Imidazo[4,5-*b*]quinoxaline Cyanines as Neurokinin Antagonists

Substance P (SP) is an undecapeptide neuromodulator that belongs to the neurokinin (NK) family, which includes the structurally related neurokinin A (NKA) and neurokinin B (NKB). On the basis of relative potencies of these agonists, three NK receptors were proposed, generally referred to as NK-1, NK-2, and NK-3.[1] Recently, three neurokinin receptors have been cloned and sequenced, which appears to validate this classification.[2] The role of these NK receptors in the pathophysiology of disease is still ill-defined, mainly due to the lack of potent, selective, and bioavailable NK antagonists. The antagonists described to date have generally involved modifications of the peptide structure of the various neurokinins, except for the recently described non-peptide quinuclidines.[3] Advances have been made in selectivity for the three NK receptors, but the bioavailability of these peptide antagonists has been the limiting factor. Thus, a receptor binding screen for potential NK antagonists from the chemical files was initiated. In particular, on the basis of the purported involvement of substance P in pain and inflammation, the biochemical screen focused on the NK-1 receptor.[4,5]

As an example of these screening results, the imidazo[4,5-*b*]quinoxaline cyanines are being reported as nonpeptide NK antagonists. The general synthesis of these compounds (Scheme I) involved displacement of 2,3-dichloroquinoxaline by ethylamine in a dipolar aprotic solvent at room temperature to give monosubstituted product 1, or by excess ethylamine at elevated temperatures to give diethyl substituted product **2** (R = C_2H_5). Monoamine 1 was treated with an alternate amine to give unsymmetrical diamines **2**. The diamines were cyclized with triethyl orthoacetate and *p*-TSOH to give quaternary intermediates **3**.[6] These versatile intermediates could be condensed with formylmethylene heterocycles (**6**) to give, after counterion exchange, the targeted cyanine compounds **5**. Alternatively, homologation of **3** under Vilsmeyer conditions ($POCl_3$, DMF) gave the (formylmethylene)imidazo[4,5-*b*]quinoxalines **4**, which were then reacted with quaternary heterocycles **7** to give, after counterion exchange, **5**.[7]

The NK-1 receptor binding assay was performed as described by Park et al. using rat forebrain tissue.[8] The

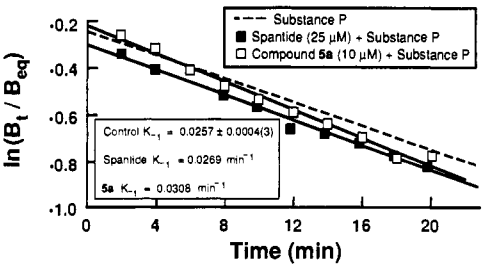

Figure 1. The effect of excess spantide or excess compound **5a** on the apparent dissociation rate of SP.

IC_{50} values were calculated from seven-point displacement curves which were run in duplicate and are reported in Table I. It was also desirable to assess the reversibility and competitiveness of this receptor interaction. The reversibility was assessed by a Scatchard analysis experiment in which increasing concentrations of test compound were used to measure the apparent affinity constant (K_d) and the number of binding sites (B_{max}) for SP. This analysis of **5a** indicated a concentration-dependent increase in the apparent K_d for SP, with no change in the B_{max} (Table II). This suggests that there is a fully reversible interaction of **5a** with the NK-1 receptor. In order to test the competitiveness of the receptor interaction, kinetic analyses of the dissociation rates for SP were investigated in the presence and absence of test compound. In Figure 1, the apparent dissociation rate (slope) of SP was uneffected by the presence of excess spantide, a known competitive SP antagonist.[9] Similar effects on the dissociation rate were also observed in the presence of excess **5a** (Figure 1). The lack of effect of **5a** on the dissociation rate of SP is indicative of a competitive interaction with the receptor.

The structure–activity relationships for receptor binding within this series of compounds were not dramatic, as shown in Table I.[10] The 6,7-dichloro substitution of the imidazo[4,5-*b*]quinoxaline system (**5b**) showed less than a 2-fold decrease in binding potency compared to that of unsubstituted **5a**. Likewise, unsymmetrical N-substituted analogues (**5c–h**), containing various tethered functional groups, produced at best an equivalent binding potency with **5a**, indicating an inability to discover additional specific binding interactions with the receptor. The most dramatic potency effects were observed among the heterocyclic variations (**5i–o**). Substitution of the indole nitrogen with functionalized alkyl groups (**5j,k**) led to decreased potencies relative to **5a** and **5i**. Other conjugated nitrogen heterocycles besides the indole were also active (**5l,m**), with the aromatic benzo moiety showing increased potency (**5m** vs **5n**). This is additionally demonstrated by the inactivity of hemicyanine **5o**. These latter two examples (**5n,o**) may indicate the necessity of a weakly basic nitrogen (via aryl and/or heteroatom conjugation) for interaction with the receptor.

Selectivity was assessed at both neurokinin and nonneurokinin receptors. In particular, **5a** showed only muscarinic 1 activity (IC_{50} = 320 nM, n = 2), while no activity was observed at concentrations of ca. 10 μM in the following receptor binding assays: α_1, α_2, and β adrenergic,

(1) Regoli, D.; Drapeau, G.; Dion, S.; Couture, R. New selective agonists for neurokinin receptors: pharmacological tools for receptor characterization. *Trends Pharm. Sci.* 1988, 290–295.
(2) Shigemoto, R.; Yokota, Y.; Tsuchida, K.; Nakanishi, S. Cloning and Expression of a Rat Neuromedin K Receptor cDNA. *J. Biol. Chem.* 1990, *265*, 623–628, and references cited therein.
(3) (a) Lowe, J. A., III WO Patent 9005525 (Pfizer, Inc.), 1990. (b) 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 Nonpeptide Antagonist of the Substance P (NK_1) Receptor. *Science* 1991, *251*, 435–437.
(4) Otsuka, M.; Yanagisawa, M. Effect of a Tachykinin Antagonist on a Nociceptive Reflex in the Isolated Spinal Cord Preparation of the Newborn Rat. *J. Physiology* 1988, *395*, 255–270.
(5) Mantyh, P. W.; Catton, M. D.; Boehmer, C. G.; Welton, M. L.; Passaro, E. P., Jr.; Maggio, J. E.; Vigna, S. R. Receptors for Sensory Neuropeptides in Human Inflammatory Diseases: Implications for the Effector Role of Sensory Neurons. *Peptides* 1989, *10*, 627–645.
(6) Brooker, L. G. S.; Van Lare, E. US Patent 3,431,111 (Eastman Kodak Co.), 1969.
(7) Mee, J. D.; Heseltine, D. W. US Patent 3,723,419 (Eastman Kodak, Co.), 1973.
(8) Park, C. H.; Massari, V. J.; Quirion, R.; Tizabi, Y.; Shults, C. W.; O'Donohue, T. L. Characteristics of ^{3}H-Substance P Binding Sites in Rat Brain Membranes. *Peptides* 1984, *5*, 833–836.
(9) Folkers, K.; Hakanson, R.; Horig, J.; Jie-Cheng, X.; Leander, S. Biological Evolution of Substance P Antagonists. *Br. J. Pharmacol.* 1984, *83*, 449–456.
(10) All new compounds have spectral data (^{1}H NMR and mass spectra) supporting their structural assignment.

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Scheme I

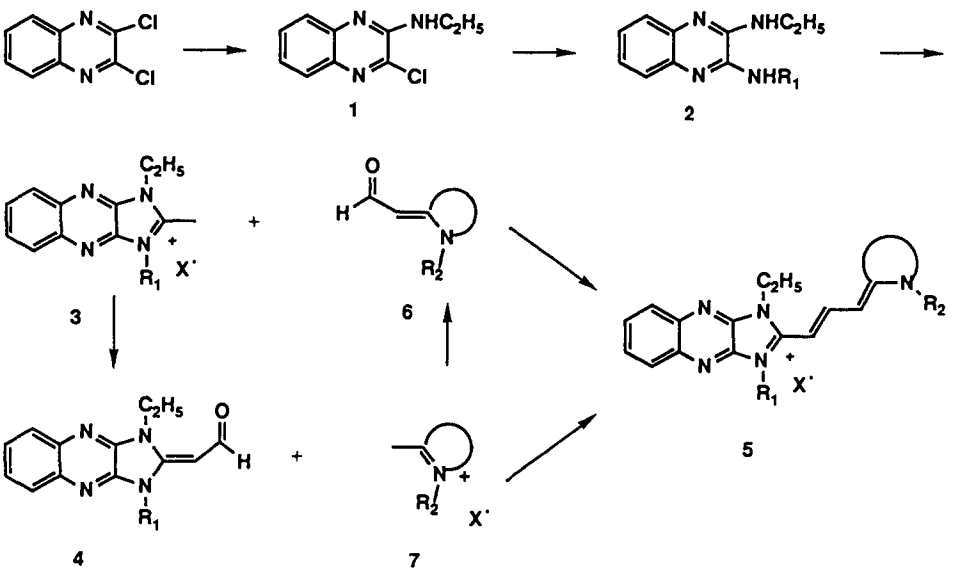
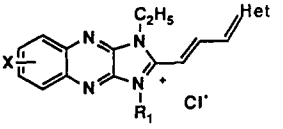
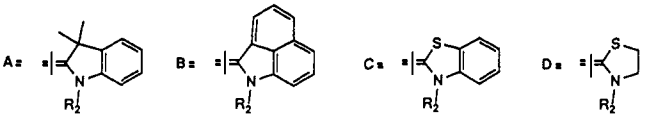


Table I. Displacement of [^{125}I]BHSP from Rat Forebrain by Imidazo[4,5-*b*]quinoxaline Cyanines



no.	X	R_1	Het	R_2	IC_{50}, nM; mean ± SEM (for 3 or *n*)
5a	H	C_2H_5	A	CH_3	510 ± 100 (5)
5b	6,7-Cl_2	C_2H_5	A	CH_3	1100 ± 350
5c	H	CH_2CONH_2	A	CH_3	2000 ± 680 (4)
5d	H	$(CH_2)_3OAc$	A	CH_3	410 ± 100
5e	H	$(CH_2)_3OH$	A	CH_3	350 ± 15
5f	H	$(CH_2)_3CO_2C_2H_5$	A	CH_3	850 ± 390
5g	H	$(CH_2)_3COOH$	A	CH_3	1400 ± 350
5h[a]	H	$(CH_2)_3N(CH_3)_2$	A	CH_3	1300 ± 250
5i	H	C_2H_5	A	C_2H_5	390 ± 26
5j[b]	H	C_2H_5	A	$(CH_2)_2OH$	1000 ± 330
5k	H	C_2H_5	A	$CH_2CON(CH_3)_2$	2600 ± 1000
5l	H	C_2H_5	B	CH_3	850 ± 85
5m	H	C_2H_5	C	CH_3	700 ± 15
5n[b]	H	C_2H_5	D	CH_3	1600 ± 360
5o[b]	H	C_2H_5			>10,000
SP					0.12 ± 0.06 (6)
spantide					500 ± 18



[a] The di-*p*-toluenesulfonate salt was prepared and tested. [b] The iodide salt was prepared and tested.

Table II. Scatchard Analysis of **5a**

concn of **5a**, μM	K_d, nM	B_{max}, fmol/mg
0	0.18, 0.15	31, 28
1.0	0.40, 0.97	39, 33
3.0	0.88, 1.49	38, 35
10.0	2.06, 3.00	35, 27

H_1 histamine, nicotinic, angiotensin II, phorbol ester, serotonin, excitatory amino acid, bradykinin, opiate, leukotriene B_4, diltiazem, bombesin, adenosine, dopamine, vasoactive intestinal peptide, phencyclidine, Arg-vasopressin, and epidermal growth factor.[11] Compound **5a** was less active in binding at the NK-2 receptor ([^{125}I]NKA in rat duodenum, IC_{50} = 1700 nM (*n* = 2)) and at the NK-3 receptor ([^{125}I]eledoisin in rat cortex, IC_{50} = 3300 nM (*n* = 2)).[12,13]

The activity of **5a** in the guinea pig ileum (gpi) contractility assay was used to determine agonist and antag-

(11) The receptor profiling was performed under contract at Nova Pharmaceutical.

(12) The NK-2 assay was performed as described: Bergstrom, L.; Beaujouan, J. C.; Torrens, Y.; Saffroy, M.; Glowinski, J.; Lavielle, S.; Chassaing, G.; Marquet, A.; D'Orleans-Juste, P.; Dion, S.; Regoli, D. ^{3}H-Neurokinin A labels a specific Tachykinin-Binding Site in the Rat Duodenal Smooth Muscle. *Mol. Pharmacol.* **1987**, *32*, 764–771.

(13) The NK-3 assay was performed under contract at Nova Pharmaceutical.

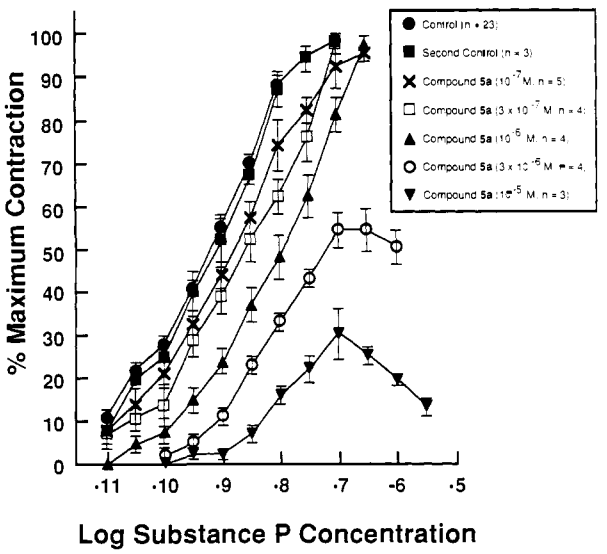


Figure 2. The effect of compound **5a** on Substance P induced contractions of guinea pig ileum.

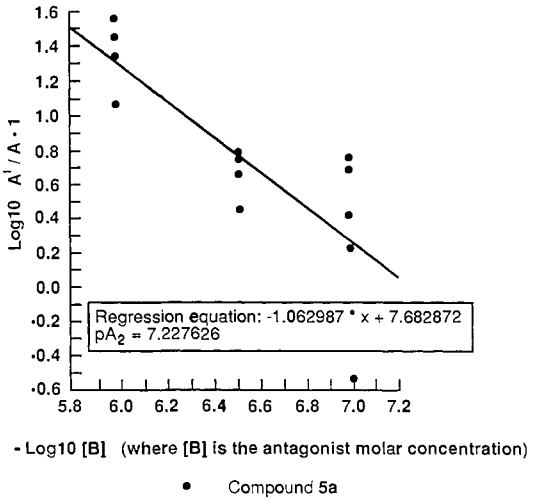


Figure 3. Schild plot of compound **5a.**

onist activities.[14] The guinea pig ileum was pharmacologically blocked with inhibitors of other spasmogens, such as prostaglandins (indomethacin), histamine (pyrilamine), or acetylcholine (atropine). At a concentration of 1 μM, atropine inhibits any indirect (NK-3)[1] muscarinic effects of the test compound, without inhibiting the NK-1 effects of SP.[15] In the absence of atropine, no spasmogenic (agonist) effect for compound **5a** due to its known NK and/or M_1 activities has been observed up to a concentration of 1 μM. Tissue pretreatment with **5a** caused an attenuation of the SP-induced contraction of gpi. A series of concentration–response curves to SP in the presence of increasing concentrations of test compound is illustrated in Figure 2. A concentration-dependent parallel, right shift from 10^{-7} to 10^{-6} M range was observed. At higher concentrations, a progressive decrease in ϵ_{max} was observed, indicative of an additional nonspecific activity. A Schild plot of these data (Figure 3) over the concentration range 10^{-7}–10^{-6} M resulted in a significant regression with slope of −1.06 and a pA_2 of 7.23 (95% confidence limits = 6.84–7.62). The specificity of this compound was tested against histamine and leukotriene D_4 induced gpi contractions. Only a nonspecific decrease in ϵ_{max} was observed with these spasmogens at concentrations greater than 10^{-6} M.

By virtue of their biochemical and pharmacological properties, it appears that members of the imidazo[4,5-*b*]quinoxaline cyanines act as competitive NK-1 antagonists. However, due to the pronounced toxic effects (loss of muscle tone, dyspnea, and cyanosis) noted at a dose (3 mg/kg iv) only slightly higher than the pharmacological dose (minimum effective dose = 1 mg/kg iv) in the rat SP-induced salivation and paw edema models[16,17] (both non-cholinergic effects[18,19]), the therapeutic relevance of NK antagonism in physiological models could not be assessed. Nevertheless, this class of compounds may provide structural information useful for the design of other nonpeptide NK antagonists.

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Supplementary Material Available: Experimental procedures for selected compounds and analytical data, including NMR values, for compound **5** (6 pages). Ordering information is given on any current masthead page.

(14) The gpi assay utilized Hartley guinea pig (M/F) terminal ileum suspended in siliconized, glass organ bath containing Tyrode's solution [(mM) 136.9 NaCl, 2.7 KCl, 0.98 $MgCl_2$, 1.80 $CaCl_2$, 0.47 $NaH_2PO_4 \cdot H_2O$, 11.9 $NaHCO_3$, 5.5 dextrose] 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 with a resting tension of 1 g. The cumulative dose–response curves for SP were constructed according to Van Rossum: Van Rossum, J. M. Cummulative Dose-Response Curves II. Techniques for the making of dose–response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.* **1963**, *143*, 299–330.

(15) Jacoby, H. I.; Lopez, I.; Wright, D.; Vaught, J. L. Differentiation of multiple neurokinin receptors in the guinea pig ileum. *Life Sci.* **1986**, *39*, 1995–2003.

(16) Wagner, L. E.; Tomczuk, B. E.; Yanni, J. M. Measurement of Tachykinin-Induced Salivation in Conscious Rats. *J. Pharmacol. Methods*, in press.

(17) Devor, M.; Papir-Kricheli, D.; Nachmias, E.; Rosenthal, F.; Gilon, C.; Chorev, M.; Selinger, Z. Substance P-induced cutaneous plasma extravasation in rats is mediated by NK1 tachykinin receptors. *Neurosci. Lett.* **1989**, *103*, 203–208.

(18) Martinez, J. R.; Martinez, A. M. Stimulatory and Inhibitory Effects of Substance P on Rat Submandibular Secretion. *J. Dent. Res.* **1981**, *60*, 1031–1038.

(19) Couture, J. L.; Drapeau, R.; Regoli, D. Capillary Permeability Induced by Intravenous Neurokinins-Receptor Characterization and Mechanism of Action. *Naunyn-Schmeideberg's Arch. Pharmacol.* **1989**, *340*, 170–179.

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