Bioorganic & Medicinal Chemistry Letters 18 (2008) 3756–3760

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological activities of reveromycin A and spirofungin A derivatives

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ARTICLE INFO

Article history:
Received 8 April 2008
Revised 8 May 2008
Accepted 9 May 2008
Available online 17 May 2008

Keywords:
Reveromycin A
Spirofungin A
Isoleucyl-tRNA synthetase
Osteoclasts
src^{ts}-NRK cells

ABSTRACT

Various derivatives of reveromycin A, an inhibitor of eukaryotic cell growth, and spirofungin A, focusing on the 5*S* hydroxyl group and C18 hemisuccinyl group, were synthesized and their inhibitory effects on both the isoleucyl-tRNA synthetase activity and the survival of osteoclasts, and activities on the morphological reversion of *src*^{ts}-NRK cells were examined. It was found that 2,3-dihydroreveromycin A is the promising derivative of reveromycin A based on the activity and stability.

Reveromycin A (**1**, Fig. 1) is a polyketide-type antibiotic isolated from the soil actinomycete *Streptomyces* as an inhibitor of mitogenic activity induced by the epidermal growth factor (EGF) in a mouse epidermal keratinocyte.[1] Reveromycin A (**1**) showed several biological activities such as the morphological reversion of *src*^{ts}-NRK cells without any noticeable cytotoxicity (EC_{50} = 1.58 μg/mL), the anti-proliferative activity against human tumor cell lines (IC_{50} = 1.3–2.0 μg/mL) as well as antifungal activity (MIC = 2.0 μg/mL, pH 3).[2] In vitro studies revealed that **1** is a selective inhibitor of protein synthesis in eukaryotic cells (IC_{50} = 40 nM).[3] In addition, the molecular target of **1** was identified as the isoleucyl-tRNA synthetase (IleRS) based on yeast genetics and biochemical studies.[4] The IC_{50} of this enzyme was 2.95 ng/mL, while the other aminoacyl-tRNA synthetases were not inhibited. Recently, it was found that **1** inhibits bone resorption by specifically inducing apoptosis in osteoclasts.[5]

Compound **1** includes the 6,6-spiroketal core bearing a hemisuccinate, two unsaturated side chains with a terminal carboxyl group and two alkyl groups in the structure.[6,7] Based on its strong biological activity as a potential drug and its synthetically challenging molecular architecture, the first asymmetric total synthesis of **1** was accomplished by our group.[8] We have also reported the chemical modification of natural reveromycin A (**1**) and its biological activities.[9] It was shown that the C5 hydroxyl group and C24 carboxyl group in **1** are particularly important for these activities from the comparison of the activities of the monoesters **3**–**5** and C5 protected derivatives **6**–**8** (Fig. 1 and Table 1). However, the C18 methoxy compound **31** among the modified derivatives was the most active and the inhibitory effect on the IleRS activity was 1/20 of that of **1**. We now report the synthesis of the circumstantial derivatives related to the C5 hydroxyl group and the C18 hemisuccinyl group in order to create more active and stable compounds, and their biological activities such as cell death inducibility of the osteoclasts in an addition to inhibition of the IleRS activity, and morphological reversion activity on *src*^{ts}-NRK cells. We have found that 2,3-dihydroreveromycin A is the promising derivative of **1**.

We synthesized derivatives of **1**, especially focusing on the stereochemistry of the C5 hydroxyl group and the polarity around the C5 hydroxyl group. First, the C5-epimer of **1**, 5-epireveromycin A (**9**), was synthesized by the Dess-Martin oxidation of **1** followed by the reduction with $NaBH_4$-$CeCl_3$ in 34% overall yield (Fig. 2 and Scheme 1). Some of the derivatives of **1** are unstable and afford a dark tar under acidic or basic conditions. It was presumed that the decom-

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doi:10.1016/j.bmcl.2008.05.054

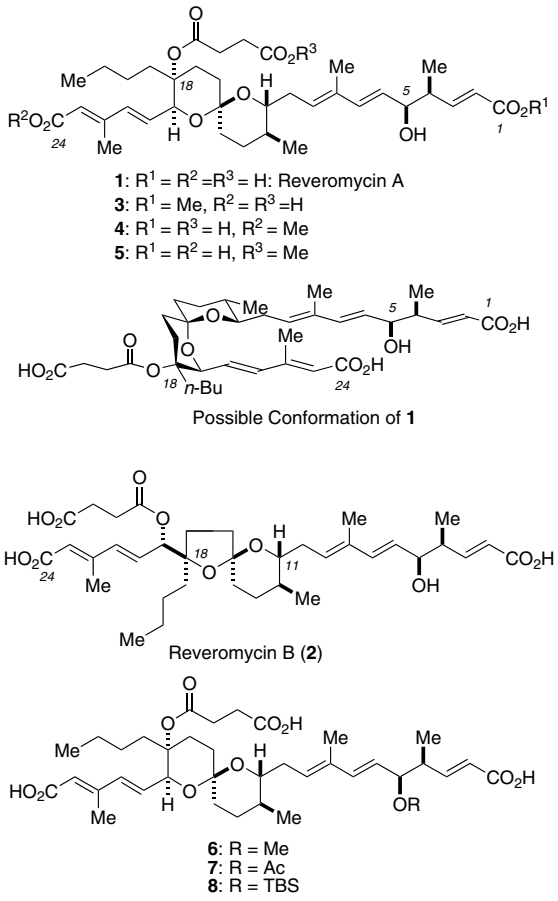


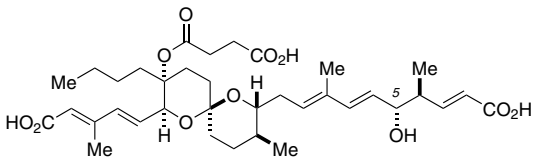
Figure 1. Structures of reveromycin A (**1**) and the derivatives.

position is a result of the β-elimination of the C5 hydroxyl group since **1** includes the γ-hydroxyl α,β-unsaturated carboxyl group on the right-side chain. 2,3-Dihydroreveromycin A (**10**) and 2,3-dihydro-5-epireveromycin A (**11**) were synthesized to prevent the β-elimination. The sulfone **20**,[8] a key intermediate for the total synthesis of **1**, was subjected to the one-pot Julia olefination[10] with an aldehyde **24** to give the allyl esters, which were then deallylated with $Pd(Ph_3P)_4$–Ph_3P–pyrrolidine[11] followed by desilylation with TBAF in DMF to afford the 2,3-dihydro derivative **10**. The aldehyde **24**, the C1–C6 segment for **10**, was prepared from the known ester **27**[12] in four steps.[13] The C5-epimer of **10**, 2,3-dihydro-5-epireveromycin A (**11**) was also synthesized with an aldehyde **25** from the one-pot Julia olefination in a similar manner for the synthesis of **10**. The aldehyde **25** was prepared from the allyl alcohol **28**[13] using the Sharpless asymmetric epoxidation with (−)-DET,[14] the Z-selective Horner–Emmons reaction,[15] and palladium-catalyzed selective hydrogenolysis of the alkenyloxirane with formic acid[16] as the key steps. The C4-hydroxyl derivative **12** might be synthesized from the sulfone **20** in a manner similar to the syntheses of **10** and **11**, and we were also interested in the polarity and structure activity relationships. Compound **12** was synthesized using a dihydroxyl aldehyde **26** prepared from 2,3-isopropylidene-L-threitol in seven steps. Reveromycin A (**1**) includes three carboxyl groups, and it was suggested that the nondissociative form of the carboxylic acids is necessary for the antifungal activity.[2] Reveromycin A (**1**) also specifically induced apoptosis in osteoclast progenitors in an acidic microenvironment, a prominent characteristic of osteoclasts.[5] We were interested in the contribution by each activity of the three carboxyl groups. Among the monoesters **3**–**5**, the C1 ester **3** showed strongest inhibitory effects on the IleRS activity and survival of the osteoclasts. Base on these results, it was suggested that the effects of the C1 carboxyl group among these three carboxyl groups are minimum. Therefore, simple compounds without the C1 carboxyl group were synthesized to verify the suggestion. The 3,5-dihydroxyl deriv-

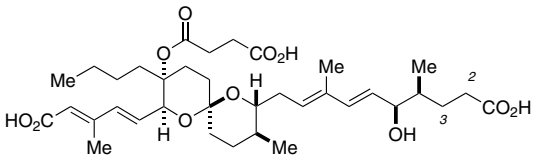
Table 1
IC_{50} on IleRS activity, cell death inducibility of osteoclasts, and morphological reversion of *src*^{ts}-NRK cells by reveromycin derivatives

Compound	Molecular weight	IC_{50} on IleRS activity (ng/mL)	Morphological reversion of *src*^{ts}-NRK cells concentration (μg/mL)						Cell death inducibility of osteoclasts (μg/mL)
			100	30	10	3	1	0.3	
1	660.79	2.95	+++[a]	+++	++	+	±	–	0.06
2	660.79	>1000	–						>15
3	702.83	211.0	+++	+++	+++	+	–		2.00
4	702.83	>1000	+++	+++	±	–	–		3.10
5	702.83	292.8	+++	+++	+++	+++	±		2.50
6	674.82	374.0	–						>15
7	702.83	>1000	+++	+++	++	–	–		0.46
8	775.05	>1000	–						5.90
9	660.79	378.3	–	–	–	–			21.5
10	662.81	11.6	+++	++	+	±			0.22
11	662.81	58.3	++	–	–	–			0.82
12	662.76	14.4	+++	+	±	–	–	–	1.01
13	620.77	22.9	+++	+	–	–	–	–	6.31
14	562.69	560.3	–	–	–	–			nt[b]
15	536.65	>1000	–	–	–	–			nt
16	496.59	>1000	–	–	–	–			nt
17	636.73	>1000	–	–	–	–			>15
18	576.68	>1000	–	–	–	–			nt
30	560.72	94.6	–						11.5
31	574.75	57.9	+++	+++	+				nt
32	620.84	497.4	+++	+	–				9.60
33	502.64	564.5	+++	+	±	–			>30
34	502.64	>1000	–	–	–	–			>30
35	602.71	>1000	tox	–	–	–			12.4

[a] Rate of reversed cells was presented as follows: +++, >80%; ++, 50–80%; +, 20–50%; ±, 10–20%; –, <10%.
[b] Not tested.



5-Epireveromycin A (**9**)



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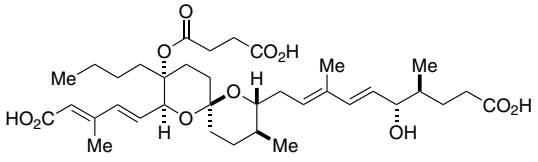
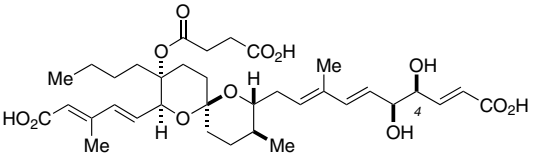


2,3-Dihydro-5-epireveromycin A (**11**)



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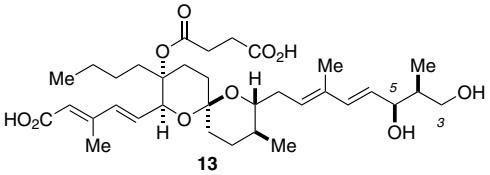

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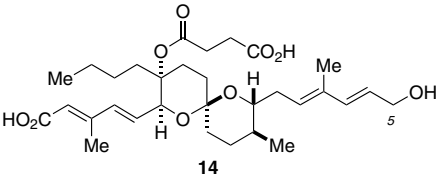

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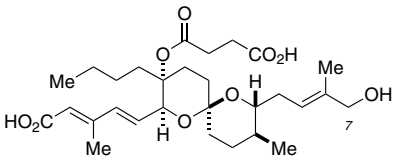

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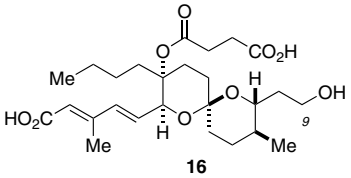

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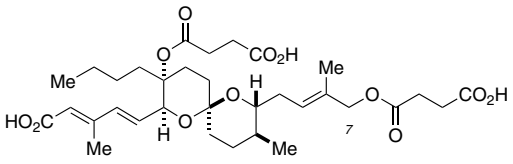

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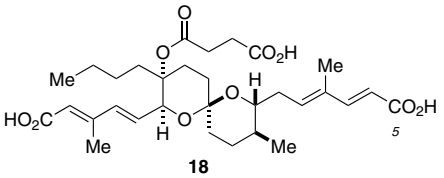

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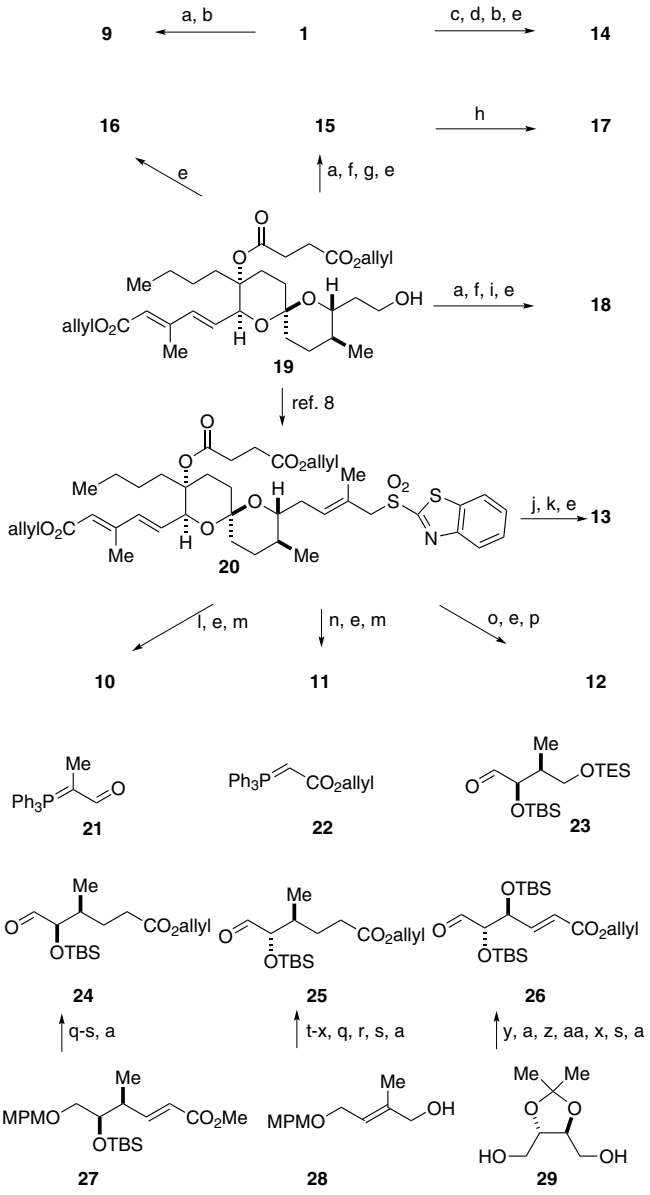
Figure 2. Reveromycin A derivatives having the modified right-side chain.

ative **13** was easily synthesized via the Julia olefination of the sulfone **20** with an aldehyde **23**[8] followed by deprotection of the silyl groups and hydrogenolysis of the allyl esters. The primary C5 hydroxyl derivative **14** was in turn prepared from the triallyl esters of **1** via the retrograde aldol reaction with NaH. The resulting unsaturated aldehyde was reduced with $NaBH_4$-$CeCl_3$ and the allyl groups were removed by the palladium (0)-catalyzed hydrogenolysis to afford **14**. The triallyl esters of **1** were prepared using the allyl alcohol and EDCI under high pressure.[9] Although the trimethyl esters of **1** were easily prepared by the esterification with $TMSCHN_2$ at room temperature, the deprotection of the dimethyl esters to obtain **14** was difficult after the retrograde aldol reaction. Since the formation of the hydrogen bond between the C7 hydroxyl group and C24 carboxyl group might be low, there is a slight contribution of the C7 hydroxyl group to the stability. The C7 hydroxyl derivative **15** without the C5 hydroxyl group was prepared from the alcohol **19**[8] in four steps including the Wittig reaction of the corresponding aldehyde with an ylide **21** followed by reduction with $Zn(BH_4)_2$ and deallylation. The hemisuccinate of the C7 hydroxyl group of **15**, a tricarboxylic acid **17**, was directly synthesized from **15** using succinic anhydride. The C5 carboxylic acid **18** was prepared from **19** via two Wittig reactions with ylides **21** and **22**. For the hemisuccinate of the C18 tertiary hydroxyl group, we have already reported the preparations and inhibitions on the IleRS activity of the hydroxyl (**30**), methoxy (**31**), and methylthiomethyl (**32**) derivatives.[9] Inhibitory effects of **30** and **31** on the IleRS activity still existed. However, the effects were 1/20–30 of that of **1**. The C18 hydrogen derivative **33**, spirofungin A, which was isolated as a secondary metabolite from *Streptomyces violaceusniger* Tü 4113, showed various antifungal activities, particularly against yeast.[17] Since spirofungin A was initially isolated as a equilibrium mixture with spirofungin B (**34**), a C15 epimer of **33**, compounds **33** and **34** were synthesized in the pure form for the study of the structure–activity relationships.[12] A C5-hemisuccinate **35** of **33**, the tricarboxylic acid as well as reveromycin A (**1**), was synthesized from **33** in three steps. The carboxyl groups of **33** were esterified with 2-(trimethysilyl)ethanol and EDCI and the C5 hydroxyl group was esterified with the *mono*-trimethysilylethyl succinate and EDCI. Finally, the trimethysilylethyl groups were cleaved with TBAF to afford the hemisuccinate **35** (see Fig. 3).

We have already reported that reveromycin A (**1**) induces the morphological reversion activity of src^{ts}-NRK cells from spherical transformed cells to flat cells at 32 °C and the molecular target of **1**



Scheme 1. Reagents and conditions: (a) Dess-Martin Periodinane, MS4A, CH_2Cl_2, (99% for **24**, 92% for **25**, 99% for **26**); (b) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, rt (34% for **9**, 22% for **14**, two steps); (c) allyl alcohol, EDCI, DMAP, CH_2Cl_2, 1.5 GPa, rt (36%); (d) NaH, THF, 0 °C; (e) $Pd(Ph_3P)_4$, Ph_3P, pyrrolidine, CH_2Cl_2, 0 °C–rt (68% for **13**, 74% for **14**, 76% for **15**, 74% for **16**, 78% for **18**); (f) **21**, toluene, 110 °C (96% for **15**, **18**, two steps); (g) $Zn(BH_4)_2$, Et_2O, 0 °C (99%); (h) succinic anhydride, DMAP, pyridine, CH_2Cl_2, rt (95%); (i) **22**, toluene, 100 °C (61%); (j) **23**, LHMDS, THF, −78 °C (90%); (k) TBAF, THF, 0 °C (79%); (l) **24**, KHMDS, THF, −78 °C (55%); (m) TBAF, DMF, rt (48% for **10**, 75% for **11**, each two steps); (n) **25**, LHMDS, THF, −78 °C (72%); (o) **26**, LHMDS, THF, −78 °C, (41%); (p) TBAF, DMPU, rt (35 % for **23**, two steps); (q) H_2, Pd/C, EtOAc, rt, (96% for **24**, 93% for **25**); (r) allyl alcohol, $ClBu_2SnOSnBu_2OH$, toluene, 110 °C, (89% for **24**, 92% for **25**); (s) DDQ, CH_2Cl_2-H_2O, rt, (95% for **24**, 95% for **25**, 99% for **26**); (t) TBHP, (−)-DET, $Ti(O\text{-}i\text{-}Pr)_4$, M-4A, CH_2Cl_2, −25 °C, (79%); (u) $(COCl)_2$, DMSO, CH_2Cl_2 then Et_3N, −78 °C; (v) $(CF_3CH_2O)_2POCH_2CO_2Me$, $KN(TMS)_2$, 18-crown-6, −78 °C, (87%, two steps); (w) $Pd_2(dba)_3{\cdot}CHCl_3$, *n*-Bu_3P, HCO_2H, Et_3N, dioxane, rt, (91%); (x) TBSCl, imidazole, DMF, rt, (94% for **25**, 94% for **26**); (y) MPMCl, NaH, THF, 0 °C–rt, (88%); (z) **22**, CH_2Cl_2, rt; (aa) TsOH, MeOH, rt–60 °C, (40%, three steps).

is the isoleucyl-tRNA synthetase (IleRS).[2,4] Recently, it was also found that **1** inhibits bone resorption by specifically inducing apoptosis in osteoclasts.[5] Therefore, we tested these biological activities of the synthesized compounds for the discovery of compounds more active and stable than **1**, and the results are shown in Table 1.

Reveromycin A (**1**) strongly inhibits the IleRS activity and the IC_{50} on this enzyme was 2.95 ng/mL. It was revealed that two of the three

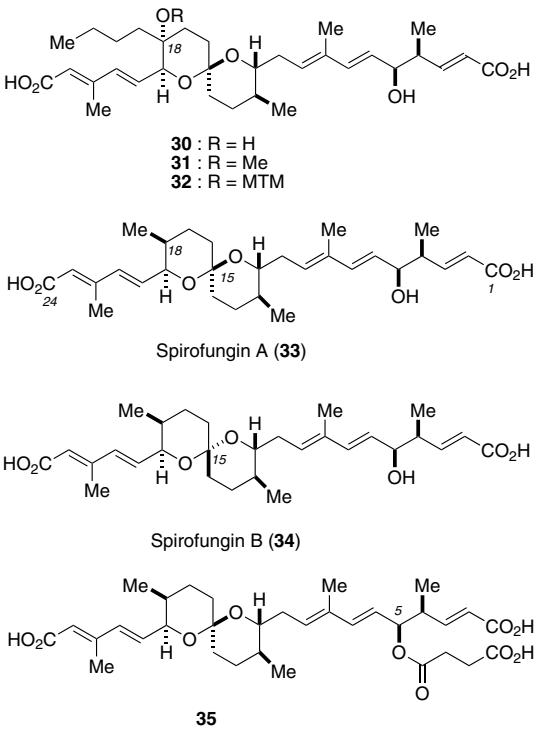


Figure 3. The C18 hemisuccinate derivatives.

carboxyl groups in **1** are essential for the activities and the free hydroxyl group at C5 and the C24 carboxyl group are particularly important for the strong activities based on the activities of the tricarboxylic acid mono- (**3**–**5**), di-, and tri-esters and C5 hydroxyl derivatives such as the methoxy (**6**), acetoxy (**7**), and silyloxy (**8**) derivatives.[9] It was anticipated that the hydrogen bond between the C5 hydroxyl group and the C24 carboxyl group might contribute to the stability and the activity of **1**. The activity of 5-epireveromycin A (**9**) was surprisingly reduced to 1/128 of that of **1** (IC_{50} = 378.3 ng/mL). It was assumed that the formation of the hydrogen bond between the C5*R* hydroxyl group and C24 carboxyl group might be difficult and even the hydrogen bond could be formed, therefore the α,β-unsaturated C1 carboxyl group was placed at a disadvantage for the reciprocal action with the target molecule. On the other hand, 2,3-dihydroreveromycin A (**10**) showed an equivalent activity to **1** (IC_{50} = 11.6 ng/mL), suggesting that the fixed conformation of C1 carboxyl group by α,β-unsaturated bond is not required for IleRS inhibition. Since **10** is free from the β-elimination, the 2,3-dihydro derivatives are promising as potent drugs. Interestingly, 2,3-dihydro-5-epireveromycin A (**11**), the 2,3-dihydrated 5-epireveromycin (**9**), recovered the activity (IC_{50} = 58.3 ng/mL). This might result from the flexibility of the conformation of the α,β-saturated C1 carboxyl group. The 4-hydroxyl derivative **12** strongly inhibited the IleRS activity as expected (IC_{50} = 14.4 ng/mL). The 3,5-dihydroxyl derivative **13** showed a strong inhibitory effect on IleRS (IC_{50} = 22.9 ng/mL) in spite of the absence of the C1 carboxyl group. Since a simple 5-hydroxyl derivative **14** showed a weak activity (IC_{50} = 560.3 ng/mL), the C3 hydroxyl group in **13** might play an important role in the activity. The primary 7-hydroxyl (**15**) and 9-hydroxyl (**16**) derivatives only slightly inhibited the IleRS activity (IC_{50} > 1000 ng/mL). The hemisuccinate **17** of the C7 hydroxyl derivative **15**, which is a tricarboxylic acid like **1** and excludes the C5 hydroxyl group, did not show any activity even at the concentration of 1000 ng/mL. The C5 carboxylic acid **18**, a tricarboxylic acid without the C5 hydroxyl group, also did not show any activity. Being related to the C18 hemisuccinyl group in **1**, the C18 hydroxyl derivative **30** and the C18

methoxy derivative **31** still inhibited the IleRS activity and the effects were 1/20–32 of the activity of **1** (IC_{50} = 94.6, 57.9 ng/mL, respectively). The C18 methoxy derivative **31** is expected to be a stable drug. The C18 MTM ether derivative **22** weakly exhibited any activities. Although a mixture of spirofungin A (**33**) and B (**34**) was reported to inhibit the growth of *Candida albicans* and *Rhodotorula rubra* (minimal concentration: 15 μg/mL),[17] the synthesized pure compound **33** very weakly inhibited the IleR activity and the effect was 1/191 of that of **1** (IC_{50} = 564.5 ng/mL). It is noteworthy that the strong inhibition of **33** on the growth of several human cancer cell lines including HL-60 and PC3 is consistent with the cell-based behavior of reveromycin A (**1**).[18] Spirofungin B **34** did not inhibit the IleRS as presumed from the structure, in which the hydrogen bond between the C5 hydroxyl group and C24 carboxyl group might be negligible. Moreover, the C5-hemisuccinate **35** of **33**, a tricarboxylic acid, did not inhibit the activity. It became clear that the C5 hemisuccinyl group in **35** decreased the inhibition activity toward IleRS, such as the C5 acetoxy (**7**) and C5 silyloxy (**8**) derivatives of **1**. Based on the effects of the IleRS activity, 2,3-dihydroreveromycin A (**10**), the 4-hydroxyl derivative **12**, and the 3,5-dihydroxyl derivative **13** were expected to be promising derivatives of **1**.

The derivatives which contain the C24 carboxyl group and the C5*S* hydroxyl group, the 2,3-dihydroxyreveromycin A (**10**) and 4-hydroxy reveromycin A (**12**), exhibited morphological reversion activities on *src*^{ts}-NRK cells at the concentration of 10 μg/mL as the C1 monoester **3** and the C4′ monoester **5**. On the contrary, the 5-epireveromycin A (**9**) did not show the morphological reversion at the concentration of 100 μg/mL, as expected from the loss of IleRS inhibition. Furthermore, the 2,3-dihydro-5-epireveromycin A (**11**) weakly exhibited the reversion (++ at 100 μg/mL). It might be due to the flexibility of the conformation on the right-side chain in **11** for the inhibition on the IleRS. The 3,5-dihydroxyl derivative **13**, a 24,4′-dicarboxylic acid, moderately showed the reversion. Spirofungin A (**33**), a 1,24-dicarboxylic acid without the C18-hemisuccinyl group as the C18 methoxy- (**31**) and C18 MTM (**32**) derivatives, strongly exhibited morphological reversion activities on *src*^{ts}-NRK cells, while **34** did not. The C5-hemisuccinate **35** of **33** did not show the reversion activity at the concentration of 100 μg/mL, while the C5 acetate **7** of reveromycin A (**1**) induced strong morphological reversion activities. It was suggested that the C5 acetate was easily hydrolyzed by cellular esterase to afford **1** after being taken into the cells, showed these activities and the C5-hemisuccinate **35** resisted hydrolysis.

Mature bone-resorbing osteoclasts mediate excessive bone loss seen in several bone disorders including osteoporosis and corticosteroid-induced bone loss. We recently reported that reveromycin A (**1**) inhibits bone resorption by specifically inducing apoptosis in the osteoclasts (IC_{50} = 0.06 μg/mL).[5] The effects of **1** on osteoclast apoptosis increased under acidic culture conditions but inhibited by neutralization. It was hypothesized that the specificity of **1** for osteoclasts might result from the acidic conditions that increased amount of the nonpolar forms of **1**, which are more cell permeable. Therefore, we focused on the carboxylic acid moieties. The tricarboxylic acid monoesters **3**, **4**, and **5** still showed strong anti-osteoclast activity (IC_{50} = 2.00–3.10 μg/mL). Furthermore, the tricarboxylic acid C5 acetate **7** had a strong inhibition (IC_{50} = 0.46 μg/mL). These compounds showed decreased or no inhibitory activity against IleRS, suggesting that the ester bond was hydrolyzed by cellular esterase and the compounds were converted into the active compound **1**. On the other hand, the effect of the tricarboxylic acid C5 TBS ether **8** was mild (IC_{50} = 5.90 μg/mL). However, it is interesting that **8**, in contrast to ester compounds **3**, **4**, **5**, and **7**, exhibited a slight effect on the morphological reversion of *src*^{ts}-NRK cells and the monocyte line RAW264 under neutral conditions (data not shown). Therefore, **8** is an attractive compound as a prodrug which was cleaved to give **1** under acidic conditions around the osteoclasts. The activity of 5-epireveromycin A (**9**) was also reduced as the inhibition on the IleRS was reduced. The 2,3-dihydroreveromycin A (**10**) inhibition was as strong as **1** (IC_{50} = 0.22 μg/mL) and even 2,3-dihydro-5-epireveromycin A (**11**) strongly induced apoptosis in the osteoclasts (IC_{50} = 0.82 μg/mL). The 4-hydroxyl derivative **12** also produced a strong inhibition (IC_{50} = 1.01 μg/mL). The inhibition effect of the 3,5-dihydroxyl derivative **13**, a dicarboxylic acid, was moderate. Surprisingly, the dicarboxylic acid, spirofungin A (**33**) and B (**34**) did not inhibit the survival of the osteoclasts. It is important to note that the hemisuccinate **35**, a tricarboxylic acid, moderately inhibited the survival of the osteoclasts although **35** did not inhibit the IleRS. Based on the effects on the survival of osteoclasts, it was shown that the tricarboxylic acid is essential to exhibit a strong activity and the C5 TBS ether **8** is an attractive compound as a prodrug.

Various derivatives of reveromycin A and spirofungin A focusing on the 5*S* hydroxyl group and C18 hemisuccinyl group were synthesized and their inhibitory effects on both the isoleucyl-tRNA synthetase activity and the survival of osteoclasts, and activities on the morphological reversion of *src*^{ts}-NRK cells were examined. 2,3-Dihydroreveromycin A (**10**) and the 4-hydroxyl derivative **12** are expected to be the attractive derivatives of **1** based on their activities and stabilities. The C5 TBS ether **8** is a promising derivative as a prodrug for osteoporosis.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (18590027) to T.S. from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the RIKEN Chemical Biology program. We thank Dr. H. Koshino for the NMR measurements and Ms. K. Harata for the mass spectrum measurements.

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