NEW VIRGINIAMYCIN M_1 DERIVATIVES: SYNTHESIS, CHOLECYSTOKININ BINDING INHIBITORY AND ANTIMICROBIAL PROPERTIES

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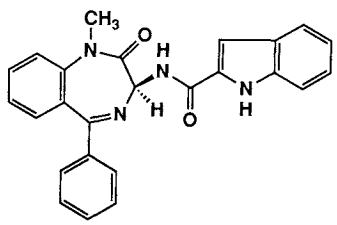
Fifteen 13-ester, 13-carbanilate and 15-hydroxy derivatives of virginiamycin M_1 were synthesized and evaluated for their abilities to inhibit a) binding to the cholecystokinin receptor subtypes in guinea-pig brain (CCK-B) and rat pancreas (CCK-A), and b) microbial growth.

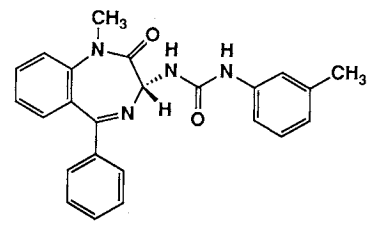
In a previous publication[1], we reported the discovery of virginiamycin M_1 and its new analogs, L-156,586, L-156,587 and L-156,906 as potent gastrin and brain cholecystokinin (CCK) antagonists from a strain of *Streptomyces olivaceus*, ATCC 53527. Of importance, the antibacterial activities and gastrin/brain CCK (CCK-B) binding affinities are separable in that series of compounds. We also noticed that few derivatives of the virginiamycin M_1 family have been reported, possibly due to the complexity of their structures and their instability[2]. Subsequently, the 13-acetate was prepared and found to selectively displace binding to the CCK-B receptor but lacked binding affinity for CCK-A receptors. Interestingly this analog showed greater inhibition for the agonist binding assay ([^{125}I]Boulton-Hunter CCK-8 ([^{125}I]BHCCK-8)) than the antagonist assay ([^{3}H]L-365,260). This property may reflect a partial agonist profile since it is shared by agonists such as CCK-8 but not by antagonists such as L-365,260. To further explore our interest in derivatives of virginiamycin M_1, we have modified the 13-hydroxyl and the 15-keto functions. In this paper, we will describe the preparation of 13-esters, 13-carbanilates and 15-hydroxy derivatives of virginiamycin M_1, as well as their CCK binding inhibition profile and antibiotic activity. The structures of these new derivatives are presented in Fig. 1.

Chemistry

Classical but effective reactions were employed. The reported yields were not optimized. Acetylation was performed at room temperature using excess acetic anhydride in pyridine. Both the mono- and

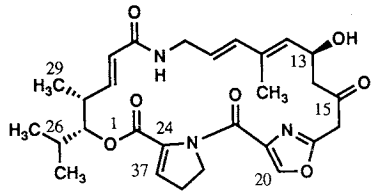


L-364,718

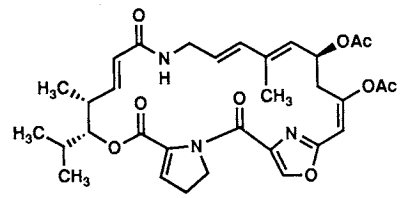


L-365,260

Fig. 1. Structures of virginiamycin M_1 (VM_1, **1**) and derivatives.



1



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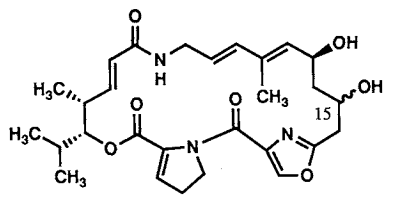
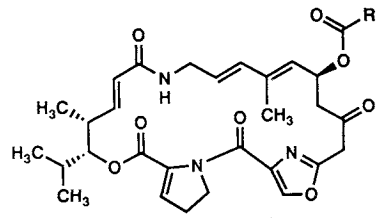


15
16, C-15 epimer



R
VM_1 esters:
2 CH_3
4 CH_2CH_3
5 *n*-Bu
6 $CH_2C_6H_5$
7 C_6H_5
VM_1 carbanilates:
8 $NHCH_3$
9 $NH(o\text{-}NO_2C_6H_4)$
10 $NH(m\text{-}NO_2C_6H_4)$
11 $NH(p\text{-}NO_2C_6H_4)$
12 NHC_6H_5
13 NH(*m*-tolyl)
14 NH(1-naphthyl)

di-acetates, **2** and **3**, were obtained and separated by flash chromatography. Other esters were prepared by using 1.05-fold excess of the respective acyl chlorides in dichloromethane at room temperature. The carbanilates were prepared using 1.1-fold excess of the respective isocyanates. These latter reactions were catalyzed by 4-dimethylaminopyridine and pyridine in anhydrous dichloromethane solutions. Sodium borohydride reduction was performed in methanol at ice-water temperature. After workup, silica gel flash chromatography was used to purify the reaction mixtures or separate the epimers. Physico-chemical characteristics, ^1H (partial) and ^{13}C NMR chemical shifts of these derivatives are described in Tables 1, 2 and 3, respectively.

Biological Activity

Radioligand binding assays were performed as described previously[1,3,4]. The results are described in Table 4. All derivatives showed reduced affinity for CCK-A receptors in rat pancreas compared with their affinity for CCK-B receptors in guinea pig brain. A number of the more active compounds including **2**, **3**, **8**, **9** and **10** showed a marked preference for the agonist binding assay ([^{125}I]BHCCKS) compared with the antagonist radioligand ([^{3}H]L-365,260). Since this profile is similar to that seen with CCK-B receptor agonists such as CCK-8S, CCK-8DS and pentagastrin but not antagonists such as L-365,260, these analogs may have partial agonist properties. These properties have not yet been confirmed in functional studies.

Agar diffusion assays were used to detect antibacterial activity[5~10]. Antibacterial activity profiles of

Table 1. Physico-chemical properties of virginiamycin M_1 derivatives.

Compound	M.F.	Exact masses by HRMS[a]		FT-IR[h] (ZnSe) ν_{max} cm^{-1}	UV[i] λ_{max}^{MeOH} nm (E%)	Rf[j]
		Found	Calculated			
2	$C_{30}H_{37}N_3O_8$	568.2639[d]	568.2658[f]	3356, 2971, 1733, 1673, 1621	226 (495), 280 (sh, 149)	0.31[k]
3	$C_{32}H_{39}N_3O_9$	609.2674[b]	609.2686	3362, 2973, 1766, 1736, 1671, 1621	213 (sh, 437), 237 (552), 280 (sh, 217)	0.22[k]
4	$C_{31}H_{39}N_3O_8$	581.2737[b]	581.2737	3345, 2973, 1733, 1673, 1621	226 (476), 280 (134)	0.25[k]
5	$C_{33}H_{43}N_3O_8$	609.3061[b]	609.3050	3355, 2966, 1733, 1673, 1621	210 (sh, 450), 226 (489), 280 (sh, 132)	0.36[k]
6	$C_{36}H_{41}N_3O_8$	643.2894[b]	643.2893	3356, 2972, 1733, 1673, 1621	213 (476), 230 (sh, 445), 280 (sh, 110)	0.28[k]
7	$C_{35}H_{39}N_3O_8$	629.2702[c]	629.2737	3357, 2971, 1727, 1673, 1621	208 (sh, 447), 232 (607), 280 (sh, 145)	0.21[k]
8	$C_{30}H_{38}N_4O_8$	582.2528[c]	582.2689	3344, 2973, 1727, 1673, 1620	224 (sh, 468), 280 (sh, 146), 334 (69)	0.29[l]
9	$C_{35}H_{39}N_5O_{10}$	690.2849[d]	690.2775[f]	3358, 2970, 1733, 1672, 1615	231 (666), 280 (sh, 192), 343 (58)	0.47[l]
10	$C_{35}H_{39}N_5O_{10}$	690.2762[d]	690.2775[f]	3341, 2971, 1730, 1671, 1619	239 (698), 280 (sh, 199), 340 (36)	0.41[l]
11	$C_{35}H_{39}N_5O_{10}$	690.2775[d]	690.2775[f]	3324, 2968, 1724, 1670, 1614	206 (263), 221 (279), 313 (114)	0.41[l]
12	$C_{35}H_{40}N_4O_8$	645.2942[d]	645.2924[f]	3338, 2968, 1730, 1671, 1618	208 (sh, 371), 239 (666), 282 (sh, 147)	0.43[l]
13	$C_{36}H_{42}N_4O_8$	681.2921[e]	681.2900[g]	3358, 2976, 1732, 1671, 1617	212 (sh, 462), 240 (661), 284 (sh, 147)	0.46[l]
14	$C_{39}H_{42}N_4O_8$	695.3090[d]	695.3081[f]	3350, 2972, 1732, 1671, 1618	229 (634), 281 (210)	0.42[l]
15	$C_{28}H_{37}N_3O_7$	527.2629[b]	527.2632	3346, 2971, 1732, 1668, 1617	217 (sh, 539), 233 (571), 280 (sh, 142)	0.28[m]
16	$C_{28}H_{37}N_3O_7$	527.2623[b]	527.2632	3339, 2973, 1733, 1668, 1619	218 (sh, 549), 235 (601), 280 (sh, 134)	0.45[m]

[a] Exact mass measurements were made on a MAT 90 or 212, or a JEOL HX110 mass spectrometer by the peak matching method using perfluorokerosene (for EI), CSI (for FAB) or Flomblin oil 2500F (for FAB) as the internal standard; [b] EI; [c] FAB; [d] FAB, with M+H observed; [e] FAB, with M+Na observed; [f] Calculated for M+H; [g] Calculated for M+Na.

[h] FT-IR data were recorded for neat samples on ZnSe crystals in a Perkin Elmer model 1750 instrument.

[i] UV data were recorded in MeOH solutions in a Beckman DU-70 spectrophotometer.

[j] E. Merck Silica gel 60F TLC plates (0.2 mm thickness) using the following mobile phases: [k] Me_2CO-hexane 40:60; [l] Me_2CO-hexane 50:50; and [m] *i*-PrOH-CH_2Cl_2 15:85.

Table 2. Proton chemical shifts[a], δ ppm (CD_2Cl_2), of virginiamycin M_1 derivatives.

Compound	H-13	Prosthetic moiety
2	5.76 (m)	1.95 (3H, s, CH_3)
3	5.66 (m)	2.00 (3H, s, CH_3), 2.20 (3H, s, CH_3), 6.23 (1H, s, H-16)
4	5.77 (m)	1.05 (3H, t, J=7.5, CH_2CH_3), 2.23 (2H, q, J=7.5, CH_2CH_3)
5	5.78 (m)	0.88 (3H, t, J=7.5, $CH_2CH_2CH_2CH_3$), 1.30 (2H, m, $CH_2CH_2CH_2CH_3$), 1.53 (2H, m, $CH_2CH_2CH_2CH_3$), 2.22 (2H, t, J=7.5, $CH_2CH_2CH_2CH_3$)
6	5.80 (m)	3.65 (2H, s, $CH_2C_6H_5$), 7.50 (5H, m, C_6H_5)
7	6.03 (m)	7.36~7.61 (5H, m, C_6H_5)
8	5.66 (m)	2.69 (3H, d, J=5, CH_3)
9	5.82 (m)	7.40~8.38 (4H, m, aromatic)
10	5.82 (m)	7.10~8.50 (4H, m, aromatic)
11	5.81 (m)	7.58 (2H, d, J=9.5), 8.12 (2H, d, J=9.5), both aromatic
12	5.80 (m)	7.00~7.40 (5H, m, aromatic)
13	5.78 (m)	2.31 (3H, s, CH_3), 6.82~7.22 (4H, m, aromatic)
14	5.85 (m)	7.42~7.88 (7H, m, aromatic)
15	4.63 (m)	—
16	4.91 (m)	—

[a] Data were recorded on a Varian XL-300 instrument at 20°C with chemical shifts reported in ppm relative to TMS at zero ppm using the solvent peak at 5.32 ppm as internal standard.

the derivatives are presented in Table 5. All compounds show some inhibition against Gram-positive bacteria, with *Micrococcus luteus* (ATCC 9341) being the most sensitive strain. Subsequent titration of these derivatives on *Micrococcus luteus* in the agar diffusion assay was performed and the results are presented in Table 6. The agar diffusion MIC was determined by extrapolation in a plot of the square of the zone diameter *versus* the log content/disc[5,7~9]. Reduced antibiotic activity was observed for the derivatives.

Experimental

General

HRMS (EI and FAB) was obtained using a Finnigan-MAT 90 or 212, or a JEOL HX110 mass spectrometer. ^1H and ^{13}C NMR spectra were obtained from CD_2Cl_2 solution on a Varian XL-300 spectrometer at 20°C. IR spectra were recorded as neat deposits on a ZnSe crystal with a Perkin Elmer model 1750 spectrophotometer. UV spectra were recorded in MeOH solutions in a Beckman DU-70 spectrophotometer. All of the radioligands were from New England Nuclear, with the exception of [^{125}I]BH-CCK-8 which came from Amersham. The cold CCK came from Peninsula Laboratories Inc. Silica gel 60 (40~63 μm particle size) for column chromatography was obtained from E. Merck. Thin layer chromatography (TLC) was performed on 250 μm E. Merck Silica gel $60F_{254}$ plates. All other chemicals were from Aldrich/Sigma Chemical Co.

Acetylation of 1

Acetic anhydride (45 ml) was added to a stirred solution of **1** (6.5 g) in anhydrous pyridine (45 ml) at room temperature. After 30 minutes, ice-cold methanol (85 ml) was added in portions. Stirring was continued for 20 more minutes followed by flash evaporation to dryness at room temperature. The residue was partitioned between dichloromethane (3 × 300 ml) and saturated NaCl (aq) (300 ml). The organic layers were pooled, dried over anhydrous Na_2SO_4, flash evaporated to dryness (dry weight = 8.1 g) and purified over a silica gel column in hexane-acetone mixtures to give 1.0 g of the monoacetate, **2**, and 1.58 g of the diacetate, **3**.

Acylation of 1 with Acyl Chlorides

Virginiamycin M_1 *n*-propionate, *n*-valerate, *n*-phenylacetate and benzoate were prepared using respective acyl chlorides.

Table 3. Carbon-13 chemical shifts[a] of virginiamycin M_1 derivatives.

Compound	Carbon-13 chemical shifts, δ ppm (CD_2Cl_2)
2	12.4, 12.9, 18.9, 19.7, 21.2, 30.2, 30.6, 38.1, 40.2, 45.1, 46.0, 51.1, 68.2, 81.7, 123.7, 125.6, 126.6, 127.5, 132.9, 136.3, 136.9, 137.2, 143.4, 145.7, 156.3, 160.8, 161.1, 167.2, 170.0, 199.3
3	11.9, 13.3, 19.0, 19.8, 21.3, 29.8, 30.4, 36.5, 38.6, 40.8, 50.6, 69.6, 82.8, 108.5, 122.1, 125.5, 127.0, 128.5, 128.6, 129.3, 133.6, 135.6, 137.2, 142.6, 143.8, 155.8, 158.4, 161.1, 161.8, 167.3, 169.0, 170.1
4	9.1, 12.4, 12.9, 18.9, 19.7, 27.8, 30.2, 30.5, 38.1, 40.3, 45.2, 45.9, 51.1, 68.0, 81.6, 123.7, 125.5, 126.7, 127.3, 132.9, 136.3, 136.8, 137.1, 143.5, 145.7, 156.3, 160.8, 161.1, 167.2, 173.4, 199.4
5	12.4, 12.9, 13.8, 18.9, 19.7, 22.5, 27.2, 30.2, 30.5, 34.3, 38.1, 40.3, 45.2, 45.9, 51.1, 68.0, 81.6, 123.7, 125.5, 126.7, 127.3, 133.0, 136.3, 136.8, 137.2, 143.6, 145.7, 156.3, 160.8, 161.2, 167.3, 172.7, 199.4
6	12.4, 12.9, 18.9, 19.7, 30.2, 30.5, 38.1, 40.4, 41.4, 45.2, 45.9, 51.1, 68.7, 81.6, 123.8, 125.4, 126.3, 127.3, 127.4, 128.8 (2×), 129.6 (2×), 129.7, 132.9, 136.3, 137.13, 137.17, 143.7, 145.6, 156.3, 160.8, 161.1, 167.5, 170.7, 199.2
7	12.4, 13.0, 18.9, 19.7, 30.2, 30.6, 38.1, 40.3, 45.3, 46.0, 51.1, 68.9, 81.6, 123.7, 125.5, 126.4, 127.6, 128.7 (2×), 129.8 (2×), 130.5, 132.9, 133.3, 136.3, 137.15, 137.25, 143.5, 145.7, 156.3, 160.9, 161.1, 165.6, 167.3, 199.3
8	12.3, 12.9, 18.9, 19.7, 27.6, 30.2, 30.5, 38.0, 40.3, 45.6, 45.9, 51.1, 68.4, 81.6, 123.7, 125.5, 127.17, 127.24, 133.1, 136.2, 136.5, 137.1, 143.5, 145.6, 156.2, 156.4, 160.8, 161.2, 167.2, 199.5
9	12.4, 13.0, 18.9, 19.7, 30.2, 30.6, 38.2, 40.2, 45.2, 46.0, 51.2, 70.0, 81.8, 121.0, 122.7, 123.8, 125.7, 126.1, 126.2, 127.9, 132.7, 135.6, 136.2, 136.4, 136.5, 137.2, 137.5, 143.4, 145.7, 152.2, 156.2, 160.8, 161.1, 167.2, 199.1
10	12.5, 13.0, 19.0, 19.7, 30.3, 30.5, 37.9, 40.8, 45.7, 45.8, 51.0, 69.2, 81.4, 113.4, 117.7, 123.9, 124.5, 125.1, 126.4, 127.5, 130.0, 133.5, 136.2, 137.1, 137.2, 140.3, 144.2, 145.5, 149.0, 152.9, 156.6, 160.7, 161.4, 167.6, 199.3
11	12.5, 13.0, 18.9, 19.7, 30.3, 30.6, 38.0, 40.5, 45.4, 45.9, 51.1, 69.6, 81.6, 118.1 (2×), 123.8 125.3 (2×), 125.4, 126.1, 127.7, 133.1, 136.3, 137.1, 137.3, 143.1, 143.9, 145.0, 145.7, 152.4, 156.4, 160.8, 161.4, 167.5, 199.1
12	12.4, 13.0, 18.9, 19.7, 30.2, 30.5, 38.0, 40.4, 45.6, 45.9, 51.1, 68.8, 81.6, 118.9, 123.4, 123.7, 125.5, 126.8, 127.4, 129.2 (2×), 133.2, 136.2, 136.8, 137.1, 138.6, 143.6, 145.6, 152.8, 156.4, 160.8, 161.3, 167.3, 199.4
13	12.4, 13.0, 18.9, 19.7, 21.5 ($CH_3C_6H_4$), 30.2, 30.6, 38.0, 40.4, 45.6, 45.9, 51.1, 68.8, 81.6, 116.0, 119.5, 123.7, 124.3, 125.5, 126.8, 127.4, 129.0, 133.2, 136.3, 136.9, 137.2, 138.4, 139.2, 143.6, 145.6, 152.7, 156.4, 160.8, 161.2, 167.3, 199.4
14	12.4, 13.0, 18.9, 19.7, 30.2, 30.5, 38.0, 40.4, 45.5, 45.9, 51.1, 69.3, 81.6, 119.7, 121.2, 123.7, 125.2, 125.5, 126.0, 126.3, 126.5, 126.7, 127.5, 128.8, 133.1, 134.4, 136.3, 137.1, 137.2, 143.5, 145.7, 153.6, 156.4, 160.8, 161.2, 167.3, 199.4
15	12.3, 13.5, 19.0, 19.7, 30.2, 30.4, 37.1, 37.8, 40.8, 44.8, 51.4, 67.2, 67.7, 81.4, 124.6, 125.0, 125.4, 133.7, 134.4, 134.8, 135.7, 137.3, 144.1, 144.3, 161.1, 161.2, 162.1, 167.2
16	11.3, 13.0, 18.8, 19.7, 30.0, 30.4, 36.0, 37.5, 41.8, 42.5, 51.1, 66.8, 67.7, 80.9, 124.9 (2×), 125.2, 133.4, 134.4, 136.1, 137.0, 137.4, 143.8, 144.2, 160.5, 161.2, 162.1, 167.0

[a] Data were recorded on a Varian XL-300 instrument at 20°C with chemical shifts reported in ppm relative to TMS at zero ppm using the solvent peak at 53.8 ppm as internal standard.

Thus, the respective acyl chloride (1.0 mmole) was added to a stirred solution of **1** (0.50 g, 0.95 mmole), pyridine (84 μl) and 4-dimethylaminopyridine (20 μg) at room temperature in anhydrous dichloromethane (10 ml) for 2 hours. Methanol (0.5 ml) was added to destroy excess reagent. The reaction mixture was partitioned between dichloromethane (3 × 50 ml) and saturated NaCl (aq) (50 ml). The pooled organic layers were dried over anhydrous Na_2SO_4 and flash evaporated to dryness (dry weight = 0.63, 0.81, 1.0 and 1.4 g, respectively). Purification over a column of 50 g silica gel in 40 ~ 80% acetone - hexane stepwise gradients yielded 0.43, 0.33, 0.29 and 0.56 g of the respective esters, **4**, **5**, **6** and **7**.

Formation of Carbanilates of **1**

Methyl, *o*-, *m*- and *p*-nitrophenyl, phenyl, *m*-tolyl and 1-naphthyl carbanilates of **1** were prepared using their respective isocyanate.

Thus, **1** (0.50 g, 0.95 mmole) in anhydrous dichloromethane was added dropwisely at room temperature to a solution of the respective isocyanate (1.05 mmole) in dichloromethane (5 ml) and toluene (5 ml).

Table 4. CCK binding inhibition of virginiamycin M_1 and derivatives.

Compound	IC_{50} (μM)					
	CCK-B (guinea pig cortex)			CCK-A (rat pancreas)		
	[^{125}I]BHCCK	[^{3}H]L-365,260	A.R.	[^{125}I]BHCCK	[^{3}H]L-364,718	A.R.
1	0.68	0.86	1	>3.0 (0%)	>3.0 (14%)	—
2	0.7	>3.0 (21%)	>25	>3.0 (5%)	>3.0 (5%)	—
3	0.8	>3.0 (13%)	>25	>3.0 (0%)	>3.0 (10%)	—
4	0.6	1.0	2	>3.0 (5%)	>3.0 (4%)	—
5	0.5	1.2	2	>3.0 (21%)	>3.0 (15%)	—
6	0.6	>3.0 (40%)	~10	>3.0 (29%)	>3.0 (14%)	—
7	0.096	0.19	2	2.3	>3.0 (16%)	—
8	0.8	>3.0 (14%)	19	>3.0 (0%)	>3.0 (19%)	—
9	0.3	3.1	10	>3.0 (3%)	>3.0 (8%)	—
10	0.4	5.2	12.9	>3.0 (32%)	>3.0 (1%)	—
11	1.6	13.1	8.7	>3.0 (10%)	>3.0 (1%)	—
12	0.3	1.6	5.3	2.3	4	1.7
13	0.4	3.0	7.5	1.9	8.0	4.2
14	0.3	2.8	9.3	1.4	4.8	3.4
15	2.1	0.14	0.1	>3.0 (5%)	>3.0 (15%)	—
16	0.4	0.53	1	>3.0 (1%)	>3.0 (5%)	—

A.R.: Agonist ratio, IC_{50} ([^{3}H]L-365,260)/IC_{50} ([^{125}I]CCK).

Table 5. Antibacterial activity of virginiamycin M_1 and derivatives.

	Inhibition zone diameters (mm) at 20 μg/disc											
	Gram+								Gram−			
Test organisms[a]:	Baci	Sta	Subt	Micr	Sta	Cory	Str	Str	Salm	Coli	Pseu	Ent
Merck No., Mb:	633	108	964	1101	2983	261	2820	2875	1287	1418	1231	835
ATCC:	—	6538P	6633	9341	—	9742	—	—	—	—	11607	—
Compound												
1	17	34	23	34	25	37	19	23	11	14	13	12
2	0	11	0	21	0	13[b]	0	0	0	0	0	0
3	0	9	0	17[c]	0	14[b]	0	0	0	0	0	0
4	11	0	21	0	12[b]	0	0	0	0	0	0	0
5	0	0	0	25	0	0	0	0	0	0	0	0
6	0	0	0	12	0	0	0	0	0	0	0	0
7	0	0	0	8	0	0	0	0	0	0	0	0
8	7[d]	19	0	27	11	22	10	16	0	0	0	0
9	0	7	0	16	0	9	0	0	0	0	0	0
10	0	0	0	8	0	0	0	0	0	0	0	0
11	0	9	0	13	0	9	0	0	0	0	0	0
12	0	0	0	14	0	0	0	0	0	0	0	0
13	0	0	0	9	0	0	0	0	0	0	0	0
14	0	0	18	9	11[e]	0	0	0	0	0	0	0
15	9[d]	27	0	35	22	30	16	30	0	8[d]	15[d]	0
16	0	22	0	35	11	26	15[c]	18	0	0	0	0

[a] Test organisms: Baci 633, *Bacillus* sp.; Sta, *Staphylococcus aureus*; Subt 964, *Bacillus subtilis*; Micr 1101, *Micrococcus luteus*; Cory 261, *Corynebacterium pseudodiphtheriticum*; Str 2820, *Streptococcus faecium*; Str 2875, *Streptococcus agalactiae*; Salm 1287, *Salmonella gallinarum*; Coli 1418, *Escherichia coli*; Pseu 1231, *Pseudomonas stutzeri*; Ent 835, *Enterobacter aerogenes*.

[b] Fuzzy zones.

[c] Slightly hazy zones.

[d] Hazy zones.

[e] Rings.

Table 6. Antibacterial activity of virginiamycin M_1 and derivatives *versus Micrococcus luteus*[a].

Compound	μg/disc								Agar diffusion MIC (μM)
	1.25	2.50	5.00	10.0	20.0	40.0	80.0	160	
1	27	31	34	36	39	NT	NT	NT	0.15
2	NZ	7	14	18	21	NT	NT	NT	3.24
3	NZ	NZ	12	15	16	NT	NT	NT	1.92
4	NZ	7	13	17	19	NT	NT	NT	2.51
5	12	16	19	21	22	NT	NT	NT	0.54
6	NT	NT	10	11	13	14	14	NT	0.41
7	NT	NT	NT	10	9	10	10	11	0.003
8	8	13	18	22	25	NT	NT	NT	1.74
9	12	15	15	16	16	NT	NT	NT	0.019
10	NT	NT	NT	8	9	10	11	11	0.82
11	NZ	NZ	7	10	12	NT	NT	NT	3.94
12	7	10	11	12	12	NT	NT	NT	0.05
13	NT	NT	NT	NZ	NZ	NZ	NZ	NZ	>243
14	NT	NT	NT	NZ	NZ	NZ	NZ	NZ	>230
15	25	27	29	31	33	NT	NT	NT	0.08
16	22	25	28	30	32	NT	NT	NT	0.24

[a] Mean of triplicate zone size (mm) after incubation at 25°C for ~18 hours.
NZ: No inhibition zone.
NT: Not tested.

Pyridine (85 μl) and dimethylaminopyridine (0.12 g) were added and the solution (except for the methyl isocyanate solution) was refluxed (steam bath) for 30 minutes or until over 90% of starting material was consumed. Methanol (1 ml) was added and reflux continued for an additional 5 minutes. The reaction mixture was poured over 50 ml saturated NaCl (aq) containing 0.5 ml conc HCl and extracted with dichloromethane (3 × 50 ml). The organic layers were washed with saturated NaCl (aq), pooled, dried over anhydrous Na_2SO_4 and flash evaporated to dryness (dry weight = 0.76, 0.73, 0.80, 0.60, 0.74, 0.80 and 0.60 g, respectively). Purification over a column of 50 g silica gel using acetone-hexane or methanol-hexane-ethyl acetate mixtures yielded 0.074, 0.24, 0.11, 0.20, 0.082, 0.10 and 0.098 g of the respective and desired carbanilates, **8**, **9**, **10**, **11**, **12**, **13** and **14**.

Sodium Borohydride Reduction of **1**

Sodium borohydride (325 mg, 8.59 mmole) in 40 ml methanolic solution was added in one shot to a stirred solution of **1** (3.84 g, 7.30 mmole) in methanol (150 ml) in an ice-water bath. Stirring was continued for 15 minutes followed by addition of acetone (10 ml) to destroy excess reagent. Flash evaporation of the reaction mixture to 65 ml was followed by addition to ice-cold saturated NaCl (aq) (500 ml). Extraction with dichloromethane (5 × 250 ml), drying the organic layers over anhydrous Na_2SO_4, and flash evaporation to dryness below 35°C, gave 3.90 g of product. Flash chromatographic purification in a column of 200 g silica gel using 10 to 20% *i*-propanol-dichloromethane mixtures for elution yielded 1.45 g of the more polar epimer, **15**, and 1.75 g of the less polar epimer, **16**.

Radioligand Binding Assays

[^{125}I]BHCCK-8 binding to rat pancreatic and guinea pig cortical preparations were performed as described previously with minor amendments[1]. CCK-B and CCK-A selective antagonist ligand assays employing [^{3}H]L-365,260 and [^{3}H]L-364,718 respectively were performed as described[3,4] with the minor modifications described below. For the [^{3}H]L-365,260 assay brain membranes were homogenized in 0.32 M sucrose (20 vol) and centrifuged at 1,000 × *g* for 10 minutes. The resulting supernatant was re-spun at 20,000 × *g* for 20 minutes. Pellets were resuspended at 1 g starting material to 60 ml of incubation buffer (20 mM HEPES, 1 mM EDTA, 150 mM NaCl and 0.25 mg/ml bacitracin, pH 7.4). Specific binding was defined with 1 μM CCK-8S using 1.5 nM radioligand. Incubations were for 40 minutes at 4°C and were terminated by filtration and washing with ice-cold 100 mM saline. For the [^{3}H]L-364,718 assay, pancreas

was homogenized in 10 mM HEPES buffer, pH 7.4, containing 0.01% trypsin inhibitor. The final tissue resuspension was 1 g starting material to 2,000 ml of assay buffer (20 mM HEPES, 1 mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl, pH 7.4, containing 0.25 mg/ml bacitracin, 0.1 mg/ml trypsin inhibitor and 2 mg/ml bovine serum albumin). Incubations were for 30 minutes at room temperature and were terminated by filtration over GF/C filters and washing with 100 mM NaCl.

Antibacterial Activity

Cultures employed in evaluating antibacterial activity included plant pathogens, human and animal opportunistic pathogens and selected laboratory strains having specific-antibiotic sensitivities or chromosomal or R-plasmid-mediated resistances. Of the 21 Gram-positive and Gram-negative bacteria tested, the present derivatives demonstrated antibacterial activity only against Gram-positive organisms, with *M. luteus* 1101 being the most sensitive. The agar diffusion MIC's were determined using the method described by BARRY[5]. Each test compound was dissolved in methanol to a concentration of 1 mg/ml. Serial dilutions of these compounds were also made to determine an agar diffusion MIC. 20 μl of each dilution was pipetted on to 6 mm paper discs, air dried and placed onto the surface of an agar plate seeded with the appropriate bacteria ($\sim 10^6$ cfu)[5~10]. Seeded agar plates were incubated overnight ($\sim$18 hours) at 25°C. Zone sizes were measured to the nearest mm. Each compound was tested in triplicate and the results shown represent the mean.

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