Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 3115–3118

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Anti-HIV Activity of Oleanolic Acid Derivatives

Yong-Ming Zhu,[a] Jing-Kang Shen,[a] Hui-Kang Wang,[b] L. Mark Cosentino[c] and Kuo-Hsiung Lee[b,*]

[a]*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, China*
[b]*Natural Products Laboratory, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7360, USA*
[c]*Biotech Research Laboratories, 217 Perry Parkway, Gaithersburg, MD 20877, USA*

Received 13 July 2001; accepted 14 September 2001

Abstract—Thirteen oleanolic acid derivatives were prepared and evaluated for anti-HIV activity in H9 lymphocytes. Saturating the C_{12}–C_{13} double bond and converting the C_{17}-carboxyl group to an aminomethyl group led to compounds **13**–**15** and **19**–**20**, respectively, which showed improved anti-HIV activity. Compound **15** was the most potent derivative with $EC_{50} = 0.0039\ \mu g/mL$ and TI = 3570.

In order to combat the debilitating disease acquired immune deficiency syndrome (AIDS), the USA FDA has approved new drugs for the treatment of human immunodeficiency virus (HIV) infection. These drugs include nucleoside analogue HIV reverse transcriptase (RT) inhibitors,[1,2] non-nucleoside RT inhibitors,[3] and protease inhibitors.[4] However, the efficacy of these HIV enzyme inhibitors is limited by the development of drug resistance and their toxic side effects.[5,6] Thus, current anti-HIV drug discovery is largely focused on new agents with novel mechanisms of action.

In our continuing search for new anti-HIV drug candidates, we previously reported that modification of betulinic acid yielded extremely potent anti-HIV compounds.[7–9] Recently, we also discovered that oleanolic acid exhibited anti-HIV activity with an EC_{50} of 1.7 μg/mL and therapeutic index (TI) of 12.8.[10] Based on this finding, oleanolic acid was also considered to be a potential anti-HIV lead triterpenoid. To explore the roles of the acyl substituent and other functional groups, we developed a method to saturate the C_{12}–C_{13} double bond and, in addition, converted the C_{17}-carboxyl to an aminomethyl group (Fig. 1).

Compounds **2**–**5**, **10**–**15**, **18**–**20** and AZT were examined for anti-HIV activity in H9 lymphocytes as shown in Table 1. Esterification of **1** at C_3-hydroxy gave **2**–**5**, which were as active or less active than the lead compound. Saturation of the C_{12}–C_{13} double bond in **1** gave **10** and led to a 3-fold increase in activity and in TI value. Esterification of **10** with appropriate anhydrides yielded compounds **11**–**15**. While 11 was less active than the parent acid (**10**), **12**–**14** were 5-fold more active than **10**, and **15** showed remarkable activity. Converting the C_{17}-carboxyl of **1** to aminomethyl gave **18**, which could not be tested due to its insolubility in DMSO. Introducing 3′,3′-dimethylglutaryl or 3′,3′-tetramethylglutaryl groups at both the C_3 and C_{28} positions of **18** produced **19**–**20**, which displayed more than a tenfold increase in activity compared with **1**.

In conclusion, oleanolic acid derivatives **12**–**15**, **19**, and **20** showed high anti-HIV activity. Compound **15** was the most active with EC_{50} and TI values of 0.0039 μg/mL and 3570, respectively. Saturation of the C_{12}–C_{13} double bond could be a major cause of anti-HIV activity enhancement, whereas a C_3 acryl side chain was essential for optimal activity. In addition, changing the C_{17}-carboxyl to aminomethyl could significantly enhance anti-HIV activity. Additional structure–activity relationship studies are in process.

A general synthesis for compounds **2**–**5** and **11**–**15** was performed according to the following procedure. Oleanolic acid or C_{12}, C_{13}-dihydrooleanolic acid was heated to reflux overnight with 2 equiv of the appropriate anhydride in anhydrous pyridine in the presence of 4-(dimethylamino)

*Corresponding author. Tel.: +1-919-962-0066; fax: +1-919-966-3893; e-mail: khlee@email.unc.edu

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00647-3

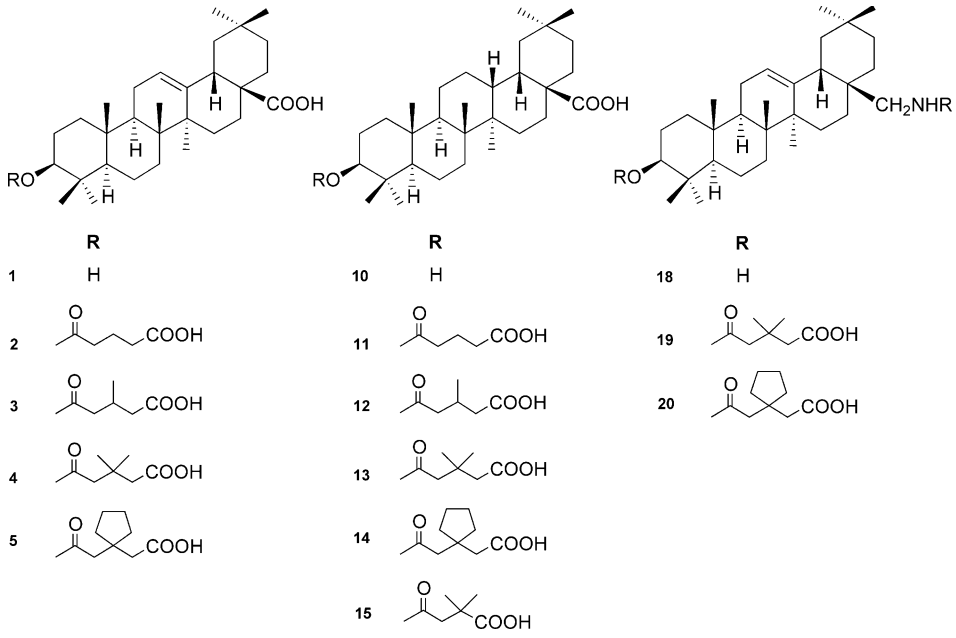


Figure 1.

Table 1. Anti-HIV activity of oleanolic acid and derivatives

Compd	Anti-HIV activity[a] EC_{50} (μg/mL)	Cytotoxicity[a] IC_{50} (μg/mL)	Therapeutic index $TI = IC_{50}/EC_{50}$
1	1.7	21.8	12.8
2	7.1	14.6	2.1
3	8.3	27.5	3.3
4	1.5	14.2	9.3
5	1.2	4.7	3.9
10	0.5	17.4	34.8
11	2.6	12.9	4.9
12	0.1	12.3	123
13	0.1	8.3	83
14	0.1	10.1	101
15	0.0039	13.7	3570
18	NT	NT	NT
19	0.1	21.2	212
20	0.1	19.5	195
AZT	0.01	500	50,000

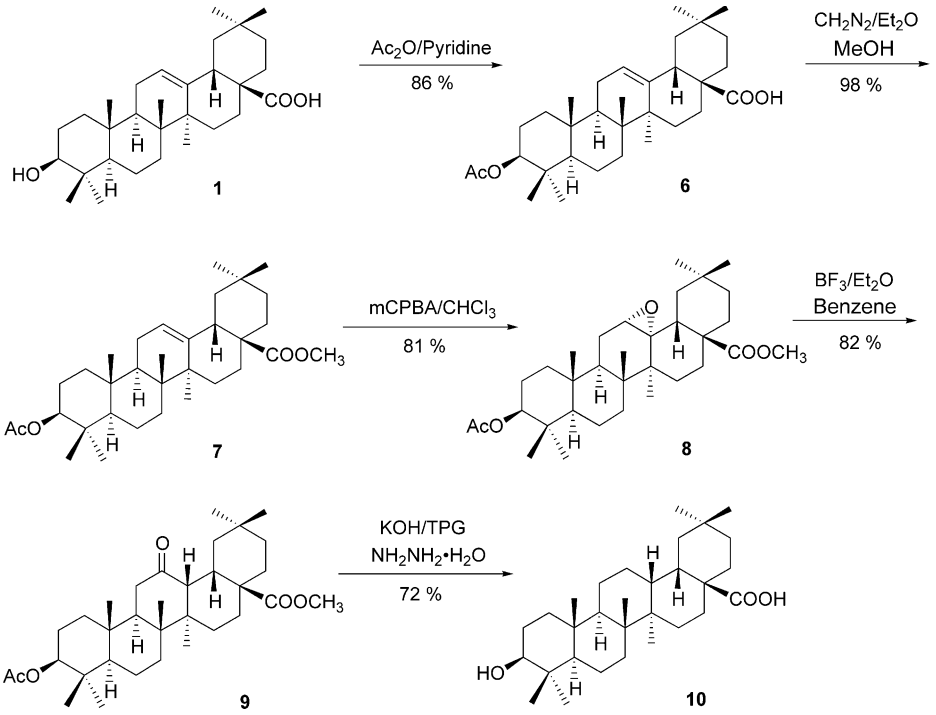
[a]NT, not tested due to solubility. All data are an average of at least two experiments.

pyridine (catalytic). When TLC indicated complete consumption of starting material, the reaction solution was diluted with $CHCl_3$ and washed with 3 N HCl solution. The organic layer was then dried over $MgSO_4$ and subjected to column chromatography. Compound **15** was obtained from preparative HPLC.[11]

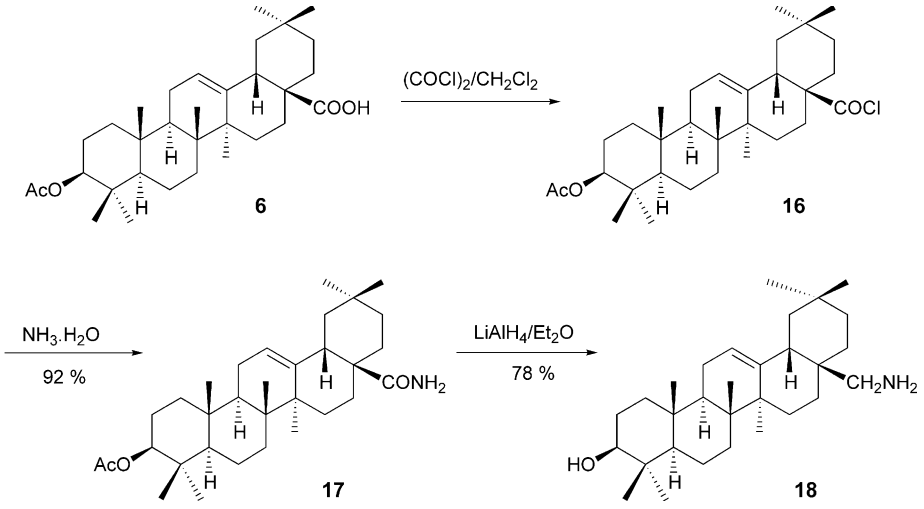
The synthesis of compound **10** is outlined in Scheme 1. Oleanolic acid was acetylated with 10 equiv of acetic anhydride in anhydrous pyridine to give 3-acetoxy-oleanolic acid (**6**) and then treated with ethereal diazomethane to yield methyl 3-acetyloleanolic acid (**7**). On oxidation with 1.2 equiv of 3-chloroperbenzoic acid in dry $CHC1_3$ at 0 °C overnight, compound **7** afforded methyl 3-acetoxy-12,13-epoxyoleanolate (**8**).[12] Compound **8** was refluxed with ethereal boron trifluoride in fresh benzene for 30 minutes to give methyl 3-acetoxy-12-oxo-13-oleanolate (**9**).[13,14] Dihydrooleanolic acid (**10**) was produced by Wolff–Kishner reduction of **9** in tripolyethylene glycol solution.

The preparation of compound **18** is illustrated in Scheme 2. 3-Acetoxyoleanolicacid (**6**) was treated with oxalyl chloride to yield 3-acetoxyoleanolic chloride (**16**). A solution of **16** in freshly distilled THF was added dropwise to concentrated ammonia solution cooled with an ice-salt bath to afford 3-acetoxyoleanolic amide (**17**). Compound **17** was refluxed with 2.0 equiv of lithium aluminum hydride in anhydrous ether to give **18**. Compounds **19** and **20** were prepared by heating **18** with 3 equiv of 3,3-dimthylglutaryl and 3,3-tetramethylglutaryl anhydride, respectively, in anhydrous pyridine in the presence of 4-(dimethylamino) pyridine (catalytic) overnight at 100 °C. The reaction mixture was worked up as for compounds **2**–**5** and **11**–**15**.

The biological evaluation of HIV-1 inhibition was carried out according to established protocols. The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum supplemented with L-glutamine at 5% CO_2 and 37 °C). Aliquots of this cell line were used in experiments only when in log-phase growth. Test samples were first dissolved in dimethylsulfoxide. The following final drug concentrations were routinely used for screening: 100, 20, 4, and 0.8 μg/mL. For active agents, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value (defined below) could be achieved. As the test sample was being prepared, an aliquot of the H9 cell line was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The stock virus used for these studies typically had a TCID 50 value of 104 Infectious Units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 Infectious Units/mL was added to the first aliquot of H9 cells. The other aliquot received only culture medium, and these



Scheme 1.



Scheme 2.

mock-infected cells were used for toxicity determinations (IC_{50} defined below). After a 4 h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24-plate containing the various concentrations of the test drug or culture medium (positive infected control negative drug control). In addition AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 for use in our in-house p24 antigen ELISA assay. P24 antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts by a Coulter Counter on the mock-infected H9 cells, which had either received culture medium (no toxicity), test sample, or AZT. If a test sample had suppressive capability and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of the test sample which was toxic to 50% of the mock-infected H9; EC_{50}, the concentration of test sample which was able to suppress HIV replication by 50%; and Therapeutic Index (TI), the ratio of IC_{50} to EC_{50}.

Acknowledgements

This investigation was supported in part by grant AI-33066 from the National Institute of Allergies and Infectious Diseases awarded to K. H. Lee.

References and Notes

1. Debyser, Z.; Pauwels, R.; Andries, K.; Declereq, E. *J. Enzyme Inhib*. **1992**, *6*, 47.
2. Barre-Sinooussi, F.; Chermann, J. C.; Rey, F. *Science* **1983**, *226*, 868.
3. Crob, P. M.; Cao, Y.; Muchmore, E.; Ho, D. D.; Norris, S.; Pav, J. W.; Shih, C. K.; Adams, J. *Nature Medicine* **1997**, *3*, 665.
4. Meek, T. D. *J. Enzyme Inhib*. **1992**, *6*, 65.
5. Schinazi, R. F.; Mead, J. R.; Feorino, P. M. *Human Retrovir*. **1992**, *8*, 963.
6. Shirahata, T.; Yarchoan, R.; O'Brien, M. C.; Husson, R. N.; Anderson, B. D.; Kojima, E.; Shimoda, T.; Broder, S.; Mitsuya, H. *Proc. Natl. Acad. Sci, U.S.A*. **1993**, *91*, 562.
7. Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. *J. Med. Chem*. **1996**, *39*, 1016.
8. Sun, I. C.; Shen, J. K.; Wang, H. K.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1267.
9. Sun, I. C.; Wang, H. K.; Kashiwada, Y.; Shen, J. K.; Cosentino, L. M.; Chen, C. H.; Yang, L. M.; Lee, K. H. *J. Med. Chem*. **1998**, *41*, 4648.
10. Kashiwada, Y.; Wang, H. K.; Nagao, T.; Kitanaka, S.; Yasuda, I.; Fujioka, T.; Yamagishi, T.; Cosentino, L. M.; Kozuka, M.; Okabe, H.; Ikeshiro, Y.; Hu, C. Q.; Yeh, E.; Lee, K. H. *J. Nat. Prod*. **1998**, *61*, 1090.
11. Compound **15**: yield 46% [after HPLC separation with *n*-hexane/ethyl acetate (10:1)], a white amorphous powder, mp 289–290 °C (MeOH–H_2O), ^{1}H NMR ($CDCl_3$, 400 MHz) δ, 0.80 (3H, s, H-25), 0.83 (9H, s, H-23, H-24, H-29), 0.90 (3H, s, H-26), 0.93 (3H, s, H-30), 0.99 (3H, s, H-27), 1.27, 1.32 [3H each, both s, 3′-(CH_3)2], 2.15 (1H, d, J = 14.2 Hz, H-18), 2.40, 2.90 (1H, each, both d, J = 15.1 Hz, H-2′), 4.52 (1H, dd, J = 11.3, 3.4 Hz), IR (KBr): 1729.9, 1697.1, 1269.0, 993.2 cm^{-1}. Anal. calcd for $C_{36}H_{58}O_6$, C 73.68, H 9.96, found C 73.81, H 9.95.
12. Boar, R. B.; Joukhadar, L.; Deluque, M.; McGhie, J. F.; Barton, D. H. R.; Arigoni, D.; Brunner, H. G.; Giger, R. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2104.
13. Farina, C.; Pinza, M. *Gazz. Chim. Ital*. **1987**, *117*, 561.
14. Boar, R. B.; Knight, D. C.; McGhie, J. F.; Barton, D. H. R. *J. Chem. Soc. (C)* **1970**, 678.