = 6.7 Hz, 1H), 3.84 (s, 3H), 3.74 (s, 2H), 3.06–3.10 (m, 2H), 2.59–2.65 (m, 4H), 2.39–2.49 (m, 2H), 2.29 (s, 3H), 2.14–2.24 (m, 1H), 1.80–1.90 (m, 2H), 1.46–1.56 (m, 8H), 1.20–1.26 (m, 2H); Anal. ($C_{28}H_{38}IN_3O_3\cdot C_2H_2O_4\cdot 2H_2O$) C, H, N.

7.3.4. *N*-(9-(4-(4′-Bromobenzylamino)butyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (2d). Obtained in quantitative yield from **1a** and 4-bromobenzaldehyde to give an off-white powder, mp 197–198 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.95 (br s, 1H), 7.44 (d, J = 8.3 Hz, 2H), 7.21 (d, J = 8.3 Hz, 2H), 7.13 (s, 1H), 6.73–6.80 (m, 2H), 5.12 (q, J = 6.8 Hz, 1H), 3.84 (s, 3H), 3.74 (s, 2H), 3.02–3.08 (m, 2H), 2.54–2.64 (m, 2H), 2.37–2.47 (m, 2H), 2.30 (s, 3H), 2.07–2.22 (m, 1H), 1.78–1.92 (m, 2H), 1.16–1.54 (m, 10H); Anal. ($C_{28}H_{38}BrN_3O_3\cdot C_2H_2O_4\cdot 2H_2O$) C, H, N.

7.3.5. *N*-(9-(4-(4′-Fluorobenzylamino)butyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (2e). Obtained in quantitative yield from **1a** and 4-fluorobenzaldehyde to give a white powder, mp 195–196 °C (dec); ^{1}H NMR (free, base, $CDCl_3$) δ 7.95 (br s, 1H), 7.29–7.34 (m, 2H), 7.13 (s, 1H), 6.97–7.07 (m, 2H), 6.73–6.80 (m, 2H), 5.12 (q, J = 6.8 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 2H), 3.04–3.08 (m, 2H), 2.56–2.65 (m, 2H), 2.38–2.48 (m, 2H), 2.29 (s, 3H), 2.09–2.19 (m, 1H), 1.79–1.90 (m, 2H), 1.19–1.56 (m, 9H); Anal. ($C_{28}H_{38}FN_3O_3\cdot C_2H_2O_4\cdot 2H_2O$) C, H, N.

7.3.6. *N*-(9-(4-(4′-Iodobenzylamino)butyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (2f). Obtained in 10% yield from **1a** and 4-iodobenzaldehyde to give a white powder, mp 159–160 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.95 (br s, 1H), 7.64 (d, J = 8.1 Hz, 2H), 7.14 (s, 1H), 7.10 (d,

J = 8.1 Hz, 2H), 6.72–6.80 (m, 2H), 5.12 (q, J = 6.7 Hz, 1H), 3.84 (s, 3H), 3.74 (s, 2H), 3.04–3.10 (m, 2H), 2.57–2.64 (m, 4H), 2.38–2.49 (m, 2H), 2.30 (s, 3H), 2.10–2.20 (m, 1H), 1.80–1.92 (m, 2H), 1.18–1.58 (m, 10H); Anal. ($C_{28}H_{38}IN_3O_3 \cdot C_2H_2O_4 \cdot 0.5\ H_2O$) C, H, N.

7.3.7. *N*-(9-(6-(3′-Bromobenzylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (3a). Obtained in 88% yield from **1b** and 3-bromobenzaldehyde to give a white powder, mp 193–194 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.95 (br s, 1H), 7.49 (s, 1H), 7.37 (d, J = 7.9 Hz, 1H), 7.22 (d, J = 7.9 Hz, 1H), 7.18 (t, J = 7.9 Hz, 1H), 7.14 (s, 1H), 6.73–6.80 (m, 2H), 5.13 (q, J = 6.7 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 2H), 3.04–3.09 (m, 2H), 2.55–2.64 (m, 4H), 2.40–2.50 (m, 2H), 2.29 (s, 3H), 2.10–2.22 (m, 1H), 1.84–1.93 (m, 2H), 1.20–1.52 (m, 14H); Anal. ($C_{30}H_{42}BrN_3O_3 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

7.3.8. *N*-(9-(6-(3′-Fluorobenzylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (3b). Obtained in 86% yield from **1b** and 3-fluorobenzaldehyde to give a white powder, mp 149–150 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.95 (br s, 1H), 7.24–7.29 (m, 1H), 7.13 (s, 1H), 7.04–7.10 (m, 2H), 6.90–6.96 (m, 1H), 6.73–6.80 (m, 2H), 5.13 (q, J = 6.5 Hz, 1H), 3.85 (s, 3H), 3.79 (s, 2H), 3.00–3.05 (m, 2H), 2.54–2.64 (m, 4H), 2.39–2.49 (m, 2H), 2.29 (s, 3H), 2.09–2.18 (m, 1H), 1.83–1.93 (m, 2H), 1.19–1.55 (m, 14H); Anal. ($C_{30}H_{42}FN_3O_3 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

7.3.9. *N*-(9-(6-(3′-Iodobenzylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (3c). Obtained in 86% yield from **1b** and 3-iodobenzaldehyde to give a white powder, mp 192–193 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.95 (br s, 1H), 7.69 (s, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.13 (s, 1H), 7.05 (t, J = 7.8 Hz, 1H), 6.72–6.79 (m, 2H), 5.13 (q, J = 6.6 Hz, 1H), 3.84 (s, 3H), 3.73 (s, 2H), 3.04–3.09 (m, 2H), 2.54–2.63 (m, 4H), 2.39–2.49 (m, 2H), 2.29 (s, 3H), 2.08–2.18 (m, 1H), 1.82–1.94 (m, 2H), 1.19–1.54 (m, 14H); Anal. ($C_{30}H_{42}IN_3O_3 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

7.3.10. *N*-(9-(6-(4′-Bromobenzylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (3d). Obtained in 85% yield from **1b** and 4-bromobenzaldehyde to give a white powder, mp 164–165 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.95 (br s, 1H), 7.44 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 7.14 (s, 1H), 6.73–6.80 (m, 2H), 5.13 (q, J = 6.8 Hz, 1H), 3.85 (s, 3H), 3.74 (s, 2H), 3.04–3.08 (m, 2H), 2.54–2.62 (m, 4H), 2.40–2.50 (m, 2H), 2.29 (s, 3H), 2.10–2.23 (m, 1H), 1.83–1.92 (m, 2H), 1.19–1.51 (m, 14H); Anal. ($C_{30}H_{42}BrN_3O_3 \cdot C_2H_2O_4 \cdot 1.5H_2O$) C, H, N.

7.3.11. *N*-(9-(6-(4′-Fluorobenzylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (3e). Obtained in 92% yield from **1b** and 4-fluorobenzaldehyde to give a white powder, mp 178–179 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.95 (br s, 1H), 7.28–7.36 (m, 2H), 7.14 (s, 1H), 6.97–7.07 (m, 2H), 6.73–6.79 (m, 2H), 5.13 (q, J = 6.8 Hz, 1H), 3.84 (s, 3H), 3.75 (s, 2H), 3.03–3.08 (m, 2H), 2.58–2.63 (m, 2H), 2.38–2.48 (m, 2H), 2.29 (s, 3H), 2.05–2.20 (m, 1H), 1.80–1.92 (m, 2H), 1.18–1.53 (m, 13H); Anal. ($C_{30}H_{42}FN_3O_3 \cdot C_2H_2O_4 \cdot 3H_2O$) C, H, N.

7.3.12. *N*-(9-(6-(4′-Iodobenzylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (3f). Obtained in 85% yield from **1b** and 4-iodobenzaldehyde to give a white powder, mp 144–145 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.95 (br s, 1H), 7.44 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 7.14 (s, 1H), 6.73–6.80 (m, 2H), 5.13 (q, J = 6.8 Hz, 1H), 3.85 (s, 3H), 3.74 (s, 2H), 3.04–3.08 (m, 2H), 2.54–2.62 (m, 4H), 2.40–2.50 (m, 2H), 2.29 (s, 3H), 2.10–2.23 (m, 1H), 1.83–1.92 (m, 2H), 1.19–1.51 (m, 14H); Anal. ($C_{30}H_{42}IN_3O_3 \cdot C_2H_2O_4 \cdot 1.5\ H_2O$) C, H, N.

7.4. General procedure for the synthesis of compounds 4a–d

A solution of 1,3-dicyclohexylcarbodiimide (154 mg, 0.74 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise to a solution of 4-halobenzoic acids (1.2 equiv) and *N*-hydroxysuccinimide (64 mg, 0.74 mmol) in CH_2Cl_2 (2 mL) at 0 °C (ice bath). After removal of the ice bath, the mixture was stirred at ambient temperature for 1 h. A solution of amine **1b** (250 mg, 0.62 mmol) in CH_2Cl_2 (5 mL) was slowly added, and the reaction mixture was stirred at ambient temperature for 3 h. The formed precipitate was filtered, the organic layer was washed with water (1× 50 mL) and then saturated aqueous K_2CO_3 (1× 50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel column chromatography (5% CH_3OH in CH_2Cl_2) to give the desired compounds. The oxalate salts were made for elemental analysis.

7.4.1. *N*-(9-(4-(4′-Chlorobenzoylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (4a). Obtained in 58% yield from 4-chlorobenzoic acid to give a white powder, mp 127–128 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.92 (br s, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 7.14 (s, 1H), 6.73–6.80 (m, 2H), 6.29 (br s, 1H), 5.14 (q, J = 6.8 Hz, 1H), 3.85 (s, 3H), 3.42–3.48 (m, 2H), 3.05–3.08 (m, 2H), 2.38–2.60 (m, 4H), 2.26 (s, 3H), 1.16–2.11 (m, 16H); Anal. ($C_{30}H_{40}ClN_3O_4 \cdot C_2H_2O_4 \cdot 2H_2O$) C, H, N.

7.4.2. *N*-(9-(4-(4′-Bromobenzoylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (4b). Obtained in 71% yield from 4-bromobenzoic acid to give a white powder, mp 152–153 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.91 (br s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.14 (s, 1H), 6.73–6.80 (m, 2 H), 6.39 (br s, 1H), 5.14 (q, J = 6.8 Hz, 1H), 3.85 (s, 3H), 3.42–3.48 (m, 2H), 3.10–3.15 (m, 2H), 2.49–2.66 (m, 4H), 2.27 (s, 3H), 1.22–2.22 (m, 16H); Anal. ($C_{30}H_{40}BrN_3O_4 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

7.4.3. *N*-(9-(4-(4′-Fluorobenzoylamino)hexyl)-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (4c). Obtained in 81% yield from 4-fluorobenzoic acid to give a white powder, mp 119–120 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.91 (br s,

1H), 7.79–7.84 (m, 2H), 6.97–7.05 (m, 3H), 6.73–6.78 (m, 2H), 6.40 (br s, 1H), 5.14 (q, $J = 6.5$ Hz, 1H), 3.85 (s, 3H), 3.41–3.47 (m, 2H), 3.18–3.22 (m, 2H), 2.53–2.74 (m, 4H), 2.27 (s, 3H), 1.28–2.17 (m, 16H); Anal. ($C_{30}H_{40}FN_3O_4 \cdot C_2H_2O_4 \cdot 1.5H_2O$) C, H, N.

7.4.4. *N*-(9-(4-(4′-Iodobenzoylamino)hexyl)-9-azabicyclo-[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (4d). Obtained in 57% yield from 4-iodobenzoic acid to give a white powder, mp 150–151 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 7.91 (br s, 1H), 7.74 (d, $J = 8.6$ Hz, 2H), 7.52 (d, $J = 8.6$ Hz, 2H), 7.13 (s, 1H), 6.73–6.77 (m, 2H), 6.38 (br s, 1H), 5.13 (q, $J = 6.8$ Hz, 1H), 3.84 (s, 3H), 3.40–3.48 (m, 2H), 3.08–3.14 (m, 2H), 2.44–2.64 (m, 4H), 2.26 (s, 3H), 1.20–2.20 (m, 16H); Anal. ($C_{30}H_{40}IN_3O_4 \cdot C_2H_2O_4 \cdot 1.5H_2O$) C, H, N.

7.5. General procedure for the synthesis of compounds 5 and 6

Primary amines **1b** or **1c** (150 mg), (+)-biotin *N*-hydroxysuccinimide ester (1.1 equiv), and triethylamine (0.1 mL) in DMF (5 mL) were stirred at 65 °C for 48 h. The mixture was allowed to cool to ambient temperature, and volatiles were removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (15% CH_3OH, 1% NH_4OH in CH_2Cl_2) to give **5** or **6**.

7.5.1. Compound 5. Obtained in 76% yield from **1b** to give a white powder; ^{1}H NMR (DMSO-d_6) δ 8.12 (br s, 1H), 7.70–7.74 (m, 1H), 7.47 (s, 1H), 6.82–6.89 (m, 2H), 6.41 (s, 1H), 6.35 (s, 1H), 4.90–4.99 (m, 1H), 4.27–4.31 (m, 1H), 4.09–4.13 (m, 1H), 3.75 (s, 3H), 2.30–3.11 (m, 13H), 2.21 (s, 3H), 1.13–2.06 (m, 22H); MS (FAB$^+$) exact mass calcd for $C_{33}H_{51}N_5O_5S$ [M+Li]$^+$: 636.3771, found: 636.3762.

7.5.2. Compound 6. Obtained in 78% yield from **1c** to give a white powder; ^{1}H NMR (DMSO-d_6) δ 8.07 (br s, 1H), 7.70–7.74 (m, 1H), 7.49 (s, 1H), 6.82–6.90 (m, 2H), 6.42 (s, 1H), 6.35 (s, 1H), 4.90–4.98 (m, 1H), 4.28–4.31 (m, 1H), 4.10–4.15 (m, 1H), 3.75 (s, 3H), 2.30–3.09 (m, 13H), 2.21 (s, 3H), 1.11–2.05 (m, 30H); MS (FAB$^+$) exact mass calcd for $C_{37}H_{59}N_5O_5S$ [M+Li]$^+$: 692.4397, found: 692.4411.

7.6. General procedure for the synthesis of compounds 7 and 8

Primary amines **1b** or **1c** (150 mg), (+)-biotinamidocaproate *N*-hydroxysuccinimidyl ester (1.1 equiv), and triethylamine (0.1 mL) in DMF (5 mL) were stirred at 50 °C for 48 h. The mixture was allowed to cool to ambient temperature, and volatiles were removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (15% CH_3OH, 1% NH_4OH in CH_2Cl_2) to give **7** or **8**.

7.6.1. Compound 7. Obtained in 88% yield from **1b** to give a white solid; ^{1}H NMR (DMSO-d_6) δ 8.11 (br s, 1H), 7.70–7.75 (m, 2H), 7.48 (s, 1H), 6.82–6.90 (m, 2H), 6.42 (s, 1H), 6.36 (s, 1H), 4.92–4.99 (m, 1H), 4.25–4.32 (m, 1H), 4.08–4.15 (m, 1H), 3.76 (s, 3H), 2.30–3.10 (m, 15H), 2.22 (s, 3H), 1.10–2.10 (m, 30H); MS (FAB$^+$) exact mass calcd for $C_{39}H_{62}N_6O_6S$ [M+Li]$^+$: 749.4612, found: 749.4628.

7.6.2. Compound 8. Obtained in 95% yield from **1c** to give an off-white solid; ^{1}H NMR (DMSO-d_6) δ 8.02 (s, 2H), 7.94 (br s, 1H), 7.14 (s, 1H), 6.73–6.80 (m, 2H), 6.26 (s, 1H), 6.14 (s, 1H), 5.09–5.18 (m, 1H), 4.49–4.54 (m, 1H), 4.30–4.34 (m, 1H), 3.85 (s, 3H), 2.40–3.30 (m, 11H), 2.29 (s, 3H), 1.21–2.23 (m, 42H); MS (FAB$^+$) exact mass calcd for $C_{43}H_{70}N_6O_6S$ [M+Li]$^+$: 805.5238, found: 805.5243.

7.7. *N*-(9-(6-(5-Dimethylamino-1-naphthalenesulfonamido)hexyl)-9-azabicyclo[3.3.1]-nonan-3α-yl)-*N*-(2-methoxy-5-methylphenyl)carbamate oxalate (9)

A solution of dansyl chloride (135 mg, 0.50 mmol) in CH_3CN (6 mL) was added dropwise to a mixture of **1b** (200 mg, 0.50 mmol) and K_2CO_3 (104 mg, 0.75 mmol) in CH_3CN (2 mL). The reaction mixture was stirred at ambient temperature for 24 h. The mixture was filtered, and volatiles were removed in vacuo. The product was purified by column chromatography (CH_3OH–CH_2Cl_2–NH_4OH 10:90:0.1) to give **9** (93%) as a yellow oil. The oxalate salt was made for analysis, mp 162–163 °C; ^{1}H NMR (free base, $CDCl_3$) δ 8.54 (d, $J = 8.6$ Hz, 1H), 8.30 (d, $J = 8.6$ Hz, 1H), 8.23–8.26 (m, 1H), 7.94 (br s, 1H), 7.50–7.58 (m, 2H), 7.18 (d, $J = 7.1$ Hz, 1H), 7.13 (s, 1H), 6.73–6.80 (m, 2H), 5.10 (q, $J = 6.7$ Hz, 1H), 4.77 (br s, 1H), 2.99–3.11 (m, 2H), 2.89 (s, 6H), 2.42–2.56 (m, 4H), 2.29 (s, 3H), 1.17–2.15 (m, 18H); MS (FAB$^+$) exact mass calcd for $C_{35}H_{48}N_4O_5S$ [M+Li]$^+$: 643.3505, found: 643.3490; Anal. ($C_{35}H_{48}N_4O_5S \cdot C_2H_2O_4 \cdot 1.25H_2O$) C, H, N.

8. Sigma receptor binding assays

Test compounds were dissolved in *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO) or ethanol and then diluted in 50 mM Tris–HCl, pH 7.4, buffer containing 150 mM NaCl and 100 mM EDTA. Membrane homogenates were made from guinea pig brain for σ_1 binding assay and rat liver for σ_2 binding assay. Membrane homogenates were diluted with 50 mM Tris–HCl buffer, pH 8.0, and incubated at 25 °C in a total volume of 150 μL in 96-well plates with the radioligand and test compounds with concentrations ranging from 0.1 nM to 10 μM. After incubation was completed, the reactions were terminated by the addition of 150 μL of ice-cold wash buffer (10 mM Tris–HCl, 150 mM NaCl, pH 7.4) using a 96-channel transfer pipette (Fisher Scientific, Pittsburgh, PA), and the samples harvested and filtered rapidly through 96-well fiber glass filter plate (Millipore, Billerica, MA) that had been presoaked with 100 μL of 50 mM Tris–HCl buffer, pH 8.0, for 1 h. Each filter was washed three times with 200 μL of ice-cold wash buffer. A Wallac 1450 MicroBeta liquid scintillation counter (Perkin-Elmer, Boston, MA) was used to quantitate the bound radioactivity.

The σ_1 receptor binding assay was conducted using guinea pig brain membrane homogenates (~300 μg

protein) and ~5 nM [^{3}H](+)-pentazocine (34.9 Ci/mmol, Perkin-Elmer, Boston, MA), incubation time was 90 min. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol.

The σ_2 receptor binding assays were conducted using rat liver membrane homogenates (~300 μg protein) and ~1 nM [^{3}H]RHM-1 (80 Ci/mmol, American Radiolabeled Chemicals Inc., St. Louis, MO) alone or ~5 nM [^{3}H]DTG (58.1 Ci/mmol, Perkin-Elmer, Boston, MA) in the presence of 1 μM (+)-pentazocine to block σ_1 sites. The incubation time was 60 min for [^{3}H]RHM-1 and 120 min for [^{3}H]DTG. Nonspecific binding was determined from samples that contained 10 μM of cold haloperidol.

Data from the competitive inhibition experiments were modeled using nonlinear regression analysis to determine the concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC_{50} value). Competitive curves were best fit to a one-site fit and gave pseudo-Hill coefficients of 0.6–1.0. K_i values were calculated using the method of Cheng and Prusoff[20] and represent mean values ± SEM. The K_d value used for [^{3}H](+)-pentazocine in guinea pig brain was 7.89 nM, for [^{3}H]DTG in rat liver was 30.73 nM, and for [^{3}H]RHM-1 in rat liver was 0.66 nM.[21]

9. Fluorescent σ_2 ligand assay

The mono-dansyl analog, **9**, was dissolved in methanol and excitation and emission spectra were determined. Fluorescent excitation and emission spectra were recorded on a spectrofluorometer (Perkin-Elmer LS 50, Wellesley, MA). Excitation spectra and emission spectra for **9** were also determined using a Zeiss two-photon microscope (LSM 510 NLO META). EMT6 cells were incubated with **9** (200 nM). To determine the maximum wavelength of excitation, cells were illuminated with wavelengths ranging from 720 nm to 860 nm at 10 nm intervals. Emission spectra were collected using a 685 nm-short pass filter. To determine the maximum emission wavelength, the excitation wavelength was set to 720 nm and emission spectra were collected using a series of filters with 10 nm bandwidth at wavelengths ranging from 457 nm to 596 nm. The emission spectra were obtained at 11 nm intervals to give images with a resolution of 512 × 512 pixels.

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