Bioorganic & Medicinal Chemistry Letters 20 (2010) 2159–2162

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Bioisostere of valtrate, anti-HIV principle by inhibition for nuclear export of Rev

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ARTICLE INFO

*Article history:*
Received 26 December 2009
Revised 1 February 2010
Accepted 9 February 2010
Available online 13 February 2010

*Keywords:*
Valtrate
Rev-export inhibitor
Anti-HIV
Iridoid
Bioisostere

ABSTRACT

Rational design by the MO calculation disclosed 5,6-dihydrovaltrate (**2**) as the bioisostere of valtrate (**1**), the Rev-export inhibitor with anti-HIV activity. The synthesis of **2** was accomplished by ingenious use of asymmetric Diels–Alder reaction and stereoselective epoxidation associated with the adjacent hydroxyl group. Because of similar biological potency to **1**, the analog **2** should be recognized as a promising scaffold for new anti-HIV agents with an unprecedented mechanism of action, inhibition for nuclear export of Rev protein, in the conventional remedy.



The acquired immunodeficiency syndrome (AIDS) is a life-threatening disease caused by HIV-1 and the HIV pandemic remains one of the most serious threats to worldwide public health.[1] Rev protein of HIV-1 was clarified to play an essential role in viral replication by constructing the structural proteins after export of the viral mRNA from the nucleus to the cytoplasm with the aid of the cargo protein CRM1.[2,3] Inhibition for nuclear export of Rev was, therefore, recognized as one of the attractive targets for new anti-HIV drugs with an unprecedented mechanism of action. In practice, leptomycin B, the first nuclear export inhibitor of Rev, was reported to show anti-HIV activity.[4,5] On the other hand, we found out valtrate (**1**) as the inhibitor for nuclear export of Rev from the medicinal plant Valerianae Radix (root of *Valeriana fauriei*) and revealed **1** to inhibit proliferation of HIV-1. Furthermore, valtrate (**1**) was shown to be linked to the Cys-529 residue in CRM1 by the covalent bond between the epoxy moiety in **1** and the thiol group in Cys-529, this indicating that the epoxy portion is the conclusive function to exert the bioactivity of **1** (Fig. 1).[6] Despite the potential biological activity as well as the elucidated mechanism of action, utilization of **1** as a scaffold toward anti-HIV agents seemed infeasible because of both scarce supply from natural resource ($6.3 \times 10^{-3}$% from crude drug) and strict limitation of derivatization of **1**; removal of the isovaleryl group at C-1 facilely brought about decomposition by way of dial with involving elimination of the acetoxy group. In this context, search for the bioisosteres of **1** was intensively considered to be important for development of new anti-HIV agents. This manuscript deals with the

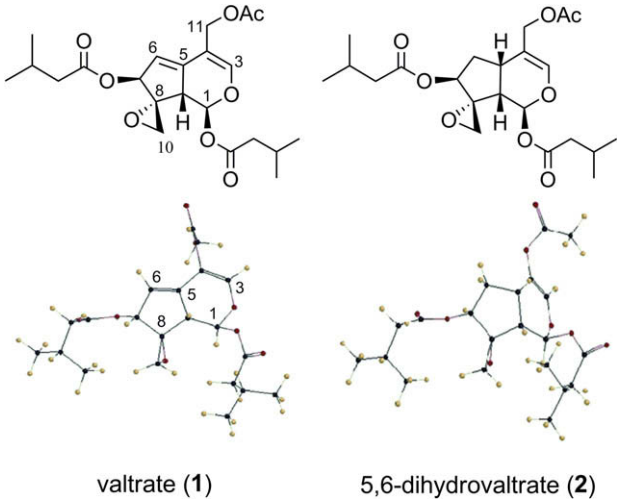


**Figure 1.** Stable conformations of valtrate (**1**) and 5,6-dihydrovaltrate (**2**) by MO calculation.

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doi:10.1016/j.bmcl.2010.02.038

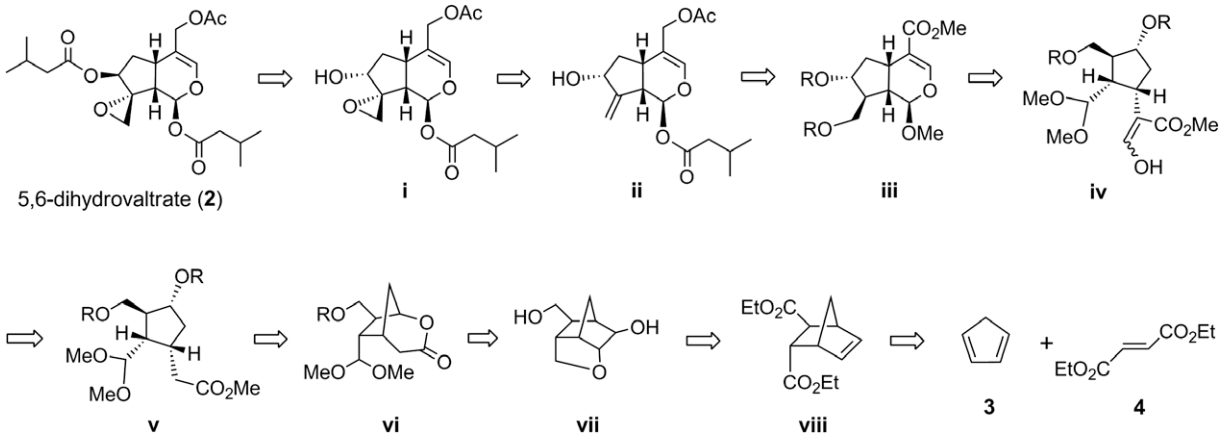
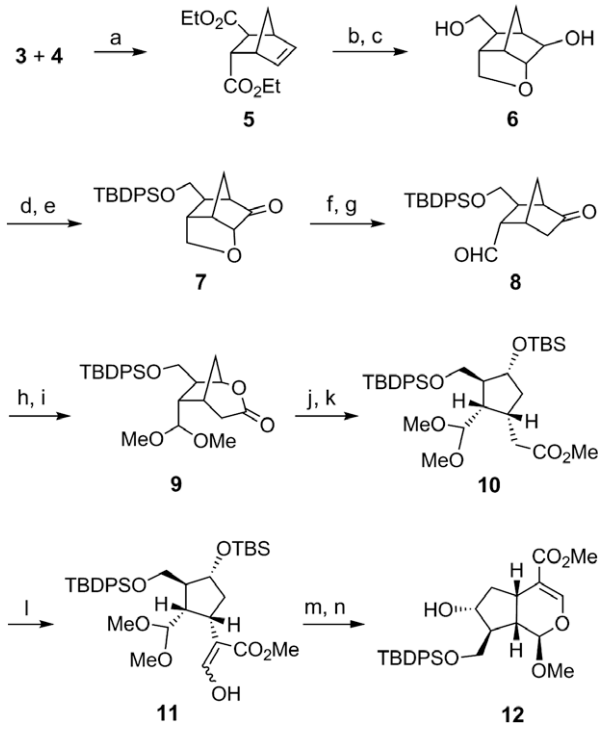



**Figure 2.** Retrosynthetic analysis of 5,6-dihydrovaltrate (**2**).

synthesis and biological property of 5,6-dihydrovaltrate (**2**), the bioisostere of valtrate (**1**), which will be anticipated to possess fairly similar steric environment around the epoxy pharmacophore to **1** through rational design by the molecular orbital calculation.

In the first instance, the most stable conformations of **1** and **2** were calculated by a semi-empirical molecular orbital method PM3. As a result of the MO calculation, no serious difference in steric environment around the C-8 epoxy moiety appeared between **1** and **2**, which intensively presented that 5,6-dihydroanalog (**2**) exhibited nearly similar inhibitory activity for nuclear export of Rev as compared with valtrate (**1**) (Fig. 1).

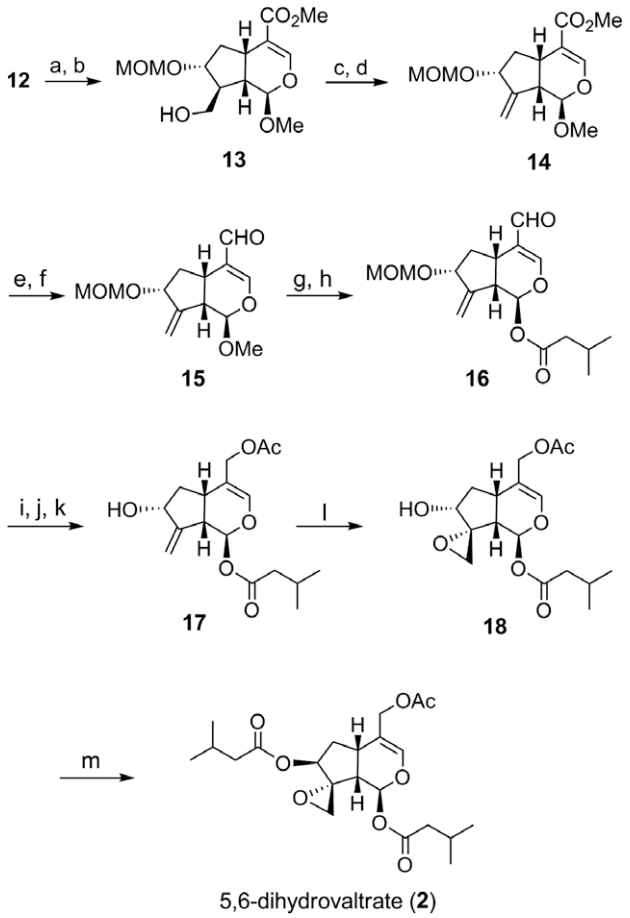
Since 5,6-dihydroanalog (**2**) was presumed to be synthesized from the highly optical pure cyclopentane **v** by way of the bicyclic methyl acetal **iii** bearing the fully oxygen-functionalized iridoid skeleton, we conducted retrosynthetic analysis of **2** as illustrated in Figure 2. Namely, the labile 1,1-disubstituted epoxy moiety would be constructed by taking advantage of the α-oriented hydroxyl group at C-7 in the late stage. The isovaleryloxy group would be introduced by Mitsunobu inversion for the hydroxyl group at C-7 in the final stage. *Exo*-olefin **ii** as the precursor of epoxide **i** would be afforded from methyl acetal **iii**. Moreover, the acetal **iii** was planned to be yielded by intramolecular transacetalization of enol **iv**, which would be obtained by condensation between the optical active cyclopentane **v** and ethyl formate. The cyclopentane **v** would be prepared by methanolysis of lactone **vi**, which would be available from cyclic ether **vii** by oxidation followed by Baeyer–Villiger rearrangement. The cyclic ether **vii** was expected to be attained by stereoselective epoxidation and intramolecular etherization from the asymmetric Deals–Alder adduct **viii** between cyclopentadiene (**3**) and diethyl fumarate (**4**) in high optical purity.




**Scheme 1.** Reagents and conditions: (a) ligand, $CH_2Cl_2$, −35 °C, quant.; (b) $LiAlH_4$, THF, 0 °C, quant.; (c) mCPBA, $CH_2Cl_2$, 90%; (d) TBDPSCl, DBU, $CH_2Cl_2$, 95%; (e) Dess–Martin periodinane, $CH_2Cl_2$, 88%; (f) Al–Hg, THF, EtOH, 92%; (g) Dess–Martin periodinane, $CH_2Cl_2$, quant.; (h) MeOTMS, TMSOTf, $CH_2Cl_2$, 80%; (i) mCPBA, $KH_2PO_4$, $CH_2Cl_2$, 81%; (j) NaOMe, MeOH, 90%; (k) TBSOTf, 2,6-lutidine, $CH_2Cl_2$, 80%; (l) LDA, HCOOEt, THF, −78 °C; (m) $BF_3{\cdot}OEt_2$, $CH_2Cl_2$, 0 °C; (n) *p*-TsOH, $CH(OMe)_3$, MeOH, three steps 54%.

The synthesis of the methyl acetal **12** with the optical active iridoid skeleton was executed as shown in Scheme 1. Asymmetric Diels–Alder reaction using Corey's chiral ligand[7] between cyclopentadiene (**3**) and diethyl fumarate (**4**) afforded the adduct **5** quantitatively with 95% ee. After reduction of the two ester functions in **5**, treatment of the resulting diol with mCPBA promoted not only epoxidation but also intramolecular etherization to give tricyclic ether **6**. The primary hydroxyl group in **6** was selectively protected as TBDPS ether and subsequent Dess–Martin oxidation[8] provided cyclic ketone **7**. Reductive cleavage of the cyclic ether in **7** with Al–Hg followed by oxidation of the resultant primary hydroxyl function afforded aldehyde **8**. The formyl group in **8** was subjected to selective acetalization by treatment with methoxytrimethylsilane (MeOTMS) in the presence of TMSOTf, then Baeyer–Villiger rearrangement by mCPBA gave seven-membered lactone **9**. Successive methanolysis of **9** with NaOMe and protection of the resulting hydroxyl group with the TBS residue provided cyclopentane **10**, which was submitted to installation of the formyl group mediated with ethyl formate and lithium diisopropyl amide to provide enol **11**. $BF_3$ catalyzed transacetalization of **11** smoothly proceeded concomitant with deprotection of the TBS function. In the last step, the partly obtained hemiacetal was entirely converted to methyl acetal **12** possessing the desired iridoid skeleton by treatment with $CH(OMe)_3$ in the presence of *p*-TsOH.

Subsequently, the synthesis of 5,6-dihydroanalog (**2**) was accomplished from **12** by ingenious use of stereoselective epoxidation associated by the adjacent hydroxyl group as displayed in Scheme 2. The secondary hydroxyl group in **12** was protected as MOM ether, then the TBDPS group was removed by treatment of




**Scheme 2.** Reagents and conditions: (a) MOMCl, *i*$Pr_2NEt$, $CH_2Cl_2$; (b) TBAF, THF; (c) $I_2$, $PPh_3$, imidazole, $C_6H_6$; (d) *t*BuOK, THF, 0 °C, four steps 40%; (e) DIBAL, $CH_2Cl_2$, −78 °C; (f) Dess–Martin periodinane, $CH_2Cl_2$, two steps 80%; (g) 10% HCl–THF, 43%, recovery of **15** 42%; (h) isovaleric acid, $Im_2CO$, DBU, $CH_2Cl_2$, 0 °C, 77%; (i) $BCl_3$, $CH_2Cl_2$, 0 °C, 65%; (j) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH; (k) AcCl, *i*$Pr_2NEt$, $CH_2Cl_2$, 0 °C, two steps 58%; (l) TBHP, $VO(acac)_2$, $C_6H_6$, 80%; (m) isovaleric acid, DEAD, $PPh_3$, $C_6H_6$, 85%.

TBAF to afford **13**. Iodination of the hydroxyl group in **13** and the following dehydrohalogenation by *t*BuOK gave *exo*-olefin **14**. After reduction of **14** with DIBAL, Dess–Martin oxidation of the resulting primary alcohol provided conjugated aldehyde **15**. Selective hydrolysis of the methyl acetal in **15** in 10% HCl–THF gave hemiacetal, which was coupled with isovaleric acid in the presence of DBU and *N*,*N*′-carbonyldiimidazole (CDI) to afford isovaleryl ester **16**. Selective removal of the MOM protection by $BCl_3$ followed by reduction by $NaBH_4$ in the presence of $CeCl_3$ led **16** to the corresponding diol, of which the primary hydroxyl group was selectively acetylated to afford **17**. Stereoselective epoxidation of **17** by *tert*-butyl hydroperoxide (TBHP) and vanadium oxyacetylacetonate [$VO(acac)_2$] mediated by the adjacent hydroxyl group at C-7[9] gave the desired α-epoxyalcohol **18**. NOE correlations between 7-H and 10-Ha, 9-H and 10-Hb in the NOESY spectrum of **18** unequivocally established the desired α-epoxy configuration. Introduction of the isovaleryloxy function to C-7 in **18** involving the steric inversion of the hydroxyl function under Mitsunobu condition[10] completed the synthesis of the target 5,6-dihydroanalog (**2**).[11]

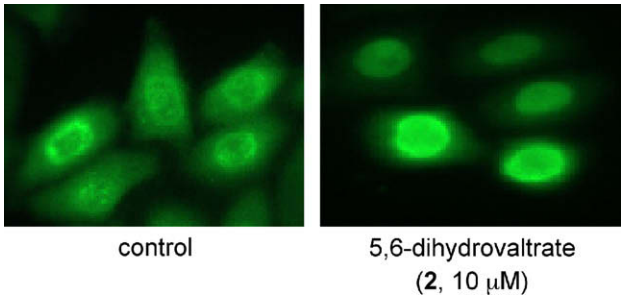



**Figure 3.** Inhibitory activity for nuclear export of Rev by **2**.

Finally, the synthesized analog (**2**) was evaluated for inhibitory activity for nuclear export of Rev protein.[12] After transfection of plasmid coding Rev tagged with human influenza haemagglutinin (HA) into HeLa cells, localization of Rev protein was examined by an indirect fluorescent antibody technique aiming at HA tag (Fig. 3). Consequently, 5,6-dihydroanalog (**2**), presumed to possess the similar steric environment around the epoxy pharmacophore to **1** by molecular orbital calculation, inhibited export of Rev from nucleus with $IC_{50}$ of 4.4 μM in practice. In addition, the analog **2** almost entirely disrupted nuclear export of Rev at the concentration of 10 μM. On the basis of these biological scores, 5,6-dihydroanalog (**2**) was undoubtedly revealed to be the bioisostere of valtrate (**1**, $IC_{50}$: 2.5 μM) as the promising scaffold of new anti-HIV agents.

In conclusion, we synthesized and disclosed 5,6-dihydrovaltrate (**2**) as the bioisostere of valtrate (**1**) with anti-HIV activity through the rational design by the MO calculation. The analog should be recognized as the promising scaffold for new anti-HIV agents with the unprecedented mechanism of action, inhibition for nuclear export of Rev protein, in the conventional remedy. Exploration for more potent analogs modifying the three acyl groups in **2** is currently under investigation in our laboratory.

## Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research (Grant No. 19590100) from the Ministry of Education, Science, Culture and Sports. The authors are grateful to the Shorai Foundation for Science and Technology financial support.

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11. *Compound* **2**: colorless oil, $[\alpha]_D^{20}$ −48.6 (*c* 2.1, MeOH), IR (KBr): 1761 (sh), 1738, 1670 $cm^{-1}$, $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$: 6.52 (1H, s, 3-H), 5.84 (1H, d, *J* = 5.5 Hz, 1-H), 4.97 (1H, dd, *J* = 6.1, 6.1 Hz, 7-H), 4.65 (1H, d, *J* = 12.2 Hz, 11-Ha), 4.46 (1H, d, *J* = 12.2 Hz, 11-Hb), 3.06 (1H, d, *J* = 5.5 Hz, 10-Ha), 2.95 (1H, ddd, *J* = 8.5, 6.1, 6.1 Hz, 5-H), 2.82 (1H, d, *J* = 5.5 Hz, 10-Hb), 2.72 (1H, dd, *J* = 8.5, 5.5 Hz, 9-H), 2.28 (1H, *J* = 13.4, 6.1, 6.1 Hz, 6-Ha), 2.21 (2H, d, *J* = 7.3 Hz, $CO_2CH_2CH(CH_3)_2$), 2.17 (2H, d, *J* = 7.3 Hz, $CO_2CH_2CH(CH_3)_2$), 2.08 (3H, s, $OCOCH_3$), 2.07 (1H, m, $CO_2CH_2CH(CH_3)_2$), 2.05 (1H, m, $CO_2CH_2CH(CH_3)_2$), 1.99 (1H, *J* = 13.4, 6.1, 6.1 Hz, 6-Hb), 0.99 (3H, d, *J* = 6.7 Hz, $CO_2CH_2CH(CH_3)_2$), 0.97 (3H, d, *J* = 6.7 Hz, $CO_2CH_2CH(CH_3)_2$), 0.96 (3H, d, *J* = 6.7 Hz, $CO_2CH_2CH(CH_3)_2$), 0.94 (3H, d, *J* = 6.7 Hz, $CO_2CH_2CH(CH_3)_2$), FAB-MS *m*/*z*: 425 $(M+H)^+$, FAB-HRMS *m*/*z*: Calcd for $C_{22}H_{32}O_8$+H: 425.2097, Found: 425.2117.
12. HeLa cells ($1.0 \times 10^5$ cells) were maintained on coverslips in 24-well microplate with 1 mL of Dulbecco's MEM medium supplemented with 10% FBS at 37 °C in 5% $CO_2$ for 24 h. Transfection of pCG-HA-Rev (plasmid encoding HA-tagged Rev protein) and pCRRE/ΔRev (plasmid encoding Gag protein) plasmids into HeLa cells were performed using PolyFect® transfection reagent kit (QIAGEN) for 16 h according to the manufacturer's instructions. After the cells were washed, each solution of tested sample at an appropriate concentration in the medium containing 1% DMSO was inoculated and the whole was incubated at 37 °C for further 12 h. Cells were rinsed with cold D-PBS (−) twice and fixed with 4% formaldehyde/D-PBS (−) for 20 min. Then the cells were defatted with MeOH under shaking for 10 min and washed with cold

D-PBS (−) thrice. After treatment with 10% FBS in Dulbecco's MEM medium for 30 min, the samples were incubated with anti-HA antibody (Roche) for 45 min followed by incubation with FITC-labeled anti-mouse IgG antibody (Vector) for 45 min. Localization of the HA-tagged Rev protein in the cells was examined under a fluorescence microscope, then image analysis was conducted by Scion image software (Scion) to determine Rev-export inhibitory activity. In the depicted pictures, several cells free from transfection displayed disperse weak fluorescence due to nonspecific binding of the antibodies.