Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3267–3270

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Facilely Accessible Multidrug Resistance Modulator Derived from Sucrose

Nobutoshi Murakami,[a] Satoru Tamura,[a] Etsuko Iwata,[a] Shunji Aoki,[a] Shin-ichi Akiyama[b] and Motomasa Kobayashi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan*
[b]*Institute for Cancer Research, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima, 890-8520, Japan*

Received 25 July 2002; accepted 27 August 2002

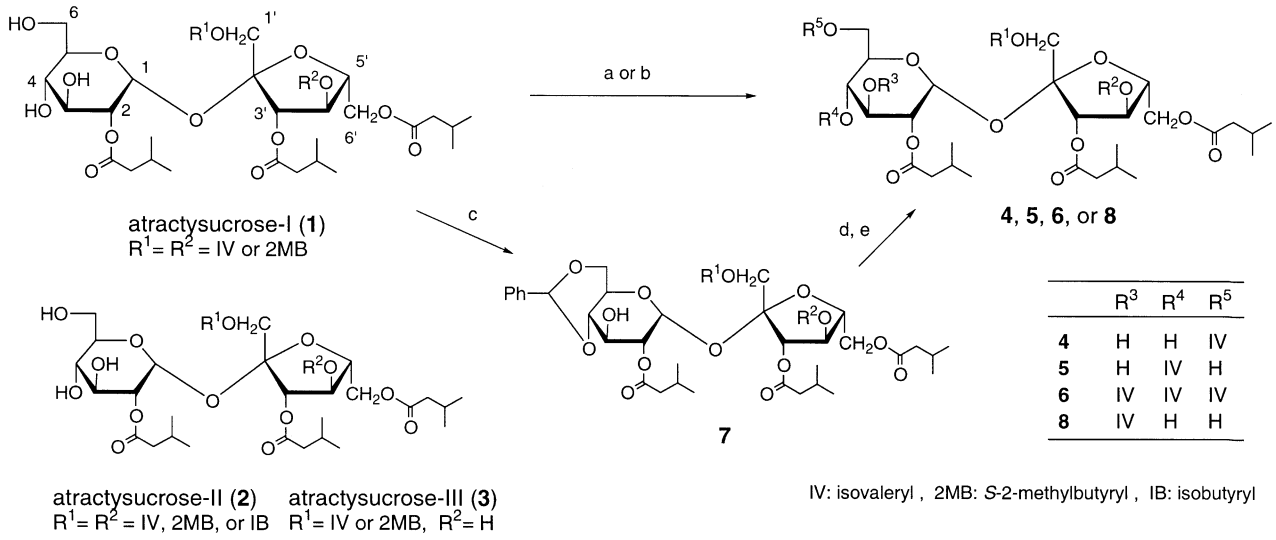
Abstract—Exploration for new MDR-modulators utilizing atractysucroses as scaffolds disclosed 2,3,4,3′,4′-*O*-pentaisovalerylsucrose (**9**) as a readily accessible medicinal lead. This lead was prepared from sucrose in 65% total yield for three steps. In addition, compound **9** exhibited more potent MDR modulating activity than verapamil, a representative modulator of MDR mediated by P-gp.

Drug resistance has been a major unsolved problem in chemotherapy for cancer patients. Especially, multidrug resistance (MDR) is a highly complicated phenomenon.[1] In many cases, MDR tumor cells acquire the resistance to many structurally and functionally unrelated anti-tumor drugs by the overexpression of P-glycoprotein (P-gp). This specific protein acts as an energy-dependent extrusion pump, which efficiently transports lipophilic chemotherapeutic agents outside tumor cells.[2,3] MDR-modulators are able to restore the inherent potency of anti-tumor agents through inhibition of the function of P-gp.[4] Thereby, exploration for MDR-modulators has been a challenging topic for anti-cancer therapeutic intervention. In this regard, we have been engaged in a search for new MDR-modulators inhibiting the transport activity of membrane proteins such as P-gp and multidrug-resistance-associated protein 1 (MRP1) from natural compounds.[5,6] During the course of this research program, we characterized new MDR-modulating oligoacylated sucroses, atractysucroses-I (**1**), II (**2**), and III (**3**), from Atractylodis Lanceae Rhizoma (So-jutsu in Japanese).[7] Analysis for contribution of the acyl residues to the MDR-modulating activity of atractysucroses brought about 2,3,4,3′,4′-penta-*O*-isovalerylsucrose (**9**). This paper communicates the readily accessible MDR-modulator **9** derived from sucrose.

*Corresponding author. Tel.: +81-6-6879-8215; fax: +81-6-6879-8219; e-mail: kobayasi@phs.osaka-u.ac.jp

Atractysucroses-I (**1**), II (**2**), and III (**3**) showed growth inhibition against MDR tumor cells overexpressing P-gp (KB-C2)[8] in the presence of 0.1 μg/mL of colchicine, a typical cytotoxic agent. Among the three congeners, **1** and **2** with five acyl residues showed similar activity, whereas **3** with four acyl residues had less potent activity than **1** and **2**. This difference led us to presume that the number and/or the location of acyl residues are crucial for the MDR-modulating activity. Thus, participation of the number and/or location of acyl residues in MDR-modulating activity was examined by derivatization of atractysucrose-I (**1**) as illustrated in Scheme 1. The condensation between **1** and 0.3 equiv of isovaleric acid by using 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDCI·HCl) and 4-dimethylaminopyridine (DMAP) furnished 6-*O*-isovaleryl (**4**) and 4-*O*-isovalerylatractysucrose-I (**5**) in 34% and 21% yields, respectively.[9] On the other hand, **1** was treated with 3.3 equiv of isovaleric acid in the presence of EDCI·HCl and DMAP to give completely acylated analogue **6** in 81% yield. Since direct introduction of an isovaleryl group to the 3-hydroxyl residue in **1** could be accomplished but only poorly, esterification at the 3-OH group was carried out after protection of the 4-OH and 6-OH groups as a benzylidene acetal. Namely, treatment of **1** with benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid monohydrate provided a benzylidene acetal **7** in 94% yield. Condensation of isovaleric acid with **7** under the same conditions as the preparation for **6** followed by removal

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00702-3



	R^3	R^4	R^5
4	H	H	IV
5	H	IV	H
6	IV	IV	IV
8	IV	H	H

Scheme 1. Synthesis of derivatives of atractysucrose-I (**1**). Reagents and conditions: (a) isovaleric acid (0.3 equiv to **1**), EDCI·HCl, DMAP, CH_2Cl_2, 34% for **4**, 21% for **5**; (b) isovaleric acid (3.3 equiv to **1**), EDCI·HCl, DMAP, CH_2Cl_2, 81%; (c) benzaldehyde dimethyl acetal, *p*-toluenesulfonic acid monohydrate, DMF, 94%; (d) isovaleric acid, EDCI·HCl, DMAP, CH_2Cl_2; (e) H_2, Pd/C, MeOH–AcOH (10:1), 92% two steps.

of the protecting group with Pd/C under hydrogen atmosphere successfully furnished 3-*O*-isovaleryl congener **8** in 92% yield for two steps.

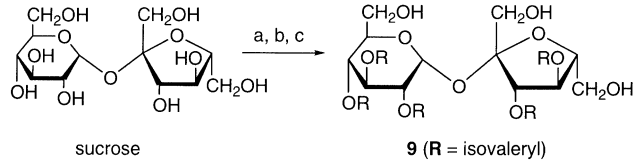
Assessment of MDR-modulating activity of all derivatives was conducted by comparison of growth inhibition against KB 3–1 cells with that against KB-C2 cells in the presence of colchicine.[10] The biological outcome is summarized in Table 1. At the concentration of 3 μg/mL or less, each monoisovalerylatractysucrose-I (**4**, **5**, **8**) reversed MDR in KB-C2 cells. Among them, 4-*O*-isovalerylatractysucrose-I (**5**) displayed as potent MDR-modulating activity as **1**, whereas 6-*O*-isovaleryl (**4**) and 3-*O*-isovaleryl congeners (**8**) showed slightly weaker activity. On the other hand, per-isovalerylatractysucrose-I (**6**) showed little activity. This result suggested that the three hydroxyl groups in **1** would not be necessarily requested for MDR-modulating activity. In addition, enhancement of lipophilicity by introduction of the three isovaleryl residues was shown to diminish biological potency fairly. These findings led us to assume that a pentaisovalerylsucrose with similar lipophilicity to atractysucrose-I (**1**) was anticipated as a potent MDR-modulator. According to this assumption, we designed a readily accessible candidate **9**, of which only the primary hydroxyl groups are free from isovaleryl functions.

Table 1. Reversal of MDR in KB-C2 cells by atractysucroses-I (**1**) and its derivatives (**4**, **5**, **6**, **8**, and **9**)

Compd	Dose	Growth inhibition (%)	
	(μg/mL)	KB-3–1[a]	KB-C2[b]
1	10	92±6	93±1
	3	21±9	76±4
	1	10±16	40±9
4	10	93±2	92±3
	3	16±16	60±10
	1	18±11	23±8
5	10	40±5	88±3
	3	10±19	79±8
	1	2±11	39±11
6	10	25±6	24±12
	3	15±15	12±11
	1	10±13	4±11
8	10	66±13	94±1
	3	16±9	53±11
	1	6±11	18±7
9	10	98±0	97±1
	3	8±11	92±1
	1	9±12	65±18

Each value presents mean±S.D. Colchicine shows no cytotoxicity against KB-C2 cells at 0.1 μg/mL.
[a]Cytotoxicity of each compound.
[b]Growth inhibition in the presence of colchicine (0.1 μg/mL).

This pentaisovaleryl candidate **9** was prepared from sucrose in three steps as shown in Scheme 2. Protection of all primary hydroxyl groups in sucrose was conducted by using *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of Et_3N and DMAP to give a tri-TBDPS ether in 68% yield. Coupling of isovaleric acid and the tri-TBDPS ether in the same manner as the preparation for **8**, and subsequent deprotection of the TBDPS groups by nBu$_4$NF in acidic medium (THF-AcOH = 4:1)[11] provided 2,3,4,3′,4′-*O*-pentaisovalerylsucrose (**9**)[12] in 95% yield for two steps. As a result of



Scheme 2. Synthesis of 2,3,4,3′,4′-*O*-pentaisovalerylsucrose (**9**). Reagents and conditions: (a) TBDPSCl, Et_3N, DMAP, DMF, 68%; (b) isovaleric acid, EDCI·HCl, DMAP, CH_2Cl_2; (c) nBu$_4$NF, THF–AcOH (4:1), 95% two steps.

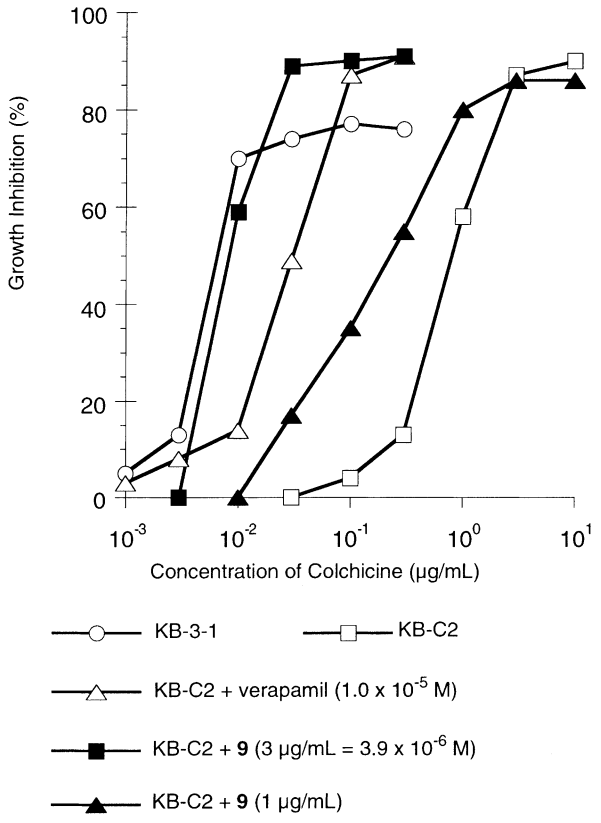


Figure 1. Reversal of MDR by 2,3,4,3′,4′-*O*-pentaisovalerylsucrose (**9**) and verapamil under various concentrations of colchicine.

evaluation for MDR-modulating activity, compound **9** showed more potent efficacy than atractysucrose-I (**1**) as shown in Table 1.

This pronounced biological potency directed us to compare activity between 2,3,4,3′,4′-*O*-pentaisovalerylsucrose (**9**) and verapamil,[13] a representative modulator of MDR mediated by P-gp. Thus, under the two concentrations of **9** (3 and 1 μg/mL), proliferation of KB-C2 cells resistant to colchicine was monitored. In both concentrations, compound **9** restored cytotoxicity of colchicine against KB-C2 cells and completely reversed colchicine-resistance at the concentration of 3 μg/mL as depicted in Figure 1. 2,3,4,3′,4′-*O*-Pentaisovalerylsucrose (**9**) restored the sensitivity of KB-C2 cells against colchicine in the lower concentration (3.9×10^{-6} M) than verapamil (1.0×10^{-5} M).[14]

In summary, we have disclosed a new readily accessible MDR-modulator, 2,3,4,3′,4′-*O*-pentaisovalerylsucrose (**9**), utilizing atractysucroses as scaffolds. It should be noted that 2,3,4,3′,4′-*O*-pentaisovalerylsucrose (**9**) was synthesized from cheaply available sucrose in 65% total yield for 3 steps.

Acknowledgements

This study was financially supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan. The authors are also grateful to the Naito Foundation, the Hoh-ansha Foundation, and the San-Ei Gen Foundation for Food Chemical Research for financial support.

References and Notes

1. Mayer, L. D.; Shabbits, J. A. *Cancer Metastasis. Rev.* **2001**, *20*, 87.
2. Gottesman, M. M.; Pastan, I.; Ambudkar, S. V. *Curr. Opin. Gen. Devel.* **1996**, *6*, 610.
3. Bellamy, W. T. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 161.
4. Ford, J. M. *Eur. J. Cancer.* **1996**, *32A*, 991.
5. Aoki, S.; Yoshioka, Y.; Miyamoto, Y.; Higuchi, K.; Setiawan, A.; Murakami, N.; Chen, Z.-S.; Sumizawa, T.; Akiyama, S.; Kobayashi, M. *Tetrahedron Lett.* **1998**, *39*, 6303.
6. Aoki, S.; Okano, M.; Matsui, K.; Itoh, T.; Satari, R.; Akiyama, S.; Kobayashi, M. *Tetrahedron* **2001**, *57*, 8951.
7. Murakami, N.; Iwata, E.; Tamura, S.; Akiyama, S.; Kobayashi, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2629.
8. Akiyama, S.; Fojo, A.; Hanover, J. A.; Pastan, I.; Gottesman, M. M. *Somat. Cell. Mol. Genet.* **1985**, *11*, 117.
9. Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962.
10. Human epidermoid carcinoma KB cells (KB-3–1) were used as the parental cell line for the present study. KB-3–1 cells were cultured in RPMI 1640 medium with 0.44 mg/mL of glutamine, 50 μg/mL of kanamycin sulfate, supplemented with 10% newborn calf serum. Multidrug resistant (MDR) KB-C2 cells were selected and maintained from KB-3–1 in the medium containing 2 μg/mL of colchicine. Reversing activity and cytotoxicity were measured by means of MTT colorimetric assay performed in 96-well plates. Equal numbers of cells (10,000 cells) were inoculated into each well with 100 μL of the culture medium. After 24 h preincubation (37 °C, 5% CO_2), 50 μL solution of the anticancer agent (colchicine) and the testing sample was added to each well. Then, the whole mixture was further incubated for 48 h under the same condition as preincubation. The cytotoxic activity of the testing sample was also examined by MTT assay using parental KB-3–1 cells. Thereafter, 25 μL of MTT solution (2 mg/mL in PBS) was added to each well and incubated for further 3 h. After removing the medium by aspiration, the resulting formazan was extracted with 200 μL of dimethylsulfoxide. The percentage of cell growth inhibition was calculated from the absorbance at 540 nm.
11. Smith, A. B., III; Ott, G. R. *J. Am. Chem. Soc.* **1996**, *118*, 13095.
12. **9**: Colorless oil, $[\alpha]_D^{22}$ +19.8°(*c* 1.08 in $CHCl_3$). IR (KBr): 3472, 1748, 1296, 1254 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) Sugar moiety δ: 5.66 (1H, d, *J* = 3.7 Hz, 1-H), 5.61 (1H, dd, *J* = 7.3, 7.9 Hz, 4′-H), 5.49 (1H, d, *J* = 7.9 Hz, 3′-H), 5.48 (1H, dd, *J* = 9.8, 10.4 Hz, 3-H), 4.97 (1H, dd, *J* = 9.8, 9.8 Hz, 4-H), 4.90 (1H, dd, *J* = 3.7, 10.4 Hz, 2-H), 4.17 (1H, ddd, *J* = 2.4, 5.5, 9.8 Hz, 5-H), 4.00 (1H, ddd, *J* = 3.1, 4.3, 7.3 Hz, 5′-H), 3.85 (1H, dd, *J* = 3.1, 12.8 Hz, 6′-Ha), 3.70 (1H, dd, *J* = 2.4, 12.8 Hz, 6-Ha), 3.65 (1H, dd, *J* = 4.3, 12.8 Hz, 6′-Hb), 3.63 (1H, d, *J* = 12.8 Hz, 1′-Ha), 3.60 (1H, dd, *J* = 5.5, 12.8 Hz, 6-Hb), 3.52 (1H, d, *J* = 12.8 Hz, 1′-Hb). Isovaleryl moiety δ: 2.39 (1H, dd, *J* = 6.9, 15.1 Hz, $COCH_2CH(CH_3)_2$), 2.32 (1H, dd, *J* = 7.5, 14.9 Hz, $COCH_2CH(CH_3)_2$), 2.09–2.21 (m, $COCH_2CH(CH_3)_2$), 1.94–2.07 (m, $COCH_2CH(CH_3)_2$), 0.99–1.01 (m, $COCH_2CH(CH_3)_2$), 0.89–0.91 (m, $COCH_2CH(CH_3)_2$). ^{13}C NMR (125 MHz, $CDCl_3$) Sugar moiety δc: 104.4 (C-2′), 89.7 (C-1), 81.2 (C-5′), 76.0 (C-3′), 72.6 (C-4′), 71.8 (C-5), 70.2 (C-2), 68.8 (C-3), 68.5 (C-4), 64.1 (C-1′), 61.5 (C-6), 60.7 (C-6′). Isovaleryl moiety δc:

171.5–173.0 ($COCH_2CH(CH_3)_2$), 42.7–43.0 ($COCH_2CH(CH_3)_2$), 25.5–25.8 ($COCH_2CH(CH_3)_2$), 22.2–22.7 ($COCH_2CH(CH_3)_2$). FABMS *m*/*z*: 785 $[M+Na]^+$. FABHRMS *m*/*z*: calcd for $C_{37}H_{62}O_{16}+Na$: 785.3935: Found 785.3936.

13. Tsuruo, T.; Iida, H.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res*. **1981**, *41*, 1967.

14. As shown in Figure 1, the incubation with 10 μg/mL of **9** in the presence of low concentration of colchicine (1×10^{-3} μg/mL) exhibited no cytotoxicity against KB C-2. However, the 10 μg/mL concentration of **9** was cytotoxic against parental KB-3–1 cells (Table 1). These findings might be ascribable to selective cytotoxic behavior of **9** against KB 3–1 cells similar to **1**.