Martinelline and Martinellic Acid, Novel G-Protein Linked Receptor Antagonists from the Tropical Plant *Martinella iquitosensis* (Bignoniaceae)

Keith M. Witherup,[*,†] Richard W. Ransom,[‡] Amy C. Graham,[§] Aurora M. Bernard,[§] Michael J. Salvatore,[§] William C. Lumma,[†] Paul S. Anderson,[||] Steven M. Pitzenberger,[†] and Sandor L. Varga[*,†]

Contribution from the Departments of Medicinal Chemistry and New Lead Pharmacology, Merck Research Laboratories, West Point, Pennsylvania 19486, and the Department of Antibiotic Discovery and Development, Merck Research Laboratories, Rahway, New Jersey 07065

Received February 9, 1995[⊗]

Abstract: Two novel pyrroloquinoline alkaloids, martinelline (**1**) and martinellic acid (**2**), were isolated from an organic extract of *Martinella iquitosensis* roots. The ethnobotanical use of the two *Martinella* species as an eye medication in South America may be explained in part by the presence of **1** and to a lesser extent **2**; this study relates the bradykinin (BK) B_1 and B_2 receptor antagonist activity of **1** and **2** as well as the affinity of **1** for histaminergic, α_1-adrenergic, and muscarinic receptors. **1** and **2** represent the only naturally occurring nonpeptide BK antagonists reported to date. The antibiotic potential, cytotoxicity, and lytic activity were also evaluated for these compounds.

Introduction

Martinella (Bignoniaceae) is a tropical genus consisting of two species, *M. iquitosensis* A. Sampaio and *M. obovata* (HBK.) Bur. & K. Schum. Their chemistry is relatively unexplored, although much has been reported for other Bignoniaceae members.[1–5] Records on the use of *Martinella* as an eye medication originate in over 13 different ethnolinguistic groups from eight South American countries.[6] A predominance of information regarding its use comes from Amazon Indian tribes of Peru, where the liana *M. obovata*, uniformly called "yuquilla", is often cultivated as the preferred treatment for eye ailments. In general, the thick fleshy root bark, with the rough outside part scraped off, is pounded and the resultant juice strained through cloth. One or two drops of this juice placed into the eyes is said to have an immediate effect on inflammation and will eventually cure conjunctivitis, a condition often caused by infection from one or several microorganisms, including Gram-positive and Gram-negative bacteria.[7] The only other ethnobotanical report concerns the use of *M. obovata* bark, together with the leaves of *Ambelania lopezii* (Apocynaceae) and the bark of *Distictella racemosa* (Bignoniaceae), for concocting an arrow poison by the Barasana Indians of Colombia.[8] Resultant to our search for bradykinin (BK) receptor antagonists from natural sources, we became interested in the organic extract of *M. iquitosensis*. The aqueous extract counterpart, which is prepared after the organic solvent extract, was inactive at the initial screening concentration of 100 μg/mL. This latter extract was therefore abandoned. Utilizing the BK assay to guide fractionation, the activity was subsequently localized to the MeOH solubles obtained by solid phase extraction of the organic CH_2Cl_2/MeOH extract (see Experimental Section). Preparative HPLC of this fraction yielded two compounds—martinelline (**1**) and martinellic acid (**2**). **1** (major component, 47% at 330 nm) and **2** (minor component, 18% at 330 nm) gave preliminary IC_{50} values of 10 and >25 μg/mL, respectively.

We report here the isolation and structural proofs for **1** and **2** (Figure 1) and discuss the potential medicinal properties thereof. To the best of our knowledge, this type of pyrroloquinoline ring system has not been previously identified in a natural product. Furthermore, **1** and **2** represent the only nonpeptide natural products identified as BK receptor antagonists. A synthetic class of nonpeptide antagonists has been reported.[9] The methyl ester of martinellic acid (**3**) was prepared to appraise the effect on receptor binding of charged (carboxylate) versus uncharged (ester) species. Since the extracts are used to treat conjunctivitis, **1** and **2** were also examined for their antibiotic as well as cytotoxic and lytic activity.

Results and Discussion

^{13}C-NMR spectroscopy revealed **1** to be a trifluoroacetic acid salt with 33 carbons for the conjugate base and 2 carbons (quartets at 158.3 and 117.1 ppm) for trifluoroacetic acid (TFA). Quantitative ^{19}F-NMR showed that 2.8 equiv of TFA were present, suggesting the presence of three basic sites in the parent molecule. The carbons of **1** can be grouped into the following

† Department of Medicinal Chemistry.
‡ Department of New Lead Pharmacology.
|| Present address: DuPont Merck Pharmaceutical Co., Experimental Station, Wilmington, DE 19880-0353.
§ Department of Antibiotic Discovery and Development.
⊗ Abstract published in *Advance ACS Abstracts*, June 1, 1995.

(1) Ngouela, S.; Tsamo, E.; Sondengam, B. L. *Planta Med.* **1988**, *54*, 476.
(2) Imakura, Y.; Kobayashi, S.; Yamahara, Y.; Kihara, M.; Tagawa M.; Murai, F. *Chem. Pharm. Bull.* **1985**, *33*, 2220.
(3) Satyavathi, M.; Radhakrishnaiah, M.; Narayana, L. L. *Curr. Sci.* **1984**, *53*, 711.
(4) Da Silveira, J.; Gottlieb, O.; De Oliveira, G. *Phytochemistry* **1975**, *14*, 1829.
(5) Duarte Weinberg, M.; Gottlieb, O.; De Oliveira, G. *Phytochemistry* **1976**, *15*, 570.
(6) Gentry, A.; Cook, K. *J. Ethnopharmacol.* **1984**, *11*, 337.
(7) Boralkar, A.; Dindore, P.; Fule, R.; Bangde, B.; Albel, M.; Saoji, A. *Indian J. Ophthalmol.* **1989**, *37*, 94.
(8) Schultes, R. *Bot. Mus. Leafl., Harv. Univ.* **1970**, *22*, 345.
(9) Sawutz, D.; Salvino, J.; Dolle, R.; Casiano, F.; Ward, S.; Houck, W.; Faunce, D.; Douty, B.; Baizman, E.; Awad, M.; Marceau, F.; Seoane, P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4693.

0002-7863/95/1517-6682$09.00/0 © 1995 American Chemical Society

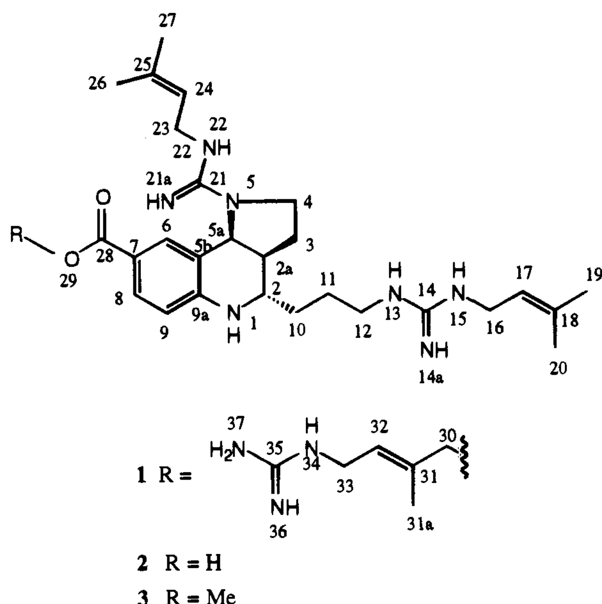
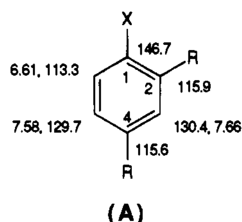


Figure 1. General structure of martinelline and martinellic acid. This numbering system is used for the NMR data.

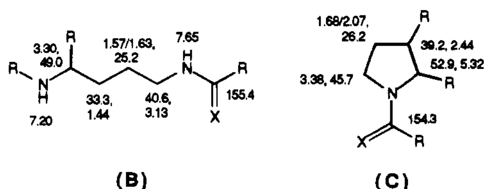
types: 10 quaternary (all in the aromatic/carbonyl regions), 3 aromatic CH, 3 CH_2, 6 CH_2X, 3 CHX, 3 olefinic CH, and 5 CH_3. From the results of carbon–proton correlation data, there are 42 carbon-bound protons. Integration of the 1D proton spectrum suggests at least 10 exchangeable proton resonances are present in the salt.

The combined results of (1) the proton 1D spectrum, (2) $^1H-^1H$ COSY, and (3) a proton-detected carbon–proton correlation experiment (HMQC) showed six cascades of protons. The first and most obvious grouping of protons belongs to a 1,2,4-trisubstituted aromatic ring (see structure A). The upfield shift



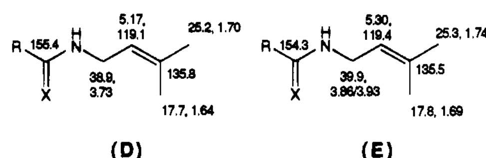
(6.61 ppm) of the proton ortho to the 1-substituent in this pattern is characteristic of either oxygen or nitrogen substitution in the 1-position. The relative downfield shifts of the remaining two protons show that a carbonyl or carbonyl-like function resides at the 4-position of the aromatic ring.

Two proton spin systems are found on 4-carbon fragments which have heteroatom substituents attached to the end carbons. From the chemical shifts of the end carbons, it is likely that the heteroatoms are nitrogens. The spin systems differ in that one is clearly part of an aliphatic chain with three sequential CH_2 units and a terminal methine (see structure B). The other spin



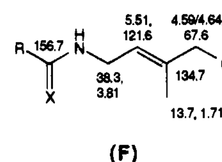
system is part of a five-membered ring where the end heteroatoms are actually a common nitrogen atom (see structure C below). The ring structure was suggested by an NOE which was observed between the methine and the methylene at the opposite end of the fragment. The ring structure was further corroborated by a long-range carbon–proton correlation (HMBC experiment) from the terminal methine proton to the methylene carbon at the other end.

The next two spin systems were found to belong to isoprenyl derivatives which differed only slightly in their chemical shifts (see structures D and E). Carbon chemical shifts and proton



couplings indicated that the attachment to each isoprenyl group is via an NH. The methyl groups of each isoprenyl group were uniquely identified by NOE enhancements with neighboring protons.

The final proton spin system is an isoprenyl derivative. As shown in structure F, one of the methyl groups was found to



be substituted with a heteroatom. The chemical shift of the carbon is indicative of oxygen substitution. The olefin was determined to be in the *E* configuration from NOE data.

The above fragments were assembled with the aid of proton-detected long-range carbon–proton correlation (HMBC) and 2D NOE (NOESY) experiments. The 5.32 ppm proton of fragment C and the 3.86/3.93 ppm methylene protons of fragment E correlate to the same downfield carbon (154.3 ppm), establishing a connection between C, E, and this carbon. This 154.3 ppm carbon is part of a guanidinium group by virtue of its chemical shift and attached exchangeable protons. Similarly, the 3.13 ppm proton of fragment B and the 3.73 ppm proton of fragment D correlate to the same guanidinium carbon (155.4 ppm). A strong NOE was observed between the 6.61 ppm aromatic proton of fragment A and the 7.20 ppm NH of fragment B, suggesting that fragment B is attached to the aromatic ring via this NH. The upfield position (6.61 ppm) of the proton ortho to the point of attachment is consistent with the suggested bond between fragments A and B. A bond between the 5.32 ppm methine of fragment C and the aromatic ring (fragment A) was deduced from correlations between the methine proton and three aromatic carbons. Another attachment to fragment C was observed in a long-range correlation between the 3.30 ppm methine proton of fragment B and the 52.9 ppm carbon of fragment C. This latter correlation fits well with fragments A, B, and C being fused to make a piperidine ring. The final attachment between fragments A and F was observed by correlations of the 4.59/4.64 ppm methylene protons of fragment F and the 7.58 and 7.66 ppm protons of fragment A to a common carbon at 165.1 ppm. The chemical shifts of the protons and carbons surrounding this point of attachment are indicative of an ester moiety (see Table 1).

The stereochemistry of the ring juncture in **1** was found to be *cis*. A large, *trans*-diaxial coupling would have been expected between the bridgehead protons if the ring juncture were *trans*. The observed coupling is 6.8 Hz, which agrees well with expectation based on models of possible *cis* conformers. The stereochemistry of the adjacent substituent in the 2-position was found to be *trans* relative to the ring juncture. A strong NOE was observed between the H-5a bridgehead

Table 1. Carbon and Proton Chemical Shifts for Martinelline and Martinellic Acid in DMSO-d_6

	compd **1**		compd **2**	
position	^{13}C (ppm)	^1H (ppm)	^{13}C (ppm)	^1H (ppm)
1		7.20 (2.9)		7.06 (3.2)
2	49.0	3.30	49.1	3.28
2a	39.2	2.44	39.3	2.43
3	26.2	2.07, 1.68	26.2	2.07, 1.69
4	45.7	3.38	45.7	3.39
5a	52.9	5.32 (68)	53.0	5.26 (6.6)
5b	115.9		115.6	
6	130.4	7.66 (1.5)	130.4	7.65
7	115.6		117.1	
8	129.7	7.58 (2.0, 8.8)	130.0	7.54 (1.7, 8.6)
9	113.3	6.61 (8.6)	113.2	6.58 (8.6)
9a	146.7		146.3	
10	33.3	1.44	33.3	1.43
11	25.2	1.57, 1.63	25.2	1.57, 1.64
12	40.6	3.13	40.7	3.13
13		7.65		7.51
14	155.4		155.4	
14a		7.44		7.42
15		7.61		7.51
16	38.9	3.73 (5.8, 5.8)	38.9	3.73 (5.6, 5.6)
17	119.1	5.17 (6 × 1.2, 6.7, 6.7)	119.1	5.17 (6 × 1.2, 6.8, 6.8)
18	135.8		135.8	
19	25.2	1.70 (~1.0)	25.2	1.70 (~1.0)
20	17.7	1.64 (~0.5)	17.8	1.64 (~0.5)
21	154.3		154.2	
21a		7.73		7.69
22		7.76		7.76
23	39.9	3.86 (5.5, 5.5, 15.4), 3.93 (6.2, 6.2, 15.4)	39.8	3.84 (5.4, 5.4, 15.6), 3.95 (5.9, 5.9, 15.6)
24	119.4	5.30 (6 × 1.2, 6.6, 6.6)	119.5	5.31 (6 × 1.2, 6.4, 6.4)
25	135.5		135.5	
26	17.8	1.69	17.9	1.69
27	25.3	1.74 (~0.7)	25.3	1.74 (~0.7)
28	165.1		167.1	
30	67.6	4.59 (13.2) 4.64 (13.4)		
31	134.7			
31a	13.7	1.71		
32	121.6	5.51 (5 × 1.2, 6.3, 6.3)		
33	38.3	3.81 (6.1, 6.1)		
34		7.83		
35	156.7			
36		7.26, broad		
37		7.26, broad		

proton and H-10 of the side chain. This NOE is only possible if H-5a and the side chain reside on the same face of the piperidine ring and they both occupy pseudoaxial positions. The H-2 to H-2a coupling was found to be small (<2 Hz) on the basis of the absence of a correlation between these protons in the $^1H-^1H$ COSY spectrum. This is in accord with H-2 and H-2a occupying pseudoequatorial positions on the piperidine ring.

Radioligand binding studies indicated that **1** interacts with BK B_1 and B_2, α_1-adrenergic, and muscarinic receptors. The results are summarized in Table 2. In each case the type of inhibition displayed by **1** was consistent with a competitive mode of interaction. **2** and **3** were found to be more than 30-fold less active than the parent ester at both BK receptor subtypes. The activity of **2** and **3** at muscarinic and α_1-adrenergic receptors was not examined.

In functional *in vitro* antagonist studies, **1** competitively blocked [des-Arg[9]]-BK and norepinephrine contractions of rabbit thoracic aorta as well as carbachol-induced contractions of guinea pig ileum. A representative experiment using [des-Arg[9]]-BK as agonist in rabbit aorta is shown in Table 3. The derived K_B values for **1** in these assays were consistent with its activities in the respective B_1, α_1-adrenergic, and muscarinic receptor binding assays. In addition, **1** also blocked histamine-stimulated contractions of isolated rabbit thoracic aorta but was without

Table 2. Martinelline Inhibition of Radioligand Binding to BK, Muscarinic, and α_1-Adrenergic Receptors

receptor	IC_{50} (nM)	receptor	IC_{50} (nM)
B_1	6400	Muscarinic	90
B_2	250	α_1-adrenergic	60

Table 3. Antagonist Activities of Martinelline in Organ Bath Assays

tissue	receptor	agonist	K_B[a] (nM)
rabbit aorta	B_1	[des-Arg[9]]-BK	2100
	α_1	norepinephrine	79
	H_1[b]	histamine	1290
	AT_1[c]	angiotensin II	NE[d]
	KCl[e]	NA	NE[d]
guinea pig ileum	M_3[f]	carbachol	100

[a] Inhibitor dissociation constant. [b] Histamine receptor. [c] Angiotensin receptor. [d] No effect. [e] 100 mM KCl used to contract tissue. [f] Muscarinic receptor.

Table 4. Minimum Inhibitory Concentration (MIC) of Martinelline and Martinellic Acid

	MIC (μg/mL)	
test organism	compd **1**	compd **2**
Proteus vulgaris	>128	>128
Bacillus subtilis	16	>128
Micrococcus luteus	128	>128
Xanthomonas campestris pv. vesicatoria	>128	>128
Escherichia coli	32	>128
Klebsiella pneumoniae	>128	>128

effects at 50 μM on angiotensin II or KCl responses in this tissue. This latter result indicates that **1** does not block plasma membrane calcium channels in smooth muscle. **1** therefore appears to be an effective inhibitor at several G-protein coupled receptor systems. It is possible that the folkloric efficacy of **1** in treating conjunctivitis may be due to its inhibitory effects at BK and histaminergic receptors, as both of these substances are known mediators of inflammatory reactions. Also, BK is a potent nociceptive agent,[10] and **1** would therefore be expected to possess analgesic activity as well. Although the ethnobotanical method of administration implies the use of an aqueous extract—juice squeezed from the pounded roots—we are confident that an aqueous extract prepared from fresh roots in this manner would contain significant amounts of **1** and **2**. Both compounds are fairly soluble in H_2O (data not shown), probably due to their positively charged guanidinium moieties.

To further correlate the use of the extracts to treat conjunctivitis, the antibiotic character of **1** and **2** was studied. Table 4 demonstrates a modicum of activity for **1** versus both Gram-positive and Gram-negative bacteria, while **2** was not active. In addition, **1** and **2** were not cytotoxic, with IC_{50} values of 75 and >100 μg/mL, respectively, or cell lytic, with MLC values of >0.1 and >1.0 mg/mL for trypan blue and red blood cell lysis, respectively. This implies that some modest Gram-positive and Gram-negative antibiotic activity by **1**, coupled with low cytotoxicity and lytic activity, may also play an important role in its reported efficacy. In conclusion, the combined effects of analgesia, antimicrobial activity, and reduction of inflammation would clearly have therapeutic value in the treatment of conjunctivitis.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer, and UV spectra were recorded on an AVIV 14DS UV−vis spectrometer. Optical rotations were

(10) Regoli, D.; Barabe, J. *J. Pharmacol.* **1980**, *32*, 1.

measured on a Perkin-Elmer 241 polarimeter. The HRFABMS data were obtained on a VG ZAB-HF spectrometer. ^{1}H-, ^{13}C-, and ^{19}F-NMR spectra of **1** and **2** were obtained on a Varian VXR-500S spectrometer operating at 499.8, 125.7, and 282.2 MHz, respectively. Approximately 12 mg of the sample was dissolved in 0.65 mL of DMSO-d_6 (99.96 atom % D, Merck Isotopes). The proton and carbon chemical shifts were measured with respect to internal TMS. All experiments were performed at 25 °C. $^{1}H-^{1}H$ COSY spectra were recorded using the standard pulse sequence.[11] Phase-sensitive NOESY experiments were performed using a 300 ms mix time.[12] Short- and long-range proton−carbon chemical shift correlation spectra were obtained using the inverse detection methods HMQC and HMBC.[13] The delay time for obtaining long-range couplings in the HMBC experiment was 65 ms. The ^{1}H-NMR spectrum of **3** (MeCN-d_3) was obtained on a Varian XL300 spectrometer and was referenced to internal TMS.

Collection and Extraction. A 17.1 g quantity of *M. iquitosensis* root was collected in Peru by Professor Walter Lewis, Washington University, and identified by Alwyn Gentry, Missouri Botanical Garden. A voucher may be examined at the Missouri Botanical Garden. The whole root was air-dried, milled, and extracted with CH_2Cl_2/MeOH (1:1) to yield 1.6 g of organic solubles. An aqueous extract was prepared subsequently which gave 2.5 g of H_2O solubles. The organic extract contained **1** and **2**. Although HPLC of the crude organic extract clearly shows the presence of **2** (data not shown), it may be an artifact of the extraction procedure since CH_2Cl_2 is known to contain trace HCl which could catalyze cleavage of the ester bond of **1** to give **2**. This conversion was not observed in nonhalogenated solvents during the time frame of NMR and HPLC analyses, respectively.

Isolation. A 0.773 g portion of the organic extract was passed through a 31 g plug of C_{18} (Vydac, 15−30 μm), eluting with 200 mL of H_2O followed by 200 mL of MeOH. The methanolic fraction (0.202 g) was subjected repeatedly (4 × 50 mg) to preparative HPLC on a Vydac C_{18}, 300 Å, 10 μm, 22.5 mm × 250 mm column, using gradient H_2O (1% TFA)/MeCN (1% TFA), 0% MeCN to 60% MeCN over 1 h (UV 330 nm), to yield 47 mg of **1** (0.6 dry wt %) and 26 mg of **2** (0.3 dry wt %).

Martinelline (1) was obtained as a pale yellow amorphous solid: $[a]_D$ +9.4° (*c* 0.02, MeOH); UV λ_{max} (H_2O, pH 7.45) 314 nm (ϵ 12 457); IR ν_{max} (KBr) 3380, 2940, 2900, 1650, 1520, 1440, 1190, 820, 790, 770, 610 cm^{-1}; high-resolution FABMS (glycerol) $[MH]^+$ *m/z* 621.4348 for $C_{33}H_{53}N_{10}O_2$ (calcd 621.4358); FABMS (glycerol) *m/z* $[MH]^+$ 621, $[MH^+ - C_6H_{11}N_3]$ 496; ^{1}H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 1.

Martinellic acid (2) was obtained as a pale yellow amorphous solid: $[a]_D$ −8.5° (*c* 0.01, MeOH); UV λ_{max} (MeOH) 300 nm (ϵ 11 617); IR ν_{max} (KBr) 3340, 2910, 1670, 1525, 1455, 1380, 1200, 830, 800, 775, 615 cm^{-1}; high-resolution FABMS (glycerol) $[MH]^+$ *m/z* 496.3389 for $C_{27}H_{42}N_7O_2$ (calcd 496.3399); FABMS (glycerol) *m/z* $[MH]^+$ 496; ^{1}H NMR (DMSO-d_6) see Table 1; ^{13}C NMR (DMSO-d_6) see Table 1.

Methyl Ester of Martinellic Acid (3). A solution of **2** (22 mg) in 200 μL of dry MeOH with 10 μL of $SOCl_2$ was maintained at 30 °C overnight. The reaction mixture was chromatographed on a Vydac C_{18}, 5 μm, 10 mm × 25 cm HPLC column using gradient H_2O (1% TFA)/MeCN (1% TFA), 20% MeCN to 60% MeCN over 40 min (UV 330 nm), to yield 6.7 mg of **3**: high-resolution FABMS (glycerol) $[MH]^+$ *m/z* 510.3558 for $C_{28}H_{44}N_7O_2$ (calcd 510.3556); ^{1}H NMR (MeCN-d_3) revealed methyl ester CH_3 proton resonances at 3.77 ppm.

Bioassays. [^{3}H][Des-Arg^{10}]-kallidin binding to BK B_1 receptors in rabbit aorta smooth muscle cells in primary culture was performed as described by Schneck *et al.*[14] [^{3}H]BK binding to guinea pig ileal B_2 receptors was performed as previously reported.[15] [^{3}H]Prazosin binding to rat cortex α_1-adrenoceptors and [^{3}H]quinuclidinyl benzilate binding to guinea pig ileum muscarinic receptors followed the methods of Battaglia *et al.*[16] and Watson *et al.*,[17] respectively. Contractile assays using strips of rabbit thoracic aorta were performed as described by Regoli *et al.*[18] Guinea pig ileum longitudinal muscle strips were prepared as detailed by Rang.[19] All dose−response curves were obtained by cumulative addition of agonist and were recorded isometrically.

Minimum Lytic Concentration (MLC). All procedures were performed under sterile conditions. Minimal lytic concentrations (MLCs) were determined in a liquid microtiter assay. A 10 mg/mL stock solution of each compound was prepared in DMSO and kept at −80 °C. Thawed stock solutions were diluted (1:10) to 1 mg/mL with a 5% (v/v) dextrose solution. From this initial concentration, 11 additional serial 2-fold dilutions of each compound were made in 5% dextrose solution in a microtiter plate, resulting in a final volume of 150 μL per well (1 mg/mL to 0.49 μg/mL). Thirty-eight microliters of a 4% solution (v/v) of defibrinated sheep blood (BBL) in 5% dextrose (v/v) was added to each concentration of compound and mixed gently. The multiwell plate was covered and incubated at 25 °C for 2 h. Lysis was demonstrated as full or partial clearing. The minimum lytic concentration (MLC) was defined as the minimum concentration required to cause lysis.[20]

Cytotoxicity Testing. Cytotoxicity determinations using 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium (MTT) were performed using mouse L1210 cells grown in Fisher's medium (Difco) plus 10% heat-inactivated fetal bovine serum. The assays were performed in microtiter plates as described by Carmichael *et al.*[21] and Twentyman and Luscombe.[22] Cell viability was also assessed using trypan blue exclusion; equal aliquots of treated L1210 cells and 0.4% trypan blue (Sigma) were mixed, and the total number of cells, as well as the proportion of viable, i.e., dye-excluding, cells, determined by microscopic observation. In both MTT and trypan blue assays, the IC_{50} indicates the concentration of compound that results in a viable cell number half the level of the control. The trypan blue assay was also used to determine the extent of lysis of L1210 cells after a 24 h incubation.

Minimal Inhibitory Concentration (MIC) Determination. Minimal inhibitory concentrations (MICs) were determined in a liquid microtiter assay. The compounds were dissolved in DMSO and diluted to 1.28 mg/mL in 10% DMSO/90% water. From this initial concentration, 10 additional serial 2-fold dilutions were made in the same diluent in a microtiter plate, resulting in a final volume of 20 μL in each well. The organisms being tested were grown in brain heart infusion broth (BHI; Difco). Stationary phase cultures (or, in the case of *Bacillus subtilis*, a spore suspension) were diluted in BHI to approximately 10^5 cfu/mL; 180 μL was added to each well. The plates were incubated at 28 °C for 24 h and visually inspected for growth. The MIC was defined as the lowest concentration of compound preventing any visible growth.

Acknowledgment. We thank Harri Ramjit and Matt Zrada, Department of Medicinal Chemistry, Merck Research Laboratories, for providing mass spectral data and UV data, respectively. We are also grateful to Florence Berg, Department of Information Research and Analysis, Merck Research Laboratories, for assisting in our literature search.

JA950447L

(11) Bax, A.; Freeman R.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 164.

(12) States, D.; Habercorn, R.; Reuben, D. *J. Magn. Reson.* **1982**, *48*, 286.

(13) Summers, M.; Marzilli, L.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285.

(14) Schneck, K.; Hess, F.; Stonesifer, G.; Ransom, R. *Eur. J. Pharmacol.* **1994**, *266*, 277.

(15) Ransom, R.; Goodman, C.; Young, G. *Br. J. Pharmacol.* **1992**, *105*, 919.

(16) Battaglia, G.; Shannon, M.; Borgunduaag, B.; Titeler, M. *J. Neurochem.* **1983**, *41*, 538.

(17) Watson, M.; Roeske, W.; Yamamura, H. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 419.

(18) Regoli, D.; Drapeau, G.; Rovero, G.; Dim, S.; Rhaleb, N.; Barabé, J.; D'Orleans-Juste, P.; Ward, P. *Eur. Pharmacol.* **1986**, *127*, 219.

(19) Rang, H. *Br. J. Pharmacol.* **1964**, *22*, 356.

(20) Thrupp, L. D. Susceptibility Testing of Antibiotics in Liquid Media. In *Antibiotics in Laboratory Medicine*; Lorian, V., Ed.; Williams & Wilkins: Baltimore, MD, 1986; pp 93−158.

(21) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.

(22) Twentyman, P. R.; Luscombe, M. *Br. J. Cancer* **1987**, *56*, 279.