Synthesis and Central Nervous System Activity of 2-Arylidene-4-aminoalkyl-2*H*-1,4-benzoxazin-3(4*H*)-ones and Related Compounds

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The synthesis and CNS activity of 2-benzylidene-4-[3-(dimethylamino)propyl]-2*H*-1,4-benzoxazin-3(4*H*)-one and 15 analogues are described. Four of these compounds were hydrogenated to give the corresponding benzyl derivatives. Several of the benzylidene compounds showed modest potency as CNS depressants and as dopamine receptor antagonists.

Since we found that a number of substituted 1,4-benzothiazines exhibited biological activity,[1] several related 1,4-benzoxazines were prepared for evaluation as potential medicinal agents. Alkylation of 2-benzylidene-2*H*-1,4-benzoxazin-3(4*H*)-one[2] (I) with 3-dimethylaminopropyl chloride yielded the N-substituted product II (Scheme I). Catalytic reduction of II yielded the N-substituted 2-benzyl-2*H*-1,4-benzoxazin-3(4*H*)-one III. The latter compound was identical with the product obtained by alkylation of 2-benzyl-2*H*-1,4-benzoxazin-3(4*H*)-one. The IR and NMR spectra of II and III were in agreement with the assigned structures.

Since II exhibited significant CNS depression in rats, the analogues shown in Table I were prepared, usually by interaction of I [or a substituted benzoxazin-3(4*H*)-one of Table IV] with the appropriate basically substituted alkyl halide (methods A and B). Compounds **7–9** were obtained by alkylation of I with $Cl(CH_2)_{3-4}Br$ and then heating the resulting intermediate with the appropriate amine (method C).

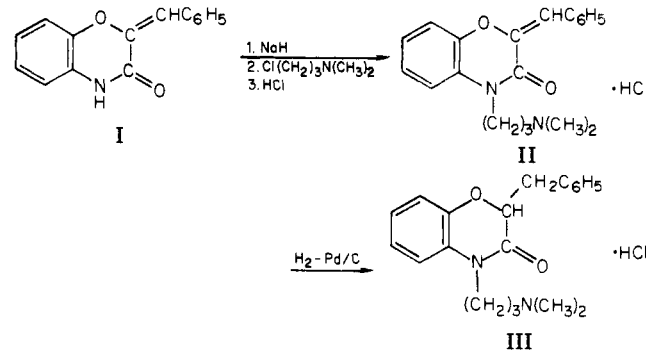
Structure–Activity Relationships. Compounds of this series were screened for CNS activity and the results are shown in Table I. The initial structural modifications of II (compound **3**), including the replacement of the dimethylamino group by other basic groups, varying the length of the basic side chain (ethylene and tetramethylene analogues) and the formation of a quaternary salt, resulted in compounds **1**, **2**, and **4–9** with decreased depressant potency. The products resulting from the introduction of a chloro, methyl, or methoxy group in the 6 position (**10–13**), the 2- and 4-chlorobenzylidene (**14**, **15**), and the 2-thienylidene analogues (**16**) were also less potent than **3**. The diethylamino and 4-methylpiperazino compounds (**5** and **6**) were slightly more potent than **3** in causing decreased grip strength or limb paralysis. Compound III (**19**) and the three analogues shown in Table II were also less potent than **3** in causing depressant or decreased reflex action. The starting material I (**17**) did not exhibit significant CNS depressant activity.

Compounds of Table I were screened as potential neuroleptic agents using a dopamine-sensitive adenylate cyclase preparation from the rat brain olfactory tubercle (Table III). Most neuroleptic agents inhibit the stimulation of this enzyme.[3] With the exception of **4** and **12**, the inhibition of dopamine-responsive adenylate cyclase activity exhibited by these compounds paralleled their CNS depressant properties. Three of the more potent inhibitors (**3**, **6**, and **12**) were tested in an avoidance procedure[4] at 25 and 50 mg/kg ip, using five rats for each compound. Compound **6** significantly reduced avoidance activity at 50 mg/kg, whereas **3** and **12** were not effective at the doses tested. Fluphenazine and clozapine caused an avoidance effect in 50% of the animals at 0.25 and 25.0 mg/kg, respectively.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer IR-621 spectrometer and the NMR spectra with Perkin-Elmer R-12B and Varian XL-100(15) spectrometers using Me_4Si as internal standard. All analyses obtained were within 0.4% of the theoretical values.

Scheme I



I II III

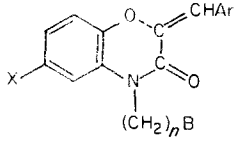
2-Benzylidene-2*H*-1,4-benzoxazin-3(4*H*)-one (I). A stirred mixture of 96.0 g (0.64 mol) of 2*H*-1,4-benzoxazin-3(4*H*)-one,[2] 106 mL (1.05 mol) of benzaldehyde, 244 mL (2.58 mol) of Ac_2O, and 122 mL (0.87 mol) of NEt_3 was refluxed for 7 h. After this mixture was cooled for 5 days, the pale yellow product was collected on a filter and washed with CH_3CN to give 41.0 g of product, mp 261–263 °C. An additional 10.1 g of material was collected after the above filtrate was allowed to stand at 5 °C for several days. The above materials were combined and crystallized from 120 mL of hot DMF–240 mL of CH_3CN to give 46.0 g (30%) of pale yellow solid, mp 264–266 °C (reported[2] mp 260–261 °C).

The 2-arylidenebenzoxazin-3-ones shown in Table IV were prepared in the above manner using the known benzoxazin-3-ones as starting materials.

An experiment based on the reference procedure gave a 10% yield of product. No product was obtained when CH_3COOH was used in place of NEt_3 in the above procedure or in the case where the reaction was carried out in DMF using $NaOCH_3$. The latter method gave a good yield of product when benzaldehyde reacted with the corresponding 1,4-benzothiazin-3(4*H*)-one.[1] The starting material was recovered in both experiments.

Method A. 2-Benzylidene-4-[3-(dimethylamino)propyl]-2*H*-1,4-benzoxazin-3(4*H*)-one Hydrochloride (II, 3). A stirred suspension of 10.3 g (0.044 mol) of I in 85 mL of Me_2SO was treated with 2.2 g (0.046 mol) of NaH (50% oil dispersion). Foaming occurred as the temperature gradually rose to 35 °C. When the temperature began to drop, the mixture was warmed to 70 °C, cooled to 25 °C, treated with 31 mL of a 2.2 N solution of 3-dimethylaminopropyl chloride (0.068 mol) in toluene, and stirred at 70–75 °C for 5 h. The mixture was cooled to room temperature and poured into 700 mL of cold water and extracted with 200 mL of Et_2O (four times). The organic phases were combined and extracted with a cold solution of 7 mL of concentrated HCl in 100 mL of H_2O, followed by 50 mL of H_2O. The aqueous phases were combined, treated with 14 g of K_2CO_3, and extracted with 100 mL of Et_2O (four times). The organic phases were combined and dried ($MgSO_4$), and the solvent was evaporated to give 11.7 g of a pale yellow solid. A sample was crystallized from diisopropyl ether to give a nearly colorless solid, mp 91–93 °C. A solution of 11.3 g of this base in 90 mL of *i*-PrOH was treated with 1 equiv of HCl in EtOH to give 10.9 g (70%) of **3**, mp 191–193 °C. Following crystallization from 100 mL of *i*-PrOH,

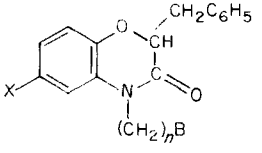
Table I. Chemical and Pharmacological Properties of 2-Arylidene-4-aminoalkyl-2*H*-1,4-benzoxazin-3(4*H*)-ones



Compd	X	Ar	*n*	B	Mp, °C	Recrystn solvent[a]	Yield, %	Formula	CNS act.,[b] ED, mg/kg ip: Depressant	Reflex
1	H	C_6H_5	2	$-NMe_2$	253–255	A	43	$C_{19}H_{20}N_2O_2 \cdot HCl$[c]	50	50
2	H	C_6H_5	2	$-NC_4H_8O$[d]	250–252	A	47	$C_{21}H_{22}N_2O_3 \cdot HCl$	>200	>200
3	H	C_6H_5	3	$-NMe_2$	196–198	B	55	$C_{20}H_{22}N_2O_2 \cdot HCl$	25	25
4	H	C_6H_5	3	$-NMe_3$	218–220	C	69	$C_{21}H_{25}N_2O_2Cl \cdot H_2O$	50	50
5	H	C_6H_5	3	$-NEt_2$	156–158	D	34	$C_{22}H_{26}N_2O_2 \cdot HCl$[c]	50	12.5
6	H	C_6H_5	3	$-NC_4H_8NCH_3$[d]	262–264	E	45	$C_{23}H_{27}N_3O_2 \cdot 2HCl$	50	12.5
7	H	C_6H_5	3	$-NC_4H_8NCH_2CH_2OH$[d]	266–268	F	10	$C_{24}H_{29}N_3O_3 \cdot 2HCl$	100	100
8	H	C_6H_5	3	$-NC_4H_8NC_6H_5$[d]	227–230	F	19	$C_{28}H_{29}N_3O_2 \cdot 2HCl$[c]	100	100
9	H	C_6H_5	4	$-NMe_2$	183–185	D	16	$C_{21}H_{24}N_2O_2 \cdot HCl$	>200	50
10	Cl	C_6H_5	3	$-NMe_2$	250–252	A	80	$C_{20}H_{21}ClN_2O_2 \cdot HCl$	>200	200
11	Cl	C_6H_5	3	$-NC_4H_8NCH_3$[d]	114–116	C	36	$C_{23}H_{26}ClN_3O_2$[e]	>200	>200
12	CH_3	C_6H_5	3	$-NMe_2$	124–126	C	36	$C_{21}H_{24}N_2O_2 \cdot C_4H_6O_4$[f]	100	100
13	CH_3O	C_6H_5	3	$-NMe_2$	232–234	A	39	$C_{21}H_{24}N_2O_3 \cdot HCl$[c]	>200	>200
14	H	2-Cl-C_6H_4	3	$-NMe_2$	222–224	B	50	$C_{20}H_{21}ClN_2O_2 \cdot HCl$	>200	100
15	H	4-Cl-C_6H_4	3	$-NMe_2$	253–255	G	48	$C_{20}H_{21}ClN_2O_2 \cdot HCl$	100	100
16	H	2-Thienyl	3	$-NMe_2$	209–211	B	48	$C_{18}H_{20}N_2O_2S \cdot HCl$	100	100
17	H	C_6H_5	0	-H (I)	264–266			$C_{15}H_{11}NO_2$	>200	>200
Meprobamate									50	25

[a] A = MeOH; B = *i*-PrOH; C = CH_3CN; D = MeCOEt; E = EtOH–MeOH; F = MeOH–Et_2O; G = EtOH. [b] All compounds were administered as 5% solutions or dispersions (containing 2 drops of Tween 80) in water at doses of 200, 100, and 50 mg/kg to young adult female rats. Compounds showing activity at 50 mg/kg were also tested at 25 and 12.5 mg/kg. The ED values are the lowest doses showing depressant (decreased motor activity and/or ataxia) or decreased reflex action (decreased grip strength or limb paralysis) in 2/2 rats. [c] Melting point of base: **1**, 89–91 °C; **5**, 60–62 °C; **8**, 129–131 °C; **10**, 132–134 °C; and **13**, 68–70 °C. Materials were crystallized from CH_3CN except **5** and **13** (diisopropyl ether). [d] NC_4H_8O = morpholino and NC_4H_8N = piperazino. [e] Because the dihydrochloride salt (mp 304–306 °C) became gelatinous on contact with water, this compound was tested as the base. The failure of this compound to show CNS activity may be due to the low solubility of the base in water. [f] $C_4H_6O_4$ = succinic acid. The HCl salt (mp 222–224 °C, from *i*-PrOH) became gelatinous on contact with water.

Table II. Chemical and Pharmacological Properties of 2-Benzyl-4-aminoalkyl-2*H*-1,4-benzoxazin-3(4*H*)-ones



Compd	X	*n*	B	Mp, °C[a]	Yield, %	Formula	CNS act.,[b] ED, mg/kg ip: Depressant	Reflex
18	H	2	$-N(CH_3)_2$	174–176	70	$C_{19}H_{22}N_2O_2 \cdot HCl$	>200	>200
19	H	3	$-N(CH_3)_2$	155–157	73	$C_{20}H_{24}N_2O_2 \cdot HCl$	100	50
20	H	3	$-NC_4H_8NCH_3$[c]	236–238	50	$C_{23}H_{29}N_3O_2 \cdot 2HCl$	>200	50
21	Cl	3	$-N(CH_3)_2$	185–187	67	$C_{20}H_{23}ClN_2O_2 \cdot HCl$	>200	100

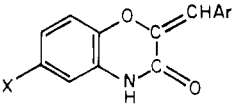
[a] Crystallization solvents: *i*-PrOH, **18**; *i*-PrOH–Et_2O, **19** and **21**; MeOH, **20**. [b] See footnote *b* of Table I. [c] NC_4H_8N = piperazino.

Table III. Dopamine Receptor Antagonist Activity of Compounds 1-17

Compd	I_{50} vs. DA-stimulated act.,[a] μM	I_{50} vs. basal act.,[b] μM
1	100	>100
2	I[c]	I[c]
3	33	600
4	650	1000
5	60	>100
6	35	450
7	54	>400
8	I[c]	I[c]
9	80	>200
10	100	>100
11	>500	>500
12	15	450
13	60	>100
14	30	170
15	30	450
16	56	>200
17	I[c]	I[c]
Fluphenazine	0.5	I[c]
Clozapine	6	I[c]

[a] Concentration of compound necessary to inhibit the stimulation by dopamine of adenylate cyclase activity by 50%. All I_{50} values are corrected for the effects of the compounds, if any, on adenylate cyclase activity in the absence of dopamine. Values are the average of 2-4 determinations. [b] Concentration of compound necessary to inhibit adenylate cyclase activity in the absence of dopamine by 50%. Values are the average of 2-4 determinations. [c] Inactive; compound inhibited adenylate cyclase activity in the absence or presence of dopamine by less than 10% at a concentration of 100 μM, except for the effect of fluphenazine on basal activity which was determined at 10 μM.

Table IV. Chemical Properties of 2-Arylidene-2*H*-1,4-benzoxazin-3(4*H*)-ones



X	Ar	Mp, °C[a]	Yield, %	Formula
Cl	C_6H_5	266-268	41	$C_{15}H_{10}ClNO_2$
CH_3	C_6H_5	269-271	18	$C_{16}H_{13}NO_2$
CH_3O	C_6H_5	237-239	21	$C_{16}H_{13}NO_3$
H	2-Cl-C_6H_4	248-250	21	$C_{15}H_{10}ClNO_2$
H	4-Cl-C_6H_4	331-333	42	$C_{15}H_{10}ClNO_2$
H	2-Thienyl	243-245	17	$C_{13}H_9NO_2S$

[a] Materials crystallized from DMF-CH_3CN.

the colorless product weighed 8.6 g (55%): mp 196-198 °C; IR (Nujol) 1687 (CO), 1638 cm^{-1} (C=C); NMR (Me_2SO-d_6) δ 2.10 (m, $CH_2CH_2CH_2$), 2.70 (s, Me_2N), 3.20 (m, $CH_2{}^+NH$), 4.10 (t, NCH_2, J = 7.0 Hz), 6.85 (s, C=CH), 7.40-7.90 (m, 9 Ar H).

Compounds 1-3, 10, and 12-16 were prepared by method A. The quaternary salt 4 was obtained by treating a solution of the base of 3 in CH_3CN (1 g/20 mL) with CH_3Cl at room temperature and allowing the mixture to stand overnight. The product crystallized from solution.

Method B. 2-Benzylidene-4-[3-(4-methyl-1-piperazinyl)propyl]-2*H*-1,4-benzoxazin-3(4*H*)-one Dihydrochloride (6). A suspension of 6.0 g (0.025 mol) of I (pulverized) in 125 mL of toluene was stirred vigorously and treated with 4.0 g (0.10 mol) of powdered NaOH. After 5 min, the mixture was treated with 13.0 g (0.034 mol) of 1-(3-bromopropyl)-4-methylpiperazine dihydrobromide[5] and then heated on a steam bath for 1 h. The mixture was cooled and treated with 50 mL of H_2O, and the product was isolated from the organic phase according to the procedure used in the preparation of II. The resulting oily base (7.3 g) was dissolved in 50 mL of EtOH and treated with 2 equiv of HCl in EtOH to give 7.3 g (65%) of 6, mp 254-256 °C. After recrystallization from a solution of 450 mL of EtOH-150 mL of MeOH, the nearly colorless material weighed 5.1 g (45%): mp 262-264 °C.

Method B was also used in the preparation of 5 and 11.

Method C. 2-Benzylidene-4-[4-(dimethylamino)butyl]-2*H*-1,4-benzoxazin-3(4*H*)-one Hydrochloride (9). Alkylation of 5.4 g (0.023 mol) of I with 6.6 g (0.039 mol) of $Cl(CH_2)_4Br$ by the procedure used in obtaining II gave 7.5 g of the intermediate chloro compound as a pale yellow oil. The latter was treated with NaI and excess $HN(CH_3)_2$ in benzene to give 6.1 g of oily base. The HCl salt was prepared in MeCOEt and recrystallized from the same solvent to give 1.3 g (16%) of colorless product, mp 183-185 °C. Compounds 7 and 8 were prepared from I in the same manner utilizing $Cl(CH_2)_3Br$ and the appropriate amine.

2-Benzyl-4-[3-(dimethylamino)propyl]-2*H*-1,4-benzoxazin-3(4*H*)-one Hydrochloride (III, 19). A suspension of 3.0 g (0.084 mol) of 3 in 100 mL of EtOH was treated with 1.0 g of Pd/C (5%) and shaken under 3 atm of hydrogen at room temperature for 4 h. The catalyst was filtered and washed with EtOH and the filtrate concentrated on a rotary evaporator to give a viscous residue. The latter was triturated with Et_2O to give 2.9 g (97%) of colorless product, mp 150-152 °C. After crystallization from a solution of 15 mL of *i*-PrOH-30 mL of Et_2O, the solid weighed 2.2 g (73%): mp 155-157 °C; IR (Nujol) 1680 cm^{-1} (CO); NMR (Me_2SO-d_6) δ 2.24 (m, $CH_2CH_2CH_2$), 2.77 (d, Me_2N, J = 3.0 Hz), 3.04 (m, $CH_2{}^+NH$), 3.14 (m, $CHCH_2$, J = 8.0, 4.0 Hz), 4.07 (t, NCH_2, J = 7.0 Hz), 4.76 (q, $CHCH_2$, J = 8.0, 4.0 Hz), 7.00-7.24 (m, 9 Ar H).

Compounds 18, 20, and 21 were obtained in a similar manner. In the case of 18, a mixture of MeOH and EtOH was used as a solvent and in the synthesis of 21, PtO_2 was used as a catalyst.

Alternative Synthesis of 19. A stirred suspension of 4.2 g (0.018 mol) of 2-benzyl-2*H*-1,4-benzoxazin-3(4*H*)-one[2] in 30 mL of DMF was treated with 0.9 g (0.019 mol) of NaH (50% oil dispersion). After the initial effervescence had subsided, the mixture was warmed to 70 °C, cooled to 25 °C, and treated with 15 mL of a 1.9 N solution of 3-dimethylaminopropyl chloride (0.028 mol) in toluene. This mixture was heated at 100-105 °C for 3 h, cooled to 25 °C, and poured into 300 mL of cold H_2O. The product was isolated according to the procedure used in the preparation of II to give 4.6 g of base. The latter gave 4.4 g (70%) of the colorless HCl salt, mp 155-157 °C. The IR spectra of 19 obtained by these two methods were identical.

Enzyme Preparation and Assay. Olfactory tubercles were dissected from the brains of male Sprague-Dawley rats (100-180 g), sacrificed by decapitation. The tissue was homogenized gently in 25 vol (w/v) of a Tris-maleate (2 mM)-EGTA (2 mM) buffer,[6] pH 7.4, and used directly in the assay.

Adenylate cyclase activity was assayed, generally in duplicate, by a combination of previously published procedures.[6,7] The assay mixture contained 2 mM $MgCl_2$, 80 mM Tris-maleate (pH 7.4), 0.6 mM ATP, 4-6 × 10^6 cpm [α-^{32}P]-ATP, 50 μL of tissue homogenate (equivalent to 2 mg of tissue, wet weight), 1 mM cAMP, and dopamine (20 μM) and antagonists, where indicated, in a final volume of 0.59 mL. The mixture was incubated for 5 min at 30 °C with shaking and then boiled for 3 min. An aliquot (0.1 mL) of a solution containing excess ATP (4 μmol) and cAMP (1.25 μmol), and [^{3}H]-cAMP (0.15 μCi) as a recovery standard, was added, and cAMP was isolated by Dowex 50 ion-exchange chromatography and treatment with nascent $BaSO_4$. The addition of 20 μM dopamine caused an increase in cyclase activity of 50-60% over that observed under basal conditions (no exogenous hormone added).

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Structure–Activity Relationships among the *O*-Acyl Derivatives of Leucomycin. Correlation of Minimal Inhibitory Concentrations with Binding to *Escherichia coli* Ribosomes

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The synthesis, antimicrobial activity, and binding to ribosomes of leucomycin and leucomycin derivatives are described. In general, the binding of the leucomycins and the leucomycin derivatives to ribosomes correlated with their antimicrobial activity. Some 2′-*O*-acyl derivatives apparently underwent gradual hydrolysis during antimicrobial assays, for their binding to ribosomes was poor compared to their relatively good antimicrobial activities. Correlation between antimicrobial activity and binding to ribosomes, their molecular site of action, provides some insight into the nature of the active molecular moieties.

We have studied the relationship between structure and microbial activity of 16-membered ring macrolide antibiotics, chiefly leucomycin and its derivatives.[1,2] It has been speculated that the presence of the aldehyde group on the lactone ring and the dimethylamino group on the mycaminose moiety may be essential for antimicrobial activity.[2,3] The combination of antimicrobial and cell-free assays permits direct evaluation of such hypotheses.

The macrolides bind to the 50S subunit of prokaryotic ribosomes and inhibit protein synthesis.[4] In addition, erythromycin derivatives and other macrolides compete for ribosomal binding sites.[5-7] Thus, the ability of a macrolide to compete with erythromycin for binding to ribosomes is related to its affinity for ribosomes. The ability to inhibit erythromycin binding in general paralleled the antibacterial activity of these derivatives.[3,7,8] However, where discrepancies between antibacterial activity and binding to ribosomes exist, other factors such as cellular permeability or modification of the antibiotic may be particularly relevant.[3,7,8]

In this paper, we describe the synthesis of acylated derivatives of leucomycin V. In addition, their antimicrobial activities (MIC) and their binding to ribosomes were evaluated.

Chemistry. The leucomycin complex is composed of ten components which differ in the groups (hydroxyl or acetyloxy) at the 3 position on the lactone ring and the acyl group at the 4″ position of mycarose.[9] These components were used as starting materials to obtain the various acyl derivatives. The acyl derivatives of leucomycin were obtained by the following general methods. The reaction of leucomycin V, 8 (LM-V), with acetic anhydride and pyridine yields tetraacetyl-LM-V, in which four hydroxyl groups (3 and 9 position on lactone ring, 2′ position on mycaminose, and 4″ position on mycarose) are acetylated in high yield. By treatment of the tetraacetate with methanol, the acetyl group at the 2′ position is selectively deacetylated because of the basicity of the dimethylamino group at the 3′ position adjacent to the hydroxyl group, yielding triacetyl-LM-V. Furthermore, instead of using acetic anhydride and pyridine, the reaction with acyl chloride in the presence of amines selectively yields only 9-*O*-acyl derivatives without acylation of the 2′-hydroxyl group. By controlling the reactivity of each hydroxyl group as described above, acetyl, propionyl, butyryl, monochloroacetyl, dichloroacetyl, 3-carboxypropionyl, 4-carboxybutyryl, and 2-methylbutyryl substitutions of the various hydroxyl groups of the leucomycin molecule were prepared. The structure of the leucomycin components and their acyl derivatives is shown in Table I. In addition, the mass spectral fragmentation peaks for each compound are summarized in Table I.

Results and Discussion

The concentrations at which [^{14}C]erythromycin binding was inhibited 50% by the leucomycin derivatives and the minimum inhibitory concentrations (MIC) are summarized in Table I. The minimal inhibitory concentration against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633, and *Klebsiella pneumoniae* ATCC 10031 was determined by the agar dilution method.

As can be seen from Table I, both 2′-*O*-acetyl-LM-A_5 (**10**) and 2′-*O*-propionyl-LM-A_5 (**11**) exhibited approximately the same antimicrobial activities against *S. aureus*, *B. subtilis*, and *K. pneumoniae* as LM-A_5 (**4**) containing a free 2′-hydroxyl. However, **4** inhibited [^{14}C]erythromycin binding to ribosomes much better than did **10** and **11**: much higher concentrations of compounds **10** and **11** (14.8 and 13.8 μM, respectively) were required to inhibit [^{14}C]erythromycin binding to ribosomes 50% than of compound **4** (1.1 μM). Thus, the decrease in affinity of 2′-*O*-acyl derivatives for ribosomes suggests that the presence of the 2′-hydroxyl of mycaminose might play a role in binding to ribosomes or that acylation of the 2′-