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# A novel microbial metabolite, activator of low density lipoprotein receptor promoter

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**Abstract**

The organic extract of the fermentation broth of a *Micromonospora* microorganism was found to contain SCH 351448 (**1**), the monosodium salt of a macrocyclic dilactone containing two identical diacids. The structure and relative stereochemistry were established by single-crystal X-ray analysis. SCH 351448 is a novel ionophoric compound and is a weak activator of low density lipoprotein receptor (LDL-R) promoter with an $IC_{50}$ of 25 μM.


LDL uptake by the LDL receptor (LDL-R) is an important mechanism for clearing serum cholesterol. LDL-R levels are regulated at the transcriptional level by the abundance of cholesterol and its metabolites in the cell membranes. High membrane cholesterol levels inhibit the cleavage of sterol response element binding proteins (SREBPs).[1] When cholesterol levels drop, SREBPs are cleaved from the membrane[2] allowing them to enter the nucleus and activate promoters containing sterol response elements, including LDL-R promoter. As part of our continuing investigation of natural products as leads for cholesterol reducing agents, screening of the ethyl acetate extracts of several microbial fermentation broths revealed that a microorganism belonging to *Micromonospora* sp. yielded an extract that displayed distinct activity in the LDL-R assay.[3] Bioassay-guided fractionation of this extract led to the isolation of **1**.

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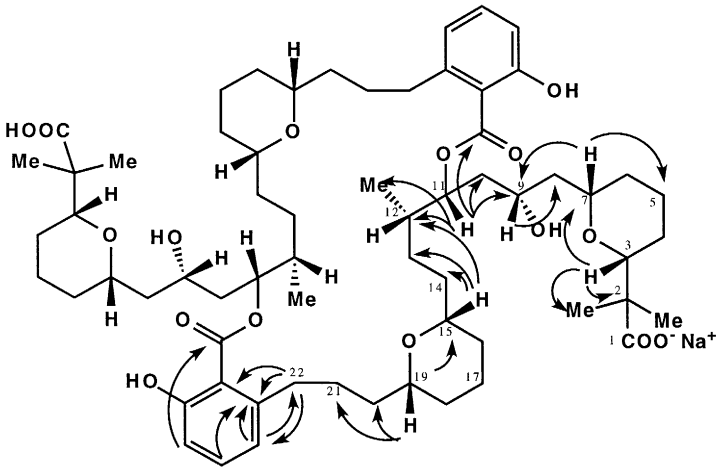

**1**

Table 1
$^{1}H$ and $^{13}C$ NMR chemical shifts for **1** ($CD_2Cl_2$)*

| Carbon # | $^{1}H$ | $^{13}C$ NMR | Carbon # | $^{1}H$ | $^{13}C$ NMR |
|---|---|---|---|---|---|
| 1 | | 178.7(s) | 17 | 1.70(m) , 1.50(m) | 24.2(t) |
| 2 | | 46.4(s) | 18 | 1.47(m) , 1.30(m) | 31.9(t) |
| 3 | 3.50(d, J= 10Hz) | 83.2(d) | 19 | 3.12(dt, J=9.2, 3 Hz) | 78.2(d) |
| 4 | 1.68(m) | 37.8(t) | 20 | 1.47(m) | 35.1(t) |
| 5 | 1.75(m), 1.50(m) | 23.5(t) | 21 | 1.80, 1.30(m) | 30.0(t) |
| 6 | 1.47(m), 1.25(m) | 32.8(t) | 22 | 3.12(m), 2.52(dt) | 36.6(t) |
| 7 | 3.58(m) | 79.1(d) | 23 | | 145.0(s) |
| 8 | 1.42(m), 1.62(m) | 43.6(t) | 24 | 6.7(dd, J= 8.0, 1Hz) | 122.3(d) |
| 9 | 3.72(t) | 67.5(d) | 25 | 7.24(dd, J= 8.0, 7.5Hz,) | 133.4(d) |
| 10 | 1.42(m) | 37.4(t) | 26 | 6.80(d, J= 1.0, 7.5Hz) | 115.8(d) |
| 11 | 5.6(dt, J= 5.5, 4.0) | 77.6(d) | 27 | | 160.1(s) |
| 12 | 2.03(m) | 36.9(d) | 28 | | 115.5(s) |
| 13 | 1.80(m), 1.30(m) | 29.5(t) | 29 | | 171.0(s) |
| 14 | 1.5(m) , 1.25(m) | 25.2 (t) | 1-Me | 1.15(s) | 19.3(q) |
| 15 | 3.12(dt, J=9.2, 3) | 78.1(d) | 1-Me | 1.19(s) | 23.1(q) |
| 16 | 1.3(m), 1.47(m) | 32.5(t) | 12-Me | 1.02(d) | 15.0(q) |

*Chemical shifts of methylenes in the upfield region may be interchangeable

A 40 L fermentation broth of *Micromonospora* sp. was extracted twice with 80 L of ethyl acetate. The organic layers were combined, dried over $Na_2SO_4$ (anhyd.) and the solvent removed. The solids from this oily extract were precipitated by dissolving it in dichloromethane and adding the solution to hexane. The resulting precipitate was filtered and the mother liquor containing hexane-soluble compounds was dried under vacuum to yield 3.0 g of solid containing all the LDL-R activity. A 1.5 g portion of this hexane-soluble extract was fractionated on a Sanki Lab's High Performance Centrifugal Partition Chromatogram

(HPCPC) using two phases of hexane:methanol (95:5) mixture.[6] Elution of active compound was monitored by activity in the LDL-R assay. The active fractions were collected and dried to furnish 674 mg of enriched complex. Separation of the active compound was achieved by reverse phase preparative HPLC on a Water's Deltapak C-18 silica column (1.9×30 cm), eluting with a mixture of methanol and water (92:8 v/v). Methanol was removed from the peak eluate, and the aqueous solution was freeze-dried to yield 40.2 mg pure SCH 351448 (**1**).

SCH 351448 (**1**) showed a sodiated molecular ion at *m/z* 1143 $(M+Na)^+$ in the FAB mass spectrum, suggesting the molecular weight of 1120 amu and thereby indicating either an even number or absence of nitrogens. The molecular formula of **1** was established as $C_{64}H_{95}O_{16}Na$ from its high resolution mass spectrum [obsd 1142.7030 and calcd 1142.6518 for $C_{64}H_{95}O_{16}Na$]. The UV spectrum (MeOH) displayed maxima at 207, 243, 283, and 312 nm while the IR spectrum in KBr showed peaks at 3433, 2856, 1703, 1668, 1461, 1295 $cm^{-1}$, suggesting the presence of ester and/or acid functionality. $^1H$ and $^{13}C$ NMR chemical shifts are listed in Table 1. The $^1H$ NMR spectrum indicated the presence of three methyls, several methylenes, and a trisubstituted aromatic ring. APT $^{13}C$ NMR identified two >C=O, one >C<, three aromatic =CH-, three aromatic =C<, six >CH-O, one -CH<, thirteen $>CH_2$, and three $-CH_3$. Two methyls were quaternary while the third was secondary as revealed by $^1H$ NMR. Although the $^{13}C$ NMR spectrum contained only 32 signals, the FABMS showed a sodiated peak at 1143 $(M+Na)^+$, leading to the conclusion that the compound was a dimer. HRMS confirmed this fact by suggesting the molecular formula $C_{64}H_{96}O_{16}$; COSY, HETCOR and HMBC provided several connectivities as shown in the structure.

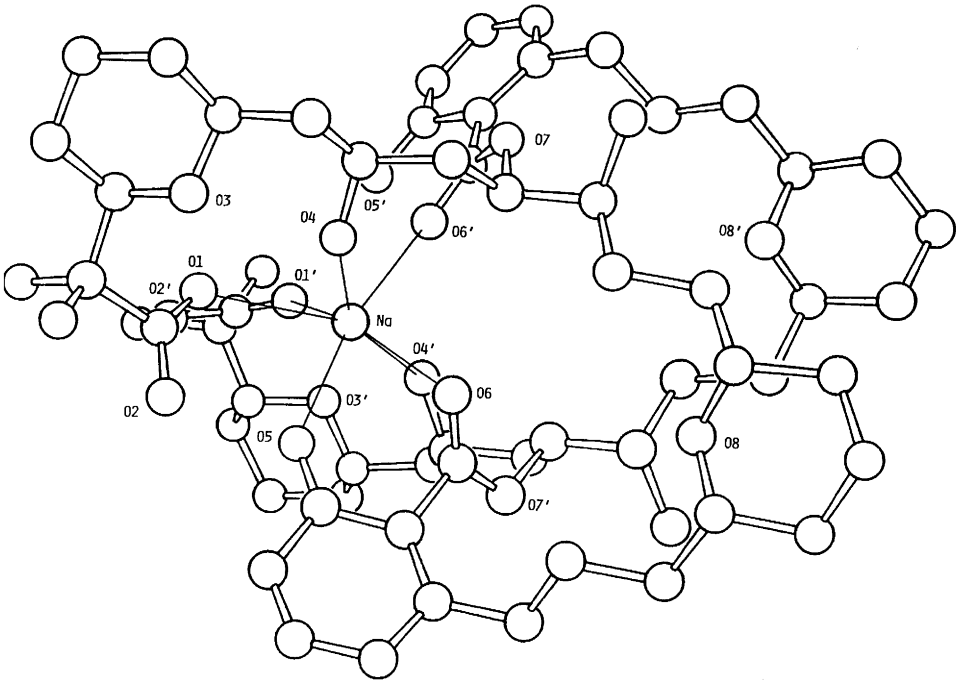


Fig. 1.

Crystallization of **1** from pyridine yielded a solvate, which was subjected to single-crystal X-ray analysis[7] to establish unambiguously the complete structure and relative stereochemistry. A view of the structure showing the solid-state conformation is presented in Fig. 1. The ionized and unionized carboxy groups of the monosodium salt of the pyran-2-(2,2-dimethylpropionic acid)-6-(2-hydroxypropyl) macrocyle substituents are intramolcularly hydrogen bonded [O2′...O1=2.535(11) Å] as are the hydroxy

groups[8] to form a quasi-ring structure which is coordinated through seven oxygen atoms to the central sodium ion.[9]

Purified SCH 351448 has an $ED_{50}$ of 25 μM in the LDL-R promoter transcription assay using hGH as a reporter gene, but it did not activate transcription of hGH from the SRα promoter.[3,5] Thus, **1** selectively activates transcription from the LDL-R promoter. In screening through chemical and natural product libraries, **1** was the only selective activator of LDL-R transcription discovered. Several cytokines have been shown to activate LDL-R transcription including oncostatin M, hepatocyte growth factor, tumor necrosis factor-a, and interleukin-1b.[10] To our knowledge, **1** is the first small molecule activator of the LDL-R promoter identified to date. Selective activators of LDL-R transcription may be able to decrease serum LDL levels by increasing LDL uptake by the LDL-R.

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3. In an effort to decrease serum LDL levels, we set up an assay for compounds, which could increase transcription from the LDL-R promoter. For the assay we placed the LDL-R promoter upstream of the human growth hormone (hGH) gene in the vector pOGH (Nichols Institute Diagnostics) to produce pLDLR-GH. pLDLR-GH was then transiently transfected into JEG-3 cells (ATCC) by electroporation. The cells were then plated in 96 well plates and incubated with compounds or natural products and 25-hydroxycholesterol overnight at 37°C and 5% $CO_2$. In the morning, the culture supernatants were taken and hGH production was measured by an ELISA assay.[4] The effects of the compounds were normalized to control wells containing 25-hydroxycholesterol (0% activation) and wells lacking 25-hydroxycholesterol (100% activation). We obtained at least a fivefold window in hGH levels between the − and + 25-hydroxycholesterol wells. An assay with cells transfected with a construct containing the SRα promoter[5] upstream of the hGH gene was also run concurrently. By comparing the results from the two assays we could find compounds which were not general transcriptional activators but rather may be specifically activating transcription from the LDL-R promoter.
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6. Hexane-rich upper phase was used as mobile phase and methanol-rich lower phase was used as lower phase. Column was rotated at 1000 rpm; flow rate: 6 mL/min; detection: UV, 225 nm.
7. *Crystal data*: $[C_{64}H_{95}O_{16}]Na\cdot 2C_5H_5N$, $M$=1301.66, monoclinic, space group $P2_1$, $a$=14.204(2) Å, $b$=20.726(3) Å, $c$=13.016(2) Å, β=110.83(1)°, $U$=3581(2) Å$^3$, $Z$=2, $D_{calcd}$=1.207 g cm$^{-3}$. The crystal structure was solved by direct methods. One of the pyridine molecules of solvation is severely disordered over several orientations. Full-matrix least-squares refinement of atomic parameters converged at $R$=0.070 ($R_w$=0.074) over 2424 reflections [$I>2.0\sigma(I)$] recorded on an Enraf-Nonius CAD-4 diffractometer (Cu–$K\alpha$ radiation, graphite monochromator, λ=1.5418 Å, $\theta_{max}$=52°). Coordinates have been deposited at the Cambridge Crystallographic Data Centre.
8. Hydrogen-bonded distances (Å): O5...O2=2.531(15), O5′...O6′=2.519(13), bifurcated O4...O1=2.940(12) and O4...O3=2.753(12) and bifurcated O4′...O1′=2.773(13) and O4′...O3′=2.785(12).
9. Na-O1=2.724(10), Na-O4=2.407(8), Na-O5=2.426(8), Na-O6=2.445(10), Na-O1′=2.402(10), Na-O4′=2.721(9), Na-O6′ 2.393(8) Å.
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