Anthelmintic Activity of 13-Alkoxy Milbemycin Derivatives

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A number of 13-alkoxy milbemycin derivatives were synthesized to evaluate their anthelmintic activity. We report the strategy for developing a potent anthelmintic product, 13-[4-(N-methanesulfonyl-N-methylamino)-phenethyloxy]milbemycin. The details of the structure-activity relationships of those derivatives are also discussed.

Milbemycin derivatives are known as potent antiparasitic agents and have attracted a great deal of interest recently. On the other hand, ivermectin, which is widely used as a potent parasiticide for livestock, is known to possess similar activity to milbemycins against endoparasites. The structures of the milbemycin derivatives and ivermectin closely resemble each other, having a 16-membered ring (Fig. 1), although ivermectin has higher activity than milbemycins.

This difference in the activity is due to the substituent at the 25-position. It is known that the lipophilicity on the substituent at the 25-position contributes to their anthelmintic activity (Table 1). Thus it seemed easier to synthesize the ivermectin derivatives than milbemycin derivatives to find more active products. However, owing to availability of substrates, we decided to develop new milbemycin derivatives, which have as strong activity as ivermectin. As it is also known that the substituent at the 13-position strongly influences the activity, a great number of substituents at the 13-position, which overcome the disadvantage of the substituent at the 25-position, have been examined. Evaluations of 13-halomilbemycin, 13-alkylmilbemycin, and 13-acyloxymilbemycin have already been reported. According to those studies, the 13-acyloxymilbemycins have quite strong activity and are thus very attractive, but the ester bond seems to be susceptible to esterase in vivo, producing the inactive 13-hydroxymilbemycin. That is why we aimed our research at 13-alkoxymilbemycins, which have an ether bond in place of the susceptible ester bond at the 13-position. The 13-alkoxymilbemycins also turned out to have strong anthelmintic activity, as reported in our previous studies.

Fig. 1. The structures of milbemycins and ivermectin.
Following this research, further detail of the structure-activity relationships was examined and 13-(4-substituted phenylethoxy)milbemycins, especially 13-[4-(N-methanesulfonyl-N-methylamino)-phenylethoxy]-milbemycin (I), proved to possess considerably high and efficient activity. That is, we finally found an ideal derivative.

We report the strategy for developing the series of 

Table 1. The relationships between the lipophilicity on the substituent at the 25-position ($R^2$) and activity.

<table>
<thead>
<tr>
<th>$R^1$</th>
<th>$R^2$</th>
<th>Dose (mg/kg)</th>
<th>Percent inhibition of growth of <em>N. brasiliensis</em> in the rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Me</td>
<td>1</td>
<td>47.3%</td>
</tr>
<tr>
<td>H</td>
<td>Et</td>
<td>1</td>
<td>81.8%</td>
</tr>
<tr>
<td>H</td>
<td>i-Pr</td>
<td>1</td>
<td>97.6%</td>
</tr>
<tr>
<td>H</td>
<td>s-Bu</td>
<td>0.25</td>
<td>86.7%</td>
</tr>
</tbody>
</table>

Scheme 1.

\[ \text{ROH} + \]

\[ \text{ROH} + \]

\[ \text{NaBH}_4, \text{MeOH} \]

\[ \text{HgI}_2, 2,6-	ext{Lutidine, Dichloroethane, b) Ag}_2\text{O, Dichloroethane, c) CuI, CF}_3\text{SO}_3\text{H, Dichloromethane} \]

a) $\text{HgI}_2$, 2,6-Lutidine, Dichloroethane, b) Ag$_2$O, Dichloroethane, c) CuI, CF$_3$SO$_3$H, Dichloromethane
derivatives to find an efficient and effective product and also describe the details of their structure-activity relationships.

Chemistry

Using our method already reported in previous papers10,11, a number of derivatives was synthesized from milbemycin A₄ via 13-iodomilbemycin (2)10) or 15-hydroxy-5-oxomilbemycin (3)11) (Scheme 1).

Biological Results and Discussion

The activity of this series of 13-alkoxymilbemycin derivatives was evaluated by oral administration to rats infected with *Nipposstrongylus brasiliensis* by use of the method described in the previous paper.

Firstly, the length of the carbon chain between the benzene ring and the oxygen atom (Fig. 2, position A) was examined (Table 2). Those results showed that the efficacy was the strongest when the number of the carbon chain was two (4, 5, 6). The efficacy clearly decreased when the chain was shorter or longer. In addition, this tendency was not dependent on the substituent at the benzene ring. Thus, the length of the carbon chain was fixed as two.

Secondly, substitution on the carbon chain was examined (Table 3). When the polar substituent was on the carbon chain, the efficacy decreased (7, 8). When the chain had a ring formation (9, 10), the efficacy did not change much compared to the straight chain. Regarding its availability and its facility in synthesis, the straight chain was chosen as a candidate.

Thirdly, the position of the substituent on the benzene ring (Fig. 2, position B) was examined (Table 4). The activity of the derivatives was maximized when the substituent on the benzene ring was at the p-position (4, 11). An amino (or substituted amino) group was chosen as a candidate to provide a greater number of potential derivatives for pursuing an ideal compound, although there did not seem to much difference in activity between the

Fig. 2. The examined position in the milbemycin derivative.

Table 2. Antiparasitic activity of derivatives which vary in the length of the carbon chain at position A in Fig. 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Efficacy (%) at dose rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25mg/kg</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2</td>
</tr>
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<tr>
<td>16</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

*: Percent inhibition of growth of *N. brasiliensis* in rats.
NT: not tested.
Table 3. Antiparasitic activity of derivatives which vary in the substituent at position A in Fig. 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Efficacy (%) at dose rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25mg/kg</td>
</tr>
<tr>
<td>7</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>82.3</td>
</tr>
<tr>
<td>8</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>27.2</td>
</tr>
<tr>
<td>9</td>
<td><img src="image3.png" alt="Structure" /> Isomer A</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td><img src="image4.png" alt="Structure" /> Isomer B</td>
<td>NT</td>
</tr>
<tr>
<td>18</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>73.5</td>
</tr>
<tr>
<td>19</td>
<td><img src="image6.png" alt="Structure" /> Me</td>
<td>86.7</td>
</tr>
<tr>
<td>20</td>
<td><img src="image7.png" alt="Structure" /> Me</td>
<td>98.4</td>
</tr>
</tbody>
</table>

*: Percent inhibition of growth of *N. brasiliensis* in rats.
NT: not tested.

Table 4. Antiparasitic activity of derivatives which vary in the position of the substituent on the benzene ring at position B in Fig. 2.

<table>
<thead>
<tr>
<th>Percent inhibition of growth of <em>N. brasiliensis</em> in rats at a dose of 0.25 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td><img src="image8.png" alt="Structure" /> H₂N-</td>
</tr>
<tr>
<td><img src="image9.png" alt="Structure" /> MeNHCONH-</td>
</tr>
</tbody>
</table>

*: Percent inhibition of growth of *N. brasiliensis* in rats.
amino group and the methoxy group (Table 2, compound 4 versus 5).

Lastly, the various N-substituted aminophenylethoxy derivatives were examined (Fig. 2, position C) (Table 5). From the evaluation, N-methanesulfonyl-N-methylamino-phenylethoxymilbemycin (1) turned out to possess considerably high and efficient activity.

### Experimental

$^1$H NMR spectra were recorded on a JNM GSX-400 spectrometer using TMS as the internal standard. Mass spectra were obtained on a JOEL FABmate.

4-(N-Methanesulfonyl-N-methylamino)phenylethoxymilbemycin (1)

4-Aminophenylethoxy-t-butyldimethylsilane

4-Nitrophenethyl alcohol (10.02 g, 60 mmol) was dissolved in DMF (70 ml), imidazole (5.44 g, 80 mmol) and t-butyldimethylsilyl chloride (12.08 g, 80 mmol) were added and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc (500 ml) and washed with H$_2$O twice, dried over anhydrous Na$_2$SO$_4$, and evaporated in vacuo. The residue was dissolved in 90% AcOH (300 ml), then the solution was cooled to 4°C and zinc dust (30 g) was added. The mixture was stirred at room temperature for 20 minutes, and then the mixture was diluted with EtOAc (700 ml) and filtered. The filtrate was washed with H$_2$O twice, dried over anhydrous Na$_2$SO$_4$, and evaporated in vacuo. The residue

### Table 5. Antiparasitic activity of derivatives which vary in the substituent on the benzene ring at position C in Fig. 2.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Efficacy (%) $^*$ at dose rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.125mg/kg</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>68.1</td>
</tr>
<tr>
<td>11</td>
<td>93.3</td>
</tr>
<tr>
<td>25</td>
<td>99.7</td>
</tr>
<tr>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td>27</td>
<td>100</td>
</tr>
<tr>
<td>28</td>
<td>92.8</td>
</tr>
</tbody>
</table>

$^*$: Percent inhibition of growth of N. brasiliensis in rats.  
NT: not tested.
was chromatographed on silica gel with the eluent (EtOAc : cyclohexane = 1 : 3) to obtain 4-aminophenylethoxy-t-butyldimethylsilane (12.55 g, 83.2% yield). (2) 4-(N-Methansulfonyl-N-methylamino)phenethyl Alcohol

4-Aminophenylethoxy-t-butyldimethylsilane was dissolved in 1,2-dichloroethane (20 ml), pyridine (2.0 ml) and methanesulfonyl chloride (1.63 ml, 21 mmol) were added. The mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc, then washed with 1 N-HCl, H2O, 4% NaHCO3, and H2O again, dried over anhydrous Na2SO4 and evaporated in vacuo. The residue was dissolved in N-methylpyrrolidone (100 ml), iodomethane (1.56 ml, 25 mmol) and sodium hydride (55%, 873 mg, 20 mmol) were added, and the mixture was stirred at room temperature for 3 hours. The reaction mixture was poured into cold diluted HCl, extracted with EtOAc, and washed with H2O, dried over anhydrous Na2SO4, and evaporated in vacuo. The residue was dissolved in MeOH (50 ml), p-toluenesulfonic acid monohydrate (50 mg) was added and the mixture was stirred at room temperature for 20 minutes. The reaction mixture was diluted with EtOAc and washed with 4% NaHCO3 and H2O again, dried over anhydrous Na2SO4, and evaporated in vacuo. The residue was dissolved in MeOH (50 ml), then sodium borohydride (1.20 g) was added and the mixture was stirred at -40°C for 10 minutes. Then the reaction mixture was diluted with acetone (20 ml), warmed to 0°C, then EtOAc was added. The solution was washed with H2O three times, dried over anhydrous Na2SO4, then evaporated in vacuo. The residue was crystallized from EtOAc and hexane to obtain 1 (8.20 g) as crystals. Then the filtrate was chromatographed on silica gel with the eluent (EtOAc : hexane = 2 : 1) to retrieve additional 1 (0.887 g) from the filtrate. Thus, the total amount of 1 was 9.09 g (84.0% yield).

13-(4-Aminophenylethoxy)-5-hydroxymilbemycin (4)

(1) 13-(4-Nitrophenylethoxy)-5-oxomilbemycin

4-Nitrophenethyl alcohol (4.35 g, 26.0 mmol) was dissolved in 1,2-dichloroethane (25 ml), copper(I) iodide (1.05 g, 5.51 mmol), trifluoromethanesulfonic acid (0.77 ml), and a solution of 15-hydroxy-5-oxomilbemycin (5.35 mmol) in 1,2-dichloroethane (5 ml) were added. The mixture was stirred at room temperature for 25 minutes. The reaction mixture was diluted with EtOAc and washed with H2O, 4% NaHCO3, and H2O again, dried over anhydrous Na2SO4, then evaporated in vacuo. The residue was chromatographed on silica gel with the eluent (EtOAc : cyclohexane = 1 : 3) to obtain 13-(4-nitrophenylethoxy)-5-oxomilbemycin (3.12 g, 82.5% yield).

(2) 13-(4-Aminophenylethoxy)-5-hydroxymilbemycin (4)

13-(4-Nitrophenylethoxy)-5-oxomilbemycin (1.685 g, 2.43 mmol) was dissolved in MeOH (33 ml) and cooled to 4°C, and then sodium borohydride (91 mg, 2.4 mmol) was added. The mixture was stirred at 4°C for 20 minutes. The reaction mixture was diluted with EtOAc, washed with H2O twice, dried over anhydrous Na2SO4, then evaporated in vacuo. The residue was dissolved in AcOH (15 ml) and zinc dust (1.5 g) was added. The mixture was stirred at room temperature for 20 minutes in a water bath. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with H2O three times, dried over anhydrous Na2SO4, and evaporated in vacuo. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain 4 (1.39 g, 86.2% yield).
13-(3,4-Dimethoxyphenylethoxy)-5-hydroxymilbemycin (5)

(1) 13-(3,4-Dimethoxyphenylethoxy)-5-oxomilbemycin

13-Iodomilbemycin (333 mg, 0.5 mmol) was dissolved in 1,2-dichloroethane (2.5 ml), 3,4-dimethoxyphenethyl alcohol (911.0 mg, 5.0 mmol) and silver oxide (1.0 g) were added. The mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with EtOAc, washed with 10% NaHCO₃, and H₂O, dried over anhydrous Na₂SO₄, then evaporated in vacuo. The residue was chromatographed on silica gel with the eluent (EtOAc:cyclohexane=1:4) to obtain 13-(3,4-dimethoxyphenylethoxy)-5-oxomilbemycin (113.3 mg, 31.4% yield).

(2) 13-(3,4-Dimethoxyphenylethoxy)-5-hydroxymilbemycin (5)

13-(3,4-Dimethoxyphenylethoxy)-5-oxomilbemycin (113.3 mg, 0.157 mmol) was dissolved in MeOH (3.7 ml) and the solution was cooled to 4 ºC, sodium borohydride (6.3 mg) was added. The mixture was stirred at 4 ºC for 30 minutes. The reaction mixture was diluted with EtOAc, washed with H₂O twice, dried over anhydrous Na₂SO₄, and evaporated in vacuo. The residue was chromatographed on silica gel with the eluent (EtOAc:cyclohexane=35:65) to obtain 5 (69.0 mg, 60.8% yield): 1H NMR (CDCl₃) δ 6.79 (1H, d, J=8.8 Hz, Ph-H), 6.75 (1H, s, Ph-H), 6.73 (1H, d, J=8.8 Hz, Ph-H), 5.70-5.84 (2H, m, C₉-H and C₁₀-H), 5.40 (1H, s, C₃-H), 5.17 (1H, m, C₁₅-H), 4.58 and 4.78 (2H, AB-q, J=15 Hz, C₂₇-CH₂), 4.29 (1H, d, J=6.2 Hz, C₅-H), 3.98 (1H, br-s, C₇-OH), 3.96 (1H, d, J=6.2 Hz, C₆-H), 3.87 and 3.86 (6H, two-s, OCH₃), 3.58 (1H, m, C₁₇-H), 3.53 and 3.30 (2H, m, C₁₃-OCH₂), 3.28 (3H, s, OCH₃), 3.03 (1H, m, C₁₃-H), 3.06 (1H, m, C₂₅-H), 2.80 (2H, m, PhCH₂), 2.17 (3H, s, CH₃CO), 1.87 (3H, s, C₄-CH₃), 1.04 (3H, d, J=6.0 Hz, C₁₃-CH₃), 0.98 (3H, dt, J=7.7 Hz, C₂₃-CH₃CH₂), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-Methoxy-2-phenylethoxy)-5-hydroxymilbemycin (7)

Derivative 7 was prepared from 13-iodomilbemycin and 2-methoxy-2-phenylethanol in a similar manner as that described for the preparation of 5: MS m/z=692 (M⁺); 1H NMR (CDCl₃) δ 7.2-7.4 (5H, m, Ph-H), 6.75 (1H, s, Ph-H), 6.73 (1H, d, J=8.8 Hz, Ph-H), 5.40 (1H, s, C₃-H), 5.17 (1H, m, C₁₅-H), 4.68 (2H, s, C₂₇-CH₂), 3.99 (1H, s, C₁₅-OH), 3.96 (1H, d, J=6.2 Hz, C₃-H), 3.55 (1H, m, C₁₅-H), 3.33 (1H, d, J=9.9 Hz, C₁₅-H), 3.28 (3H, s, OCH₃), 3.03 (1H, m, C₂₅-H), 1.87 (3H, s, C₂₅-CH₂), 1.10 (3H, d, J=6.2 Hz, C₁₂-CH₃), 0.98 (3H, t, J=7.3 Hz, C₂₅-CH₃CH₂), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-Hydroxy-2-phenylethoxy)-5-hydroxymilbemycin (8)

(1) 13-[2-Phenyl-2-(tetrahydro-pyran-2-yloxy)ethyloxy]-5-oxomilbemycin

13-Iodomilbemycin (500 mg, 0.75 mmol) was dissolved in 1,2-dichloroethane (2.5 ml), 2-phenyl-2-(tetrahydro-pyran-2-yloxy)ethanol (836 mg, 3.75 mmol), 2,6-lutidine (0.09 ml, 0.78 mmol), and mercury(II) iodide (511 mg, 1.125 mmol) were added. The mixture was stirred at room temperature for 90 minutes. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with 20% sodium iodide twice, 10% NaHCO₃, 0.5M citric acid, and H₂O, dried over anhydrous Na₂SO₄, and evaporated in vacuo. The residue was chromatographed on silica gel with the eluent (EtOAc:cyclohexane=1:4) to obtain 13-[2-phenyl-2-(tetrahydro-pyran-2-yloxy)ethyloxy]-5-oxomilbemycin (349 mg, 52.9% yield).

(2) 13-(2-Hydroxy-2-phenylethoxy)-5-hydroxymilbemycin (8)

13-Iodomilbemycin (333 mg, 0.5 mmol) was dissolved in 1,2-dichloroethane (2.5 ml), 2-phenyl-2-(tetrahydro-pyran-2-yloxy)ethanol (836 mg, 3.75 mmol), 2,6-lutidine (0.09 ml, 0.78 mmol), and mercury(II) iodide (511 mg, 1.125 mmol) were added. The mixture was stirred at room temperature for 90 minutes. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with 20% sodium iodide twice, 10% NaHCO₃, 0.5M citric acid, and H₂O, dried over anhydrous Na₂SO₄, and evaporated in vacuo. The residue was chromatographed on silica gel with the eluent (EtOAc:cyclohexane=1:4) to obtain 13-[2-phenyl-2-(tetrahydro-pyran-2-yloxy)ethyloxy]-5-oxomilbemycin (349 mg, 52.9% yield).
2-phenylethoxy)-5-oxomilbemycin. A part of the residue (178 mg, 0.234 mmol) was dissolved in MeOH (7.0 ml), cooled to 4°C, and then sodiumborohydride (9.4 mg) was added. The mixture was stirred at 4°C for 15 minutes. The reaction mixture was diluted with EtOAc, washed with H2O twice, dried over anhydrous Na2SO4, then evaporated in vacuo. The residue was chromatographed on ODS, with the eluent (80% acetonitrile) to obtain 8 (173 mg, quantitative): MS m/z=660 (M-H2O); 1H NMR (CDCl3) δ 7.25~7.40 (5H, m, Ph-H), 5.40 (1H, s, C2-H), 5.18 (1H, m, C3-H), 4.88 (0.5H, dd, J=4.0 and 8.0 Hz, PhCH), 4.83 (0.5H, dd, J=3.3 and 8.8 Hz, PhCH), 4.69 (2H, s, C27-CH2), 4.29 (1H, br-s, C7-OH), 3.96 (1H, d, J=6.2 Hz, C6-H), 3.56 (1H, m, C5-H), 4.00 (1H, br-s, C7-OH), 3.96 (1H, d, J=6.2 Hz, C6-H), 3.60 (1H, m, C17-H), 3.36 (1H, d, J=9.9Hz, C13-H), 3.25~3.43 (2H, m, C2-H and C13-OCH2), 3.24 (0.5H, d, J=9.9Hz, C13-OCH2), 3.17 (0.5H, d, J=9.9Hz, C13-OCH2), 3.25-3.43 (2H, m, C2-H and C13-OCH2), 3.27 (1H, s, C4-CH3), 2.82 (3H, d, J=6.6 Hz, C24-CH3); and 10 (25 mg) was chromatographed on ODS with the eluent (80% acetonitrile) to obtain 9 (24 mg): MS m/z=715 (M-CH3NH2); 1H NMR (CDCl3) δ 6.90~7.20 (3H, m, Ph-H), 6.19 (1H, br-s, NH), 5.41 (1H, s, C7-OH), 5.24 (1H, m, C15-H), 4.70~4.67 (2H, AB-q, J=14.7 Hz, C27-CH2), 4.01 (1H, s, C2-OH), 3.96 (1H, d, J=6.2 Hz, C6-H), 3.60 (1H, m, C17-H), 3.36 (1H, d, J=9.9 Hz, C13-H), 3.27 (1H, m, C2-H), 2.82 (3H, d, J=4.8 Hz, N-CH3), 1.88 (3H, s, C27-CH3), 1.03 (3H, d, J=6.3 Hz, C15-H), 1.00 (3H, t, J=7.3 Hz, C25-CH2CH3), 0.82 (3H, d, J=6.2 Hz, C24-CH3).

13-{5-[(N-Methylcarbamoyl)-amino]indan-2-yl oxy}-5-hydroxymilbemycin (9) and (10)

13-(5-Nitro-2-indanyloxy)-5-oxomilbemycin (130 mg, 0.189 mmol) was dissolved in 1,2-dichloroethane (1.5 ml), methyl isocyanate (0.189 mmol) was added. The mixture was stirred at room temperature for 90 minutes. Then the reaction mixture was evaporated in vacuo. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain 9 (24 mg): MS m/z=715 (M-CH3NH2); 1H NMR (CDCl3) δ 6.90~7.20 (3H, m, Ph-H), 6.19 (1H, br-s, NH), 5.41 (1H, s, C7-OH), 5.24 (1H, m, C15-H), 4.70~4.67 (2H, AB-q, J=14.7 Hz, C27-CH2), 4.01 (1H, s, C2-OH), 3.96 (1H, d, J=6.2 Hz, C6-H), 3.60 (1H, m, C17-H), 3.36 (1H, d, J=9.9 Hz, C13-H), 3.27 (1H, m, C2-H), 2.82 (3H, d, J=4.8 Hz, N-CH3), 1.88 (3H, s, C27-CH3), 1.03 (3H, d, J=6.3 Hz, C15-H), 1.00 (3H, t, J=7.3 Hz, C25-CH2CH3), 0.82 (3H, d, J=6.6 Hz, C24-CH3); and 10 (25 mg) MS m/z=715 (M-CH3NH2); 1H NMR (CDCl3) δ 6.90~7.20 (3H, m, Ph-H), 6.19 (1H, br-s, NH), 5.41 (1H, s, C7-OH), 5.24 (1H, m, C15-H), 4.70~4.67 (2H, AB-q, J=14.7 Hz, C27-CH2), 4.01 (1H, s, C2-OH), 3.96 (1H, d, J=6.2 Hz, C6-H), 3.60 (1H, m, C17-H), 3.36 (1H, d, J=9.9 Hz, C13-H), 3.27 (1H, m, C2-H), 2.82 (3H, d, J=4.8 Hz, N-CH3), 1.88 (3H, s, C27-CH3), 1.03 (3H, d, J=6.3 Hz, C15-H), 1.00 (3H, t, J=7.3 Hz, C25-CH2CH3), 0.82 (3H, d, J=6.6 Hz, C24-CH3).

13-(5-Amino-2-indanyloxy)-5-hydroxymilbemycin (11)

Derivative 11 was prepared from 4 in a similar manner as that described for the preparation of 9-(3).
2.77 mmol) was dissolved in MeOH (37 ml) and cooled to 4°C, and then sodium borohydride (91 mg, 2.4 mmol) was added. The mixture was stirred at 4°C for 15 minutes. The reaction mixture was diluted with EtOAc, washed with H2O twice, dried over anhydrous Na2SO4, then evaporated in vacuo. The residue was chromatographed on ODS with the eluent (85% acetonitrile) to obtain 13-(4-nitrobenzyloxy)-5-hydroxymilbemycin (1.432 g, 74.6% yield).

(3) 13-(4-Aminobenzyloxy)-5-hydroxymilbemycin (12)

13-(4-Nitrobenzyloxy)-5-hydroxymilbemycin (1.2 g, 1.73 mmol) was dissolved in 90% AcOH (12 ml), zinc dust (1.2 g) was added. The mixture was stirred at room temperature for 20 minutes in a water bath. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with H2O four times, dried over anhydrous Na2SO4, then evaporated in vacuo. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain 12 (1.092 g, 94.9% yield): MS m/z=663 (M+); 1H NMR (CDCl3) δ 7.09 (2H, d, J=8.4 Hz, Ph-H), 6.60 (2H, d, J=8.4 Hz, Ph-H), 5.40 (1H, s, C3-H), 4.68 (2H, s, C27-H), 4.34 and 4.06 (2H, AB-q, J=11.4 Hz, PhCH2), 4.28 (1H, m, C5-H), 3.96 (1H, s, C7-OH), 3.95 (1H, d, J=6.2 Hz, C6-H), 3.65 (2H, br-s, NH2), 3.60 (1H, m, C17-H), 3.30 (1H, d, J=9.8 Hz, C13-H), 3.27 (1H, m, C2-H), 3.09 (1H, m, C25-H), 1.87 (3H, s, C4-CH3), 1.06 (3H, d, J=6.6 Hz, C12-CH3), 0.99 (3H, t, J=7.1 Hz, C25-CH2CH3), 0.84 (3H, d, J=6.6 Hz, C24-CH3).

13-[3-(3,4-Dimethoxyphenyl)propyloxy]-5-hydroxymilbemycin (15)

Derivative 15 was prepared from 13-iodomilbemycin and 3-(3,4-dimethoxyphenyl)propyl alcohol in a similar manner as that described for the preparation of 5: MS m/z=736 (M+); 1H NMR (CDCl3) δ 7.20–7.35 (5H, m, Ph-H), 5.70–5.85 (2H, m, C9-H and C10-H), 5.40 (1H, m, C3-H), 4.68 (2H, s, C27-CH2), 4.30 (1H, m, C5-H), 3.95 (1H, d, J=6.2 Hz, C6-H), 3.87 and 3.86 (6H, two-s, OCH3), 3.58 (1H, m, C17-H), 3.28 (1H, m, C2-H), 3.20 (1H, d, J=9.8 Hz, C13-H), 1.88 (3H, s, C4-CH3), 1.13 (3H, d, J=6.6 Hz, C12-H), 0.99 (3H, t, J=7.3 Hz, C25-CH2CH3), 0.83 (3H, d, J=6.2 Hz, C24-CH3).

13-[4-(Acetylamino)benzyloxy]-5-hydroxymilbemycin (16)

Derivative 16 was prepared from 12 in a similar manner as that described for the preparation of 6: 1H NMR (CDCl3) δ 0.84 (3H, d, J=6.5 Hz, C-24 CH3), 0.99 (3H, t, J=7.3 Hz, C-25 CH2CH3), 1.08 (3H, d, J=6.4 Hz, C-12 CH3), 1.87 (3H, s, C-4 CH3), 2.18 (3H, s, acetyl H), 3.09 (1H, m, C-25 CH2), 3.58 (1H, m, C-17 H), 3.96 (1H, d, J=6.2 Hz, C-6 H), 4.29 (1H, d, J=5.9 Hz, C-5 H), 4.67 and 4.70 (2H, ABq, J=14.5 Hz, C-27 H), 7.31 (2H, d, J=8.2 Hz, Ph-H), 7.47 (2H, d, J=8.2 Hz, Ph-H).

13-[3-(4-N-Acetylaminophenyl)propyloxy]-5-hydroxymilbemycin (17)

The derivative 17 was prepared from 13 in a similar manner as described for the preparation of 6.

13-(2-Phenylethoxy)-5-hydroxymilbemycin (18)

Derivative 18 was prepared from 13-iodomilbemycin and phenethyl alcohol in a similar manner as that described for the preparation of 5: MS m/z=662 (M+); 1H NMR (CDCl3) δ 7.20–7.35 (5H, m, Ph-H), 5.40 (1H, m, C3-H), 4.68 (2H, s, C27-CH2), 4.30 (1H, m, C5-H), 3.95 (1H, d, J=6.2 Hz, C6-H), 3.94 (1H, d, J=10.0 Hz, C13-H), 2.87 (2H, m, PhCH2), 1.87 (3H, s, C4-CH3), 1.04 (3H, d, J=6.6 Hz, C12-CH3), 0.98 (3H, t, J=7.3 Hz, C25-CH2CH3), 0.82 (3H, d, J=6.2 Hz, C24-CH3).

13-(1-Methyl-2-phenylethoxy)-5-hydroxymilbemycin (19)

Derivative 19 was prepared from 13-iodomilbemycin and 1-methyl-2-phenylethanol in a similar manner as that described for the preparation of 5: MS m/z=676 (M+); 1H NMR (CDCl3) δ 7.10–7.40 (5H, m, Ph-H), 5.40 (1H, m, C3-H), 4.68 (1H, s, C27-CH3), 4.66 (1H, s, C25-CH3), 4.28 (1H, m, C5-H), 3.95 (0.5H, d, J=6.2 Hz, C24-CH3), 3.94 (0.5H, d, J=6.2 Hz, C24-CH3).
d, J=6.2 Hz, C₂₅-H), 3.34 (0.5H, d, J=9.9 Hz, C₁₃-H), 3.28 (0.5H, d, J=9.9 Hz, C₁₃-H), 3.10 (0.5H, m, C₂₅-H), 3.06 (0.5H, m, C₂₅-H), 1.87 (3H, s, C₄-CH₃), 0.84 (1.5H, d, J=6.2 Hz, C₂₄-CH₃).

13-(2-Methyl-2-phenylethoxy)-5-hydroxymilbemycin (20)

Derivative 20 was prepared from 13-iodomilbemycin and 2-methyl-2-phenylethanol in a similar manner as that described for the preparation of 5: MS m/z=676 (M⁺); ¹H NMR (CDCl₃) δ 7.15-7.35 (5H, m, Ph-H), 5.40 (1H, s, C₃-H), 5.15 (1H, s, C₁₅-H), 4.67 (2H, s, C₂₇-CH₂), 4.29 (1H, m, C₁₇-H), 3.96 (1H, s, C₇-OH), 3.95 (1H, d, J=5.9 Hz, C₆-H), 3.18 (1H, d, J=9.9 Hz, C₁₅-H), 3.05 (1H, m, C₂₅-H), 2.84 (3H, d, J=5.1 Hz, NCH₃), 1.83 (3H, s, C₄-CH₃), 1.02 (3H, d, J=6.6 Hz, C₁₂-CH₃), 0.97 (3H, t, J=7.3 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-(2-Aminophenyl)ethyloxy)-5-hydroxymilbemycin (21)

Derivative 21 was prepared from 15-hydroxy-5-oxomilbemycin and 2-nitrophenethyl alcohol in a similar manner as that described for the preparation of 4: MS m/z=677 (M⁺); ¹H NMR (CDCl₃) δ 7.0-7.1 (2H, m, Ph-H), 6.7-6.8 (2H, m, Ph-H), 5.7-5.8 (2H, m, C₉ and C₁₀-H), 5.40 (1H, s, C₃-H), 5.39 (1H, s, C₃-H), 4.67 (2H, s, C₂₇-CH₂), 4.29 (1H, d, J=6.2 Hz, C₂₅-H), 3.98 (3H, d, J=6.6 Hz, C₁₂-CH₃), 0.98 (3H, t, J=7.3 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-(3-Aminophenyl)ethyloxy)-5-hydroxymilbemycin (22)

Derivative 22 was prepared from 15-hydroxy-5-oxomilbemycin and 3-nitrophenethyl alcohol in a similar manner as that described for the preparation of 4: MS m/z=677 (M⁺); ¹H NMR (CDCl₃) δ 7.14 (1H, m, Ph-H), 6.7-6.8 (3H, m, Ph-H), 5.40 (1H, s, C₃-H), 5.15 (1H, s, C₁₅-H), 4.68 (2H, s, C₂₇-CH₂), 4.29 (1H, m, C₁₇-H), 3.06 (1H, m, C₂₅-H), 2.83 (3H, d, J=4.8 Hz, NCH₃), 1.87 (3H, s, C₄-CH₃), 1.03 (3H, d, J=6.6 Hz, C₁₂-CH₃), 0.98 (3H, t, J=7.5 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-(2-N-Methylcarbamoylaminophenyl)ethyloxy)-5-hydroxymilbemycin (23)

The derivative 23 was prepared from 21 in a similar manner as that described for the preparation of 9-(3): MS m/z=703 (M⁻CH₃NH₂⁺); ¹H NMR (CDCl₃) δ 7.0-7.5 (4H, m, Ph-H), 5.40 (1H, s, C₃-H), 5.15 (1H, s, C₁₅-H), 4.67 (2H, s, C₂₇-CH₂), 4.29 (1H, m, C₁₇-H), 3.96 (1H, s, C₇-OH), 3.95 (1H, d, J=5.9 Hz, C₆-H), 3.18 (1H, d, J=9.9 Hz, C₁₅-H), 3.05 (1H, m, C₂₅-H), 2.84 (3H, d, J=5.1 Hz, NCH₃), 1.83 (3H, s, C₄-CH₃), 1.02 (3H, d, J=6.6 Hz, C₁₂-CH₃), 0.97 (3H, t, J=7.3 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-(3-N-Methylcarbamoylaminophenyl)ethyloxy)-5-hydroxymilbemycin (24)

Derivative 24 was prepared from 22 in a similar manner as that described for the preparation of 9-(3): ¹H NMR (CDCl₃) δ 7.22 (1H, m, Ph-H), 7.17 (1H, s, Ph-H), 7.07 (1H, d, J=8.1 Hz, Ph-H), 6.96 (1H, d, J=7.3 Hz, Ph-H), 6.25 (1H, s, NH), 5.40 (1H, s, C₃-H), 5.16 (1H, m, C₁₅-H), 4.29 (1H, m, C₁₅-H), 4.00 (1H, s, C₇-OH), 3.96 (1H, d, J=5.8 Hz, C₇-OH), 3.21 (1H, d, J=9.6 Hz, C₁₅-H), 3.06 (1H, m, C₂₅-H), 2.83 (3H, d, J=4.8 Hz, NCH₃), 1.87 (3H, s, C₄-CH₃), 1.03 (3H, d, J=6.6 Hz, C₁₂-CH₃), 0.98 (3H, t, J=7.5 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-(4-Methoxycarbonylaminophenyl)ethyloxy)-5-hydroxymilbemycin (25)

Derivative 25 was prepared from 4 and methyl chloroformate in a similar manner as that described for the preparation of 6: MS m/z=764 (M⁺); ¹H NMR (CDCl₃) δ 8.80 (1H, s, NH), 7.54 (2H, d, J=8.8 Hz, Ph-H), 7.22 (2H, d, J=8.8 Hz, Ph-H), 5.40 (1H, s, C₇-OH), 5.16 (1H, m, C₁₅-H), 4.68 (2H, s, C₂₇-CH₂), 4.29 (1H, m, C₁₅-H), 3.70 (3H, s, OCH₃), 3.20 (1H, d, J=9.9 Hz, C₁₅-H), 3.06 (1H, m, C₂₅-H), 2.83 (2H, m, PhCH₂), 1.87 (3H, s, C₄-CH₃), 1.03 (3H, d, J=6.6 Hz, C₁₂-CH₃), 0.98 (3H, t, J=7.3 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-(2-(4-Methanesulfonylaminophenyl)ethyloxy)-5-hydroxymilbemycin (26)

Derivative 26 was prepared from 4 and methanesulfonyl chloride in a similar manner as that described for the preparation of 6.

13-(2-(4-Cyanoacetylaminophenyl)ethyloxy)-5-hydroxymilbemycin (27)

Cyanoacetic acid (42.5mg, 0.50mmol) was dissolved in 1,2-dichloroethane (2.5 ml) and the solution was cooled to 4°C. Then pyridine (0.05 ml), 2-chloroformyl-1,2,4-
triazolo[4,3-a]pyridin-3-one\textsuperscript{12} (100 mg, 0.50 mmol) and 4 (203 mg, 0.30 mmol) were added. The mixture was stirred at room temperature for 90 minutes then at 35°C for an extra hour. The reaction mixture was diluted with EtOAc, washed with 1 N-HCl, H₂O, 4% NaHCO₃, and H₂O again, dried over anhydrous Na₂SO₄, then evaporated in vacuo. The residue was chromatographed on ODS with the eluent (80% acetonitrile) to obtain crude 27. The crude was chromatographed on silica gel with the eluent (EtOAc:cyclohexane=3:1) to obtain pure 27 (120 mg, 53.7% yield): 1H NMR (CDCl₃) δ 7.70 (1H, s, NH), 7.40 (2H, d, J=8.4 Hz, Ph-H), 7.20 (2H, d, J=8.4 Hz, Ph-H), 5.40 (1H, s, C₃-H), 5.17 (1H, m, C₁₅-H), 4.68 (2H, s, C₂₇-H), 4.29 (1H, m, C₅-H), 3.98 (1H, s, C₇-OH), 3.95 (1H, d, J=6.2 Hz, C₆-H), 3.54 (2H, s, NCCH₂), 3.20 (1H, d, J=9.9 Hz, C₁₃-H), 3.06 (1H, m, C₂₅-H), 2.82 (2H, m, PhCH₂), 1.87 (3H, s, C₄-CH₃), 1.03 (3H, d, J=6.6 Hz, C₁₂-CH₃), 0.98 (3H, t, J=7.3 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

13-[2-(4-Methoxyacetylaminophenyl)ethyloxy]-5-hydroxymilbemycin (28)

Derivative 28 was prepared from 4 and methoxyacetyl chloride in a similar manner as that described for the preparation of 6: MS m/z=749 (M⁺); 1H NMR (CDCl₃) δ 8.19 (1H, s, NH), 7.47 (2H, d, J=8.4 Hz, Ph-H), 7.17 (2H, d, J=8.4 Hz, Ph-H), 5.40 (1H, m, C₁₅-H), 4.68 (2H, s, C₂₇-H), 4.29 (1H, d, J=6.6 Hz, C₂₅-H), 3.95 (1H, s, C₇-OH), 3.91 (1H, d, J=6.6 Hz, C₂₄-CH₃), 3.85 (3H, t, J=7.3 Hz, C₂₅-CH₂CH₃), 0.82 (3H, d, J=6.6 Hz, C₂₄-CH₃).

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References