Synthesis and Substance P Antagonist Activity of Naphthimidazolium Derivatives

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The synthesis of unsymmetrical naphth[2,3-*d*]imidazolium and bridged naphth[2,3-*d*]imidazolium derivatives and their substance P (SP) antagonist activity are described. All compounds were evaluated for their ability to displace SP from neurokinin-1 (NK-1) receptor sites using standard receptor binding methodology (rat forebrain membrane). 1,3-Diethyl-2-[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium chloride (**7a**), a representative compound in this series, was further evaluated for SP antagonist activity in a guinea pig ileum contractility assay. In vivo SP antagonist activity of **7a** was demonstrated using SP-induced salivation and paw edema models performed in rats.

Introduction

Substance P (SP) is an undecapeptide neurotransmitter and is implicated in several important disease states, particularly in pain and inflammation.[1,2] However, the therapeutic significance of a SP antagonist has not been fully explored, primarily due to the lack of orally available, nonpeptide SP antagonists except for the recently disclosed nonpeptidic quinuclidines.[3] During ongoing efforts to discover novel, nonpeptide SP antagonists, we have prepared several imidazo[4,5-*b*]quinoxaline derivatives,[4] represented by general structure I (Figure 1). With a goal to prepare more potent SP antagonists and to understand the structure–activity relationship of these compounds, we were interested in the synthesis of the more electron donating[5] naphth[2,3-*d*]imidazolium ring system represented by general structure II (Figure 1). The target compounds II also serve as carbon isosteres of I. The preparation of these compounds and their biological activities as SP antagonists are described here.

Chemistry. A series of unsymmetrical compounds containing the unbridged, naphth[2,3-*d*]imidazole nucleus were prepared from 1-ethyl-2-methyl-1*H*-naphth[2,3-*d*]-imidazole (**3**, Scheme I, Table I). This heterocycle (**3**) was obtained by the condensation of 2,3-diaminonaphthalene (**1**) with triethyl orthoacetate in *N*,*N*-dimethylformamide to obtain **2** followed by alkylation with ethyl iodide. Quaternization was accomplished by heating the monosubstituted 1*H*-naphth[2,3-*d*]imidazole **3** with the desired alkyl halide, yielding either the 1,3-diethyl **4a** or the 1-ethyl 3-hydroxyethyl **4b** quaternary salt. The condensation of these quaternary salts with an acetanilidovinyl intermediate[6,7] (**5**) was achieved either with dry pyridine/triethylamine[8] or acetonitrile/DBU to give quaternary iodide salts **6a–e** (Scheme I, Table I). In an effort to improve solubility, several of these compounds were converted to the corresponding chloride salts (**7a–d**, Scheme I, Table I) using an ion-exchange resin.

Attempts to condense 1-ethyl-3-(2-hydroxyethyl)-2-methyl-1*H*-naphth[2,3-*d*]imidazolium iodide (**4b**) with acetanilidovinyl intermediate **5** using the conditions outlined in Scheme I were unsuccessful. Condensation occurred only after **4b** was acylated in situ using acetic anhydride, yielding the acylated quaternary salt **6c** (Table I). Partial hydrolysis occurred during ion exchange, yielding a 2:1 mixture of acylated and nonacylated quaternary chloride salts (**7c** and **7d**, respectively, Table I) which were separated by fractional crystallization.

Preparation of 1,3-diethyl-2-[3-(1-methylbenz[*cd*]indol-2(1*H*)-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]-imidazolium chloride (**9**) involved condensation of **4a** with [1-methylbenz[*cd*]indol-2(1*H*)-ylidene]acetaldehyde[9–11] (**8**) in refluxing acetic anhydride followed by ion exchange (Scheme I, Table I).

Quaternary derivatives containing the alkyl-bridged naphth[2,3-*d*]imidazole nucleus (**13a–e**, Scheme II, Table II) were prepared by reacting either a 1,2,3,4-tetrahydro-1*H*-naphth[2′,3′:4,5]imidazo[1,2-*a*]pyridinium or a 2,3-dihydro-1*H*-naphtho[2,3-*d*]pyrrolo[1,2-*a*]imidazolium quaternary salt (**12**) with the desired acetanilidovinyl intermediate[6,7] **5** using triethylamine in dry pyridine or ethanol.[12] The requisite bridged naphth[2,3-*d*]imidazole quaternary salts **12** were prepared by condensation of 2,3-diaminonaphthalene **1** with the appropriate δ or γ-substituted lactone[13] **10** followed by quaternization using

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(1) Regoli, D.; Drapeau, G.; Dion, S.; Couture, R. New Selective Agonists for Neurokinin Receptors: Pharmacological Tools for Receptor Characterization. *Trends Pharmacol. Sci.* **1988**, *9*, 290–295.
(2) Otsuko, M.; Yanagisawa, M. Effect of a Tachykinin Antagonist on a Nociceptive Reflex in the Isolated Spinal Cord-Tail Preparation of the New-born Rat. *J. Physiol. (London)* **1988**, *395*, 255–270.
(3) Snider, R. M.; Constantine, J. W.; Lowe, J. A., III; Longo, K. P.; Lebel, W. S.; Woody, H. A.; Drozda, S. E.; Desai, M. C.; Vinick, F. J.; Spencer, R. W.; Hess, H.-J. A Potent Nonpeptidic Antagonist of the Substance P (NK1) Receptor. *Science* **1991**, 435–437.
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(5) James, T. H. Sensitizing and Desensitizing Dyes. *The Theory of the Photographic Process*, 4th ed.; MacMillan Publishing Co.: New York, 1977; pp 194–234.
(6) For a review on the preparation of acetanilidovinyl intermediates: Hamer, F. M. The Cyanine Dyes and Related Compounds. *The Chemistry of Heterocyclic Compounds*; Interscience Publishers: New York, 1964; Vol. 18, pp 116–147.
(7) Piggott, H. A.; Rodd, E. H. Improvements in and Relating to the Manufacture of Intermediates and Dyes from Heterocyclic Nitrogen Compounds. GB Patent 344,409 (ICI, Ltd.) 1929; GB 354,898 (ICI, Ltd.), 1930.
(8) Stetsenko, A. V.; Yarmolyuk, S. N. Benzoimidacyanines. *Ukr. Khim. Zh. (Russ. Ed.)* **1981**, *47* (9), 964–966.
(9) Rozhinskii, Y. I.; Mostoslavskii, M. A. N-Substituted Naphthostyryl Derivatives. I. Synthesis of N-Alkyl-naphthostyryls. *Zh. Org. Khim.* **1972**, *8*, 2177–2181.
(10) Mikhailenko, F. A.; Vasilenko, N. P.; Kachkovskii, A. D.; Rozhinskii, Y. I. Effect of Polar Substituents and the Length of the Polymethine Chain on the Color of Cyanine Dyes of the Benzo[c,d]indole Series. *Zh. Org. Khim.* **1982**, *18*, 435–441.
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(13) Mosby, W. L. Reactions of δ-Valerolactone with *ortho*- and *peri*-Naphthylenediamines. *J. Org. Chem.* **1959**, *24*, 419–421.

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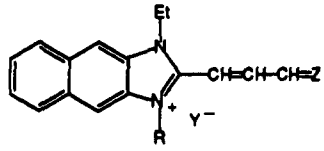
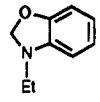
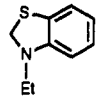
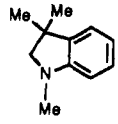
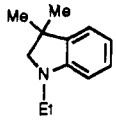
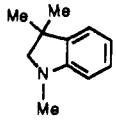
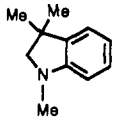
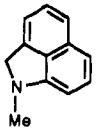
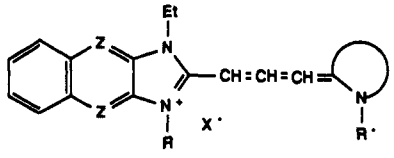
Table I. Physical and Biochemical Results of Unsymmetrical Naphth[2,3-*d*]imidazolium Derivatives



no.	z	R	Y⁻	% yield[a]	mp, °C	formula	IC_{50},[b] nM
6d		Et	I	31	285–86	$C_{27}H_{28}IN_3O$	310 ± 9
6e		Et	I	39	265–66	$C_{27}H_{28}IN_3S$	320 ± 30
7a		Et	Cl	95.6	236–38	$C_{29}H_{32}ClN_3$	390 ± 62[c]
7b		Et	Cl	88.2	160–63	$C_{30}H_{34}ClN_3$	360 ± 60
7c		CH_2CH_2OAc	Cl	51.6	200–02	$C_{31}H_{34}ClN_3O_2$	410 ± 23
7d		CH_2CH_2OH	Cl	31.6	226–28	$C_{29}H_{32}ClN_3O$	450 ± 52
9		Et	Cl	71.4	265–68	$C_{30}H_{28}ClN_3$	2000 ± 120
substance P							0.12 ± 0.06

[a] Yield of last step. [b] Concentration necessary for 50% inhibition (IC_{50}) are means ± SEM for three determinants unless otherwise noted. See Experimental Section for procedure. [c] Same as in footnote *b* with the exception that four determinants were used in the calculation of IC_{50}.



I. Z = N, Imidazo[4,5-b]quinoxaline derivatives
II. Z = C, Naphth[2,3-d]imidazole derivatives
R, R' = alkyl, X⁻ = chloride and iodide

Figure 1. Generic structure for imidazo[4,5-*b*]quinoxalinium and naphth[2,3-*d*]imidazolium quaternary salts.

an alkyl halide or tosylate.[14]

Biological Results and Discussion

Target compounds were evaluated in a NK-1 receptor binding assay using rat forebrain tissue.[15] The reversibility

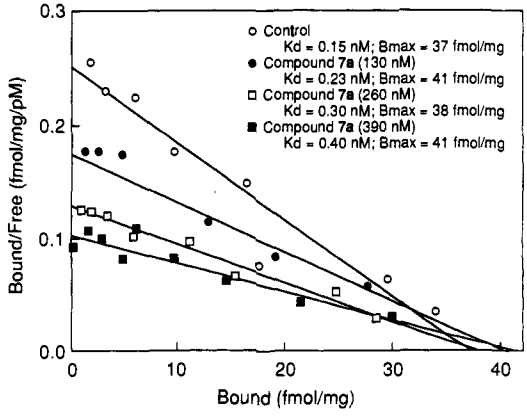
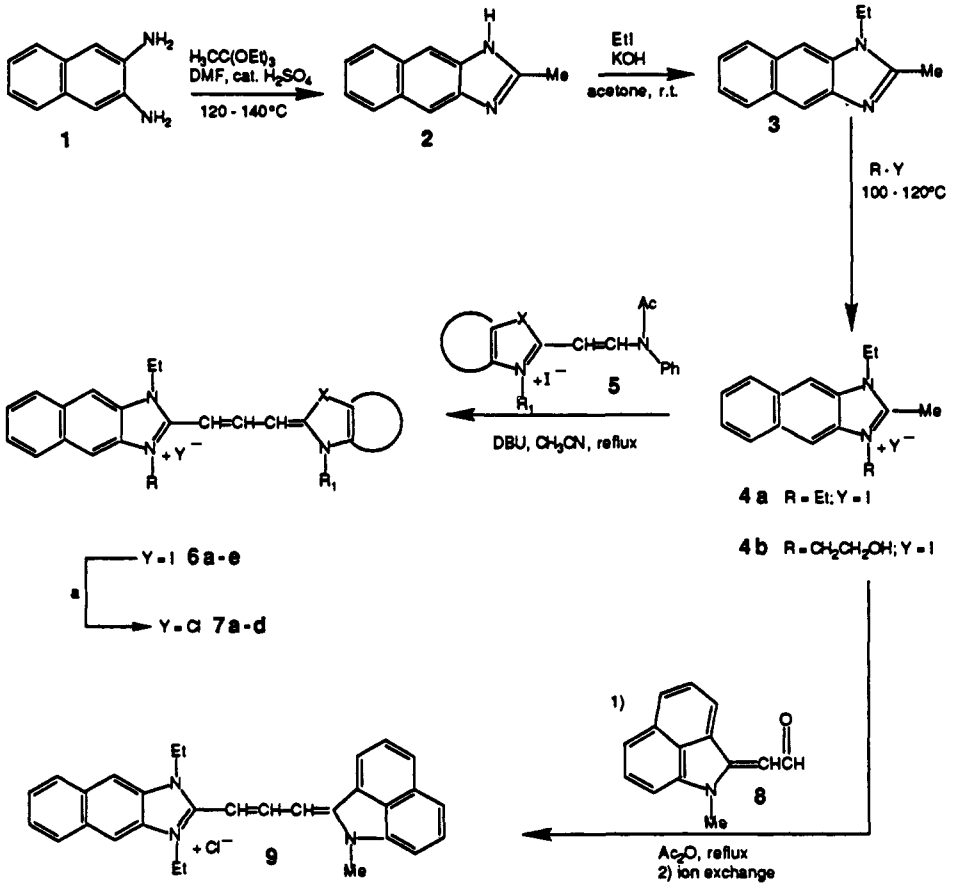


Figure 2. ^{125}I-BH-substance P binding to rat forebrain with **7a**. Scatchard analysis.

of the compound–receptor interaction was established by Scatchard analyses. The results of this analysis for compound **7a** indicated a concentration-dependent increase in the apparent K_d for SP with no change in B_{max} (Figure 2). This suggests that there is a fully reversible interaction of **7a** with the NK-1 receptor. To further investigate the nature of the interaction of **7a** with the NK-1 receptor, the dissociation rate of SP was investigated in the presence

(14) Lincoln, L. L.; Brooker, L. G. S. Photographic Sensitizing Dyes of the Merocyanine and Styryl Types in Silver Halide Photographic Emulsions. US Patent 3,397,981 (Eastman Kodak Company), 1968.
(15) Park, C. H.; Massari, V. J.; Quirion, R.; Tizabi, Y.; Shultz, C. W.; O'Donohue, T. L. Characteristics of ^{3}H-Substance P Binding Sites in Rat Brain Membranes. *Peptides* 1984, *5*, 833–836.

Scheme I[a]



[a] (a) Ion exchange.

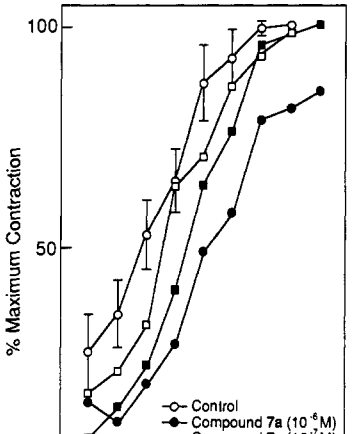


Figure 3. Effect of compound **7a** on substance P induced contractility of isolated guinea pig ileum.

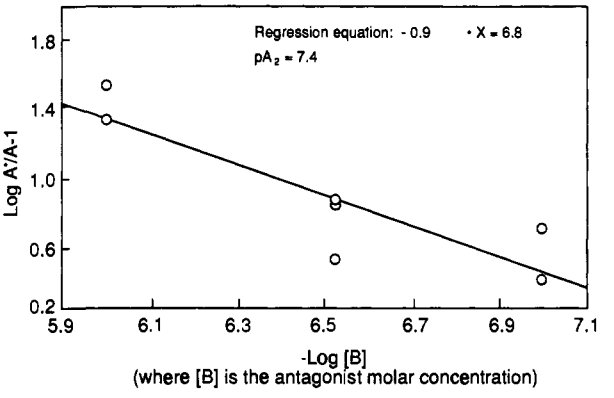


Figure 4. Schild plot of compound **7a**.

and absence of test compound **7a** (data not shown). In the presence of 2.5 and 10 μM compound **7a**, the rate of dissociation was increased by 1.3- and 1.5-fold, respectively, when compared to the results in the absence of test compound. This suggests either the interaction of **7a** with the receptor is not strictly competitive or that some nonspecific interaction is occurring at these relatively high concentrations of test compound.

The structure–activity relationships observed in this class of compounds were based on potency in the receptor binding assay (Tables I and II). The nonbridged naphthimidazoliums (**6d,e** and **7a–d**, Table I) showed comparable binding potencies. Thus, introduction of hydrogen bonding substituents within the naphth[2,3-*d*]imidazole nucleus or changing the heterocycle on the "right-hand portion" of the molecule had no effect on binding potency. However, incorporation of a bulky benz[*cd*]indole moiety as in **9** resulted in an approximately 4-fold decrease in binding potency. Within the 1,3-diethyl imidazoquinoxaline series, a 2-fold decrease in binding potency had occurred when the benz[*cd*]indole nucleus was incorporated into the molecule.[4] Within the bridged naphth[2,3-*d*]imidazole series (Table II), the benzothiazole heterocycle (**13c**) was the most potent in binding with an IC_{50} = 180 nM. Analogues **13a**, **13b**, and **13d** showed only marginal activity.

To determine agonist/antagonist activity of this class of compounds, **7a** was selected for further evaluation in

Scheme II

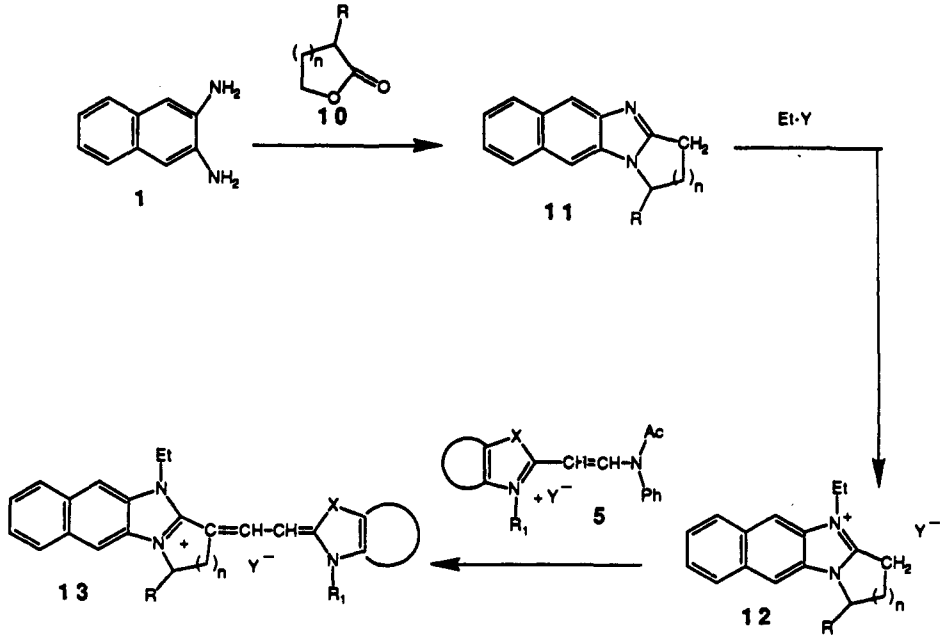
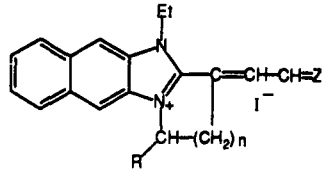
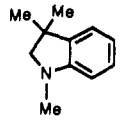
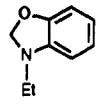
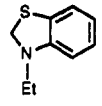
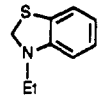
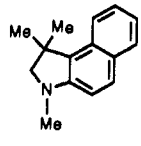


Table II. Physical and Biochemical Results of Unsymmetrical Bridged Naphth[2,3-*d*]imidazolocarbocyanine Dyes



no.	Z	R	*n*	% yield[a]	mp, °C	formula	IC_{50},[b] nM
13a		H	1	44	283–284	$C_{29}H_{30}IN_3$	790 ± 150[c]
13b		H	1	63	219–221	$C_{27}H_{26}IN_3O$	4200 ± 850
13c		Me	1	54	278–279	$C_{28}H_{28}IN_3S$	180 ± 15
13d		H	2	36	281–282	$C_{28}H_{28}IN_3S$	1300 ± 180
13e		Me	1	10	292–293	$C_{34}H_{34}IN_3$	310 ± 56
substance P							0.12 ± 0.06

[a] Yield of last step. [b,c] See Table I for explanation of footnote.

a guinea pig ileum (gpi) contractility assay.[16] No agonist activity was observed with this compound. Tissue pretreatment with **7a** caused a dose-dependent (10^{-8} M to 10^{-8} M) parallel right shift in the SP dose–response curve (Figure 3). Higher concentrations produced progressive

(16) VanRossum, J. M. Cumulative Dose-Response Curves II. Techniques for the Making of Dose–Response Curves in Isolated Organs and the Evaluation of Drug Parameters. *Int. Pharmacodyn.* **1963**, *143*, 299–330.

Table III. GPI Contractility and in Vivo Data

compd	IC_{50}[b]	pA_2 (95% CL)	slope	rat salivation: ED_{50} (mg/kg, iv)
7a	390 ± 62[c]	7.45 (6.85–8.05)	−0.92	0.48
14[a]	510 ± 100[d]	7.23 (6.84–7.62)	−1.06	1.73
spantide	500 ± 18	6.37 (6.04–6.70)	−0.95	1.25

[a] Figure 1: I; imidazoquinoxaline analogue of **7a**. [b] See Table I for explanation of footnote. [c] See Table I for explanation of footnote. [d] Same as footnote *c* with the exception that five determinants were used in the calculation of IC_{50}.

decreases in ϵ_{max}. The pA_2 value was calculated from Schild analyses (Figure 4) of the contractility data, and the results are summarized in Table III. This pA_2 of 7.42 for compound **7a** is more potent than the calculated pA_2 based on its binding potency. Thus, the IC_{50} of **7a** (390 ± 31 nM), which corresponds to a K_i of 195 (50% of IC_{50}), correlates to a calculated pA_2 value of 6.71. With 95% confidence limits, these pA_2 values differ in potency only by approximately 5-fold. This discrepancy may be due to the basic differences of these two assays like whole tissue/membrane fractions, neuronal/non-neuronal tissue, and physiological/nonphysiological buffers. Compound **7a**, with a pA_2 of 7.42, was as potent an antagonist as the corresponding imidazoquinoxaline 14 (pA_2 = 7.23) and was more potent than the reference peptide antagonist spantide[17] (pA_2 = 6.37).

The in vivo SP antagonist activity of **7a** was determined in rats using the SP-induced salivation[18] and paw edema assays.[19] The ED_{50}'s of this compound (**7a**), the corresponding imidazoquinoxaline analogue (**14**), and spantide are given in Table III. From the data, compound **7a** was found to be more potent than both spantide and 14 in inhibiting SP-induced salivation. In addition, **7a** was found to be active in inhibiting SP-induced paw edema following po administration. The minimum effective dose (MED) for **7a** in the edema assay was 31.6 mg/kg. This was comparable to the MED obtained for the imidazoquinoxaline analogue 14 (31.6 mg/kg). Further investigation of in vivo SP antagonist activity of **7a** revealed that during intravenous (iv) administration at doses of 3 mg/kg, the animals experienced losses of muscle tone, dyspnea, and cyanosis as toxicological effects.

Conclusions

The biochemical and pharmacological data obtained for the naphth-1*H*-imidazoliums indicate that they are NK-1 receptor antagonists. This was confirmed by their inhibitory activities in SP-induced salivation and paw edema assays. Even though their binding potency and tissue contractility were comparable to the corresponding imidazoquinoxalines[4] I (Figure 1), these compounds were approximately 4-fold more potent in the rat salivation assay (Table III). Due to the apparent toxic effects of **7a** in physiological models at doses only slightly higher than the effective pharmacological doses, the therapeutic relevance of this class of SP antagonists could not be assessed. As a result, no further work is currently planned within this series of compounds.

Experimental Section

Melting points were recorded using a Thomas-Hoover or Mel-Temp melting point apparatus and are uncorrected. Proton NMR spectra were obtained in either deuterated dimethyl sulfoxide or deuterated chloroform using a General Electric QE-300 spectrometer. Chemical shifts were reported in ppm downfield from internal standard (tetramethylsilane). Elemental analyses were obtained on all intermediates and are within ±0.4% unless otherwise noted. Final compounds were difficult to analyze by combustion analysis[20] and were characterized from spectroscopic data only (proton NMR, FDMS, and UV–vis spectroscopy), and these were found to be homogeneous by HPLC. Mass spectra were obtained in either electron impact (EI) or field desorption (FD) modes. Ultraviolet–visible spectra for final dyes were obtained in acetonitrile (HPLC grade, Baker) using a Perkin-Elmer Lambda 9 UV/vis/nir spectrophotometer. HPLC retention times were obtained using either a Hewlett-Packard 1090 liquid chromatograph or a Waters 990 3D-Chromatograph. The Hewlett-Packard 1090 liquid chromatograph was equipped with a 25-cm Kodak Supelco column (supelcosil, LC-8), a programmable solvent gradient system and a Hewlett-Packard diode array detector. A tetrabutylammonium phosphate ion pair buffer system served as the mobile phase. Retention times are given for an eluent flow rate of 1.5 mL/min. The Waters 990 3D-Chromatograph was equipped with a 15-cm Hamilton PRP-1 column, a programmable solvent gradient system, and a Hewlett-Packard diode array detector. An ammonium acetate buffer system (pH = 4.65) was used as the mobile phase. Retention times are given for an eluent flow rate of 2.0 mL/min. All reactions were carried out under nitrogen atmosphere. Anhydrous magnesium sulfate was used as a drying reagent, and solutions were concentrated using a rotary evaporator.

2-Methyl-1*H*-naphth[2,3-*d*]imidazole (2). To a stirred solution of 6.32 g (40.0 mmol) of 2,3-diaminonaphthalene (1) in 80 mL of *N*,*N*-dimethylformamide, 8.43 g (52.0 mmol) of triethyl orthoacetate was added dropwise over a 3-min period. The reaction mixture was then treated with a catalytic amount of concentrated sulfuric acid (0.16 mL) and heated at 120 °C for 2 h. Heating was continued at 140 °C for 2 h. The resulting brown solution was cooled to room temperature, poured into 200 mL of ice/water, and neutralized to a pH between 7 and 8 using saturated sodium bicarbonate solution. The precipitated solid was collected by filtration, washed with water, and dried under vacuum yielding 6.92 g of a tan solid. This solid was slurried in 250 mL of boiling dichloromethane and filtered hot, followed by stirring in boiling acetonitrile. The rose-colored solid was collected by filtration and dried to yield 5.0 g (68.7%) of **2**: mp 275 °C dec (lit.[21] mp 286 °C). Anal. ($C_{12}H_{10}N_2 \cdot 0.1H_2O$) C, H, N.

1-Ethyl-2-methyl-1*H*-naphth[2,3-*d*]imidazole (3). To a stirred suspension of 5.61 g (100.0 mmol) of potassium hydroxide in 100 mL of acetone was added 3.64 g (20.0 mmol) of **2** in portions, and stirring was continued at room temperature until the reaction mixture became homogeneous (30 min). The reaction mixture was treated dropwise with 1.7 mL (22.0 mmol) of iodoethane and stirred continually at room temperature for 3 h. The insolubles were removed by filtration, washed thoroughly with acetone, and the combined filtrates were concentrated. The oily brown residue was treated with 200 mL of water and extracted with ethyl acetate (3 × 75 mL). The combined organic layers were washed with saturated sodium chloride, dried, filtered, and concentrated. Crystallization from anhydrous ether yielded 2.26 g (53.8%) of a light brown solid (**3**): mp 91–93 °C (lit.[21] mp 52 °C). Anal. ($C_{14}H_{14}N_2 \cdot 0.1H_2O$) C, H, N.

1,3-Diethyl-2-methyl-1*H*-naphth[2,3-*d*]imidazolium Iodide (4a). A solution of 2.10 g (10.0 mmol) of **3** and 15 mL of iodoethane was heated to 100 °C in an oil bath for 4 h. The slurry was cooled to room temperature and treated with 30 mL of anhydrous ether. The resulting solid was collected by filtration and dried overnight, yielding 3.45 g (94.3%) of a pale yellow solid (**4a**): mp 295 °C dec; ^{1}H NMR (DMSO) δ 1.46 (t, 3 H), 3.02 (s, 3 H), 4.58 (q, 2 H), 7.61 (m, 2 H), 8.13 (m, 2 H), 8.60 (s, 2 H). Anal. ($C_{16}H_{19}IN_2$) C, H, N.

1-Ethyl-3-(2-hydroxyethyl)-2-methyl-1*H*-naphth[2,3-*d*]-imidazolium Iodide (4b). This quaternary salt was prepared as described for **4a** with the following modifications: freshly distilled 2-iodoethanol was used, reaction temperature was 120 °C, final reaction mixture was slurried in acetone yielding a white solid (**4b**) (26.9%): mp 288–290 °C; ^{1}H NMR (DMSO) δ 1.46 (t, 3 H), 3.00 (s, 3 H), 3.88 (m, 2 H), 4.61 (m, 4 H), 5.11 (t br, 1 H), 7.63 (m, 2 H), 8.15 (m, 2 H), 8.57 (s, 1 H), 8.62 (s, 1 H). Anal. ($C_{16}H_{19}IN_2O$) C, H, N.

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1,3-Diethyl-2-[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Iodide (6a). A slurry of 0.55 g (1.5 mmol) of **4a** and 0.67 g (1.5 mmol) of 2-(2-acetanilidovinyl)-1,3,3-trimethyl-3*H*-indolium iodide[7] in 10 mL of acetonitrile was heated to reflux and treated dropwise with 0.27 g (1.8 mmol) of DBU, yielding a bright red-orange, homogeneous solution. The reaction mixture was heated at reflux for 30 min and then cooled to 0 °C overnight. The precipitated solid was collected by filtration, washed thoroughly with ether, and dried, yielding 0.70 g (85.4%) of a fluffy, red solid (**6a**): mp 219–221 °C; ^{1}H NMR (DMSO) δ 1.54 (t, 6 H, 7.15 Hz), 1.61 (s, 6 H), 3.38 (s, 3 H), 4.53 (q, 4 H, 7.25 Hz), 6.07 (d, 1 H, 12.48 Hz), 6.49 (d, 1 H, 14.6 Hz), 7.04 (t, 1 H, 7.29 Hz), 7.13 (d, 1 H, 7.68 Hz), 7.29 (t, 1 H, 7.69 Hz), 7.43 (d, 1 H, 7.47 Hz), 7.56 (m, 2 H), 7.95 (t, 1 H, 13.5 Hz), 8.09 (m, 2 H), 8.39 (s, 2 H); MS *m/e* 422; UV λ_{max} (CH_3CN) = 502.2 nM, ϵ (CH_3CN) = 7.60 × 10^4.

1,3-Diethyl-2-[3-(1,3-dihydro-3,3-dimethyl-1-ethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Iodide (6b). To a stirred solution of 0.21 g (0.5 mmol) of 2-(2-acetanilidovinyl)-3,3-dimethyl-1-ethyl-3*H*-indolium iodide[7] in 5 mL of acetonitrile 0.14 mL (1.5 mmol) of acetic anhydride was added. The resulting mixture was heated to reflux for 3 h. An additional 0.09 mL (1.0 mmol) of acetic anhydride was added, and heating was continued for 1 h. The reaction was cooled to room temperature overnight, yielding the crude acetanilidovinyl intermediate.[7] To this solution 0.18 g (0.5 mmol) of **4a** was added in 1 portion. The mixture was heated to reflux, treated dropwise with 0.09 g (0.6 mmol) of DBU, and heated at reflux for an additional 1.5 h. The reaction mixture was cooled in a refrigerator overnight. The resulting solid was collected by filtration and washed with cold acetonitrile, followed by ether to yield 0.22 g (78.6%) of a fluffy, red-orange solid (**6b**): mp 237–239 °C; ^{1}H NMR (DMSO) δ 1.24 (t, 3 H, 6.96 Hz), 1.55 (t, 6 H, 7.11 Hz), 1.61 (s, 6 H), 3.93 (br q, 2 H, 6.99 Hz), 4.52 (br q, 4 H, 7.04 Hz), 6.15 (d, 1 H, 12.32 Hz), 6.52 (d, 1 H, 14.58 Hz), 7.05 (t, 1 H, 7.43 Hz), 7.13 (d, 1 H, 8.01 Hz), 7.29 (t, 1 H, 7.67 Hz), 7.44 (d, 1 H, 7.57 Hz), 7.55 (m, 2 H), 7.95 (t, 1 H, 13.54 Hz), 8.09 (m, 2 H), 8.38 (s, 2 H); MS *m/e* 436; UV λ_{max} (CH_3CN) = 501.2 nm, ϵ (CH_3CN) = 7.78 × 10^4.

1,3-Diethyl-2-[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Chloride (7a). A solution of 1.1 g (2.0 mmol) of **6a** in 75 mL of methanol was treated with 11.0 g of amberlite IRA-400 chloride ion-exchange resin, and the mixture was allowed to stir at room temperature for 1 h. The resin was removed by filtration and washed with methanol. The combined filtrates were treated with 11.0 g of fresh resin and stirred for 1 h. This process of stirring and filtering was repeated two times. Finally, the filtrate was concentrated, yielding crude **7a** which was purified by suspending the solid in hot toluene and filtering to yield 0.88 g (95.6%) of a fine red solid (**7a**): mp 236–238 °C; ^{1}H NMR (DMSO) δ 1.54 (t, 6 H, 7.13), 1.61 (s, 6 H), 3.39 (s, 3 H), 4.53 (br q, 4 H, 7.05 Hz), 6.08 (d, 1 H, 12.47 Hz), 6.51 (d, 1 H, 14.54 Hz), 7.04 (t, 1 H, 7.36 Hz), 7.13 (d, 1 H, 7.85 Hz), 7.28 (t, 1 H, 7.69 Hz), 7.43 (d, 1 H, 7.13 Hz), 7.56 (m, 2 H), 7.94 (t, 1 H, 13.53 Hz), 8.08 (m, 2 H), 8.39 (s, 2 H); MS *m/e* 422; UV λ_{max} (CH_3CN) = 498.8 nm, ϵ (CH_3CN) = 7.56 × 10^4. HPLC retention time = 24.57 min (Hewlett-Packard system).

1,3-Diethyl-2-[3-(1,3-dihydro-3,3-dimethyl-1-ethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Chloride (7b). This compound was prepared by the method outlined for **7a** and was recrystallized from acetonitrile/anhydrous ether, yielding 0.75 g (88.2%) of a red solid (**7b**): mp 160–163 °C; ^{1}H NMR (DMSO) δ 1.24 (t, 3 H, 7 Hz), 1.55 (t, 6 H, 7.12 Hz), 1.61 (s, 6 H), 3.93 (br q, 2 H, 7.12 Hz), 4.52 (br q, 4 H, 6.96 Hz), 6.15 (d, 1 H, 12.4 Hz), 6.52 (d, 1 H, 14.5 Hz), 7.05 (t, 1 H, 7.4 Hz), 7.13 (d, 1 H, 7.9 Hz), 7.29 (t, 1 H, 7.71 Hz), 7.44 (d, 1 H, 7.2 Hz), 7.56 (m, 2 H), 7.95 (t, 1 H, 12.8 Hz), 8.08 (m, 2 H), 8.38 (s, 2 H); MS *m/e* 436; UV λ_{max} (CH_3CN) = 502.2 nm, ϵ (CH_3CN) = 7.60 × 10^4; HPLC retention time = 11.14 min (Waters system).

3-(2-Acetoxyethyl)-1-ethyl-2-[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Iodide (6c). To a stirred slurry of 0.11 g (0.3 mmol) of **4b** in 3 mL of acetonitrile, 0.06 g (0.6 mmol) of acetic anhydride was added in 1 portion. The resulting mixture was heated at reflux until the reaction mixture became homogeneous. At this point 0.13 g (0.3 mmol) of 2-(2-acetanilidovinyl)-1,3,3-trimethyl-3*H*-indolium iodide[7] was added in 1 portion followed by the dropwise addition of 0.10 g (0.66 mmol) of DBU. The resulting red-orange solution was heated at reflux for an additional 1.5 h and then cooled to 0 °C. The precipitated solid was collected by filtration, washed with anhydrous ether, and dried under vacuum, yielding 0.12 g (66.7%) of a fluffy, orange solid (**6c**): mp 248–250 °C; ^{1}H NMR (DMSO) δ 1.60 (m, 9 H), 1.83 (s, 3 H), 3.38 (s, 1 H), 4.53 (br m, 4 H), 4.77 (br t, 2 H), 6.03 (d, 1 H), 6.52 (d, 1 H), 7.03 (t, 1 H), 7.13 (d, 1 H), 7.28 (t, 1 H), 7.43 (d, 1 H), 7.77 (m, 2 H), 7.98 (t, 1 H), 8.10 (br m, 2 H), 8.38 (s, 1 H), 8.41 (s, 1 H); MS *m/e* 480; HPLC retention time = 10.75 min (Waters system).

3-(2-Acetoxyethyl)-1-ethyl-2-[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Chloride (7c) and 1-Ethyl-3-(2-hydroxyethyl)-2-[3-(1,3-dihydro-1,3,3-trimethyl-2*H*-indol-2-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Chloride (7d). Ion exchange of **6c** was achieved using the procedure outlined for **7a**. Proton NMR of the crude product showed it to be a 2:1 mixture of acylated and nonacylated dyes, respectively. This mixture (0.55 g, 1.2 mmol) was slurried in 8 mL of acetonitrile, heated to reflux, and treated dropwise with 0.11 mL (1.2 mmol) of acetic anhydride. The resulting mixture was heated at reflux for an additional 1 h and then cooled to 0 °C. The precipitated solid was collected by filtration, washed with ether, and dried, yielding 0.18 g (31.6%) of a fine orange solid (**7d**): mp 226–228 °C; ^{1}H NMR (DMSO) δ 1.53 (t, 3 H), 1.61 (s, 6 H), 3.38 (s, 3 H), 4.06 (br m, 2 H), 4.55 (br m, 4 H), 5.48 (br m, 1 H), 6.02 (d, 1 H), 6.48 (d, 1 H), 7.03 (t, 1 H), 7.12 (d, 1 H), 7.27 (t, 1 H), 7.43 (d, 1 H), 7.57 (m, 2 H), 8.10 (br m, 2 H), 8.38 (m, 3 H); HPLC retention time = 13.56 min (Hewlett-Packard system). The filtrate was concentrated, and the residue was redissolved in 10 mL of acetonitrile and treated with 3 drops of DBU to decompose any excess acetic anhydride. The solution was then treated with anhydrous ether to the cloud point, chilled, and filtered, yielding 0.32 g (51.6%) of a fine orange solid (**7c**): mp 200–202 °C; ^{1}H NMR (DMSO) δ 1.60 (m, 9 H), 1.83 (s, 3 H), 3.40 (s, 3 H), 4.53 (br m, 4 H), 4.78 (br t, 2 H), 6.07 (d, 1 H), 6.53 (d, 1 H), 7.05 (t, 1 H), 7.15 (d, 1 H), 7.30 (t, 1 H), 7.45 (d, 1 H), 7.57 (m, 2 H), 7.98 (t, 1 H), 8.10 (br m, 2 H), 8.40 (s, 1 H), 8.42 (s, 1 H); HPLC retention time = 16.83 min (Hewlett-Packard system).

1,3-Diethyl-2-[3-(1-methylbenz[*cd*]indol-2(1*H*)-ylidene)-1-propenyl]-1*H*-naphth[2,3-*d*]imidazolium Chloride (9). A slurry of 0.31 g (1.5 mmol) of [1-methylbenz[*cd*]indol-2-(1*H*)-ylidene]acetaldehyde (**8**)[9-11] and 1,3-diethyl-2-methyl-1*H*-naphth[2,3-*d*]imidazolium iodide (**4a**) in 10 mL of acetic anhydride was stirred at 130 °C in an oil bath for 1.5 h. The resulting mixture was cooled to room temperature, and the precipitated solid was collected by filtration, dried, and recrystallized from 50 mL ethanol, yielding 0.53 g (63%) of a dark red solid. This iodide salt was converted to the corresponding chloride salt via the procedure outlined for **7a**. Crystallization induced from ethanol/ether yielded 0.30 g (71.4%) of a fine, dark blue solid (**9**): mp 265–268 °C dec; ^{1}H NMR (DMSO) δ 1.60 (t, 6 H), 3.58 (s, 3 H), 4.63 (br m, 4 H), 6.58 (d, 1 H), 6.93 (d, 1 H), 7.10 (d, 1 H), 7.46 (m, 2 H), 7.60 (m, 2 H), 7.77 (t, 2 H), 7.97 (d, 1 H), 8.13 (m, 3 H), 8.33 (t, 1 H), 8.50 (s, 1 H); MS *m/e* 430; HPLC retention time = 24.38 min (Hewlett-Packard system).

Substance P Receptor Binding Method. ^{125}I-Bolton Hunter-Substance P (^{125}I-BH-SP 2200 Ci/mmol) was purchased from New England Nuclear. Receptor binding was performed according to Park et al.[15]

The forebrain (whole brain minus cerebellum) of a male Sprague–Dawley rat was homogenized in 20 volumes of ice-cold 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl), pH 7.4, at 4 °C, 5 mM KCl, and 120 mM NaCl (wash buffer) with a Tekmar Tissumizer Mark II. The homogenate was centrifuged at 48,000*g* for 10 min, and then the resulting pellet was resuspended in 50 mM Tris·HCl, pH 7.4, at 4 °C, 10 mM ethylenediaminetetraacetic acid (EDTA), and 300 mM KCl, and incubated for 30 min at 4 °C. The homogenate was then centrifuged as above and centrifuged twice more in 50 mM Tris·HCl (pH 7.4) at 25 °C. The final pellet was resuspended in 60 volumes of Tris buffer.

The binding assay mixture, 0.25 mL, contained 75–125 μg of membrane protein, 0.1 nM ^{125}I-BH-SP, test compound in 50 mM Tris·HCl, pH 7.4 at 25 °C, 0.02% bovine serum albumin, 2 μg/mL

chymostatin, 4 μg/mL leupeptin, 40 μg/mL bacitracin, and 1.2 mM $MnCl_2$. Nonspecific binding was defined with 1 μM SP. All assays were run in duplicate or triplicate, and the reaction mixtures were incubated 20 min at 25 °C. The assay mixtures were then diluted with 2 mL of wash buffer and filtered through GF/C glass fiber filters presoaked in 0.01% polyethylenimine. The filters were washed eight times more with 2 mL of wash buffer. The radioactivity trapped on the filters was counted in a Packard Cobra gamma counter. The competition curve data were analyzed by computer nonlinear least squares best fit of the data to the Hill equation which determines the IC_{50} values from at least seven concentrations of the test compound (1×10^{-10} M to 1×10^{-5} M).

Isolated Guinea Pig Ileum Contractility Method. Hartley guinea pigs of either sex (250–500 g) were killed by CO_2 inhalation. The point at which the terminal ileum enters the cecum was located. The ileum was cut, and a section approximately 30 cm in length was removed. The ileum was dissected free of fat and connective tissue, and the terminal 5 cm was discarded. Individual sections, approximately 2.0 cm in length, were then cut beginning at the terminal end and suspended in a jacketed, 10-mL, siliconized, glass organ bath containing Tyrode's solution of the following composition in mM: 136.9 NaCl, 2.7 KCl, 0.98 $MgCl_2$, 1.80 $CaCl_2$, 0.47 $NaH_2PO_4H_2O$, 11.9 $NaHCO_3$, 5.5 dextrose. The Tyrode's solution was supplemented with 10^{-6} M indomethacin, pyrilamine, and atropine, and bacitracin (40 mg/L). The tissues were maintained at 37 °C and gassed with 95% O_2–5% CO_2. A resting tension of 1 g was applied to the tissue, and the preparations were allowed to equilibrate for 60 min with washes every 15 min. Isometric contractions were recorded using Grass FT-03 force displacement transducers attached to a Grass Model 7E polygraph.

A submaximal concentration (10 nM) of the agonist SP was then added to each tissue, and the individual response was recorded. This was repeated every 15 min until a reproducible response was obtained (usually three to four additions). Thirty minutes following stimulation of the tissues, cumulative dose–response curves for SP were constructed according to the method of Van Rossum.[16] Tissues were then washed and allowed to restabilize for 30–45 min with washing every 10 min. Test compounds were then added to the tissues 3 min before repeating the cumulative dose–response curves. A shift to the right of the SP dose–response without a significant reduction of the maximal contractile response suggested competitive antagonism of SP by the test compound.

Rat Salivation Method. Fed male Sprague–Dawley rats (200–300 g) were weighed and placed into groups of six using random number tables generated by the SAS program, Proc Plan. Conscious rats were injected in vivo, via the lateral tail vein, with either the vehicle (0.9% saline w/v) or sialogogue. The volume of injection for the vehicle and compounds was 1.0 mL/kg. Compounds were injected on a μg/kg basis. The group of rats injected with saline (0.9% w/v) served as the baseline control. The rat's oral cavity was swabbed immediately after in vivo injection by placing and holding a preweighed absorbant foam cube (5/16 in., Texwipe Company, Upper Saddle River, NJ) sublingually for 10 sec using a Triceps foam pencil (Texwipe Company, Upper Saddle River, NJ). Foam cubes were reweighed immediately after use. The difference between the initial weight of the cube and the weight of the cube after use represents saliva secreted.

Data were analyzed with Dunnett's *t*-test[22] that compares several treated groups with a control group. Regression analysis was used to determine dose–response and relative potency.[23]

Substance P Induced Paw Edema Method. Fasted male Sprague–Dawley rats were randomized into groups of six. The volume of the hind paw of each rat was determined plethysmographically by means of a mercury filled vessel connected to a pressure transducer (Grass p23A) that in turn was connected to strip chart recorder (linear, Cole Parmer Model No. 55). The instrument was calibrated so that 1 mL of fluid produced a pen deflection of 25 mm. The test compounds were dissolved or suspended in aqueous 0.5% Tween 80. Sonification was used to facilitate solubilization or reduce particle size. Rats were dosed orally with the tween 80 vehicle or test compounds 1 h prior to the subcutaneous injection of 0.1 mL of a solution containing 100 μg/mL of SP (Cambridge Research Biochemicals, Cambridge, MA) in 0.9% saline. Fifteen minutes after SP injection the hind paw volume was measured as described, and edema was determined by the difference in the two volume measurements. Results were expressed as edema (mL). The data were analyzed with Dunnett's *t*-test.[22] Regression analysis was used to determine dose–response.[23] The protocol for this experiment was reviewed by the animal care review committee at Eastman Kodak Co. and all animal care and use associated with this procedure meets NIH guidelines.

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