Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3549–3552

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Lupane Triterpenes and Derivatives with Antiviral Activity

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Received 3 March 2003; revised 24 April 2003; accepted 4 July 2003

Abstract—Betulin and betulinic acid have been modified at the C-3 and C-28 positions and the antiviral activity of derivatives has been evaluated in vitro. It was found that simple modifications of the parent structure of lupane triterpenes produced highly effective agents against influenza A and herpes simplex type 1 viruses.

Introduction

Betulin **1** and betulinic acid (BA) **3** are pentacyclic triterpenes of lupane type of natural origin isolated from various plants. The content of betulin in the outer bark of white birches trees widespread in the northern latitudes of the world, varies between 10 and 35% depending on the growth conditions, age, season and so on.[1] BA was originally extracted from the tropical tree, *Ziziphus mauritiama Lam* (*Rhamnaceae*)[2,3] and from the leaves of *Syzigium claviflorum* and *Aerva javanica* (*Amaranthaceae*). BA can be easily prepared from an available bioprecursor, betulin, by a two-step synthetic process.[4] BA showed selective cytotoxicity against human melanoma, neuroectodermal and malignant brain tumor cell lines,[5,6] induced apoptosis in human neuroblastoma cell lines.[7] BA and its derivatives have been identified as a potent and selective HIV-1 inhibitor.[2,8–11] Specific inhibitors of HIV-1 with a new mode of action were found among a series of ϖ-undecanoic amides of BA. It was revealed that this class of compounds interfere with HIV-1 entry in the cells at a posbinding step.[10] The esterification of betulin and BA with 3′,3′-dimethylglutaryl and 3′,3′-dimethylsuccinyl groups potentiated the anti-HIV activity of compounds.[9,12,13] Amino acid conjugates of BA are of interest as inhibitors of human melanoma and fibrosarcome.[14] Betulin and BA have antiviral properties also against herpes simplex viruse type 1.[15] This paper describes the synthesis and antiviral evaluation of new betulin and BA derivatives against influenza A and herpes simplex type I viruses in vitro. It was envisioned that a series of simple modifications of the triterpenoids parent structures could produce a number of potentially important new derivatives with antiviral activity.

Materials and Methods

Betulin **1** was extracted from the birch bark of the Betula pendula Roth collected in Ufa, Russia with 2-propanol–water mixture (9:1, v/v) according to ref 16. Betulonic acid **2** was prepared from betulin 90–92% by using Jones' oxidation (CrO_3/H_2SO_4/acetone).[4] BA **3** was synthesized from **2** by $NaBH_4$ reduction in 2-propanol (92% yield) and recrystallized in hot methanol to pure 3β isomer with mp 290–291 °C. Lit.:[17] 291–292 °C.

Antiviral activity was tested in cell cultures with influenza A/FPV/Rostock/34 (H7N1) and herpes simplex type 1 (HSV-1, strain 1C) viruses as described early.[18,19] Study of the antiviral activity against influenza A virus was carried out in chicken embryo cells (CEC) by plaque reduction assay method. Investigations of antiviral activity against HSV-1 were carried out by inhibition of viral cytopathic effect (CPE) in rhabdomyosarcoma (RD) cell line. The compounds tested were predissolved in 10% ethanol and then were prepared in consecutive 2-fold dilutions in maintenance medium (medium 199, Sigma Chemical Co).

A virus titer reduction in comparison with control, 50% effective concentration (EC_{50}), and maximum nontoxic concentration (MNTC) to EC_{50} ratio of tested substances were calculated as antiviral activity criteria.

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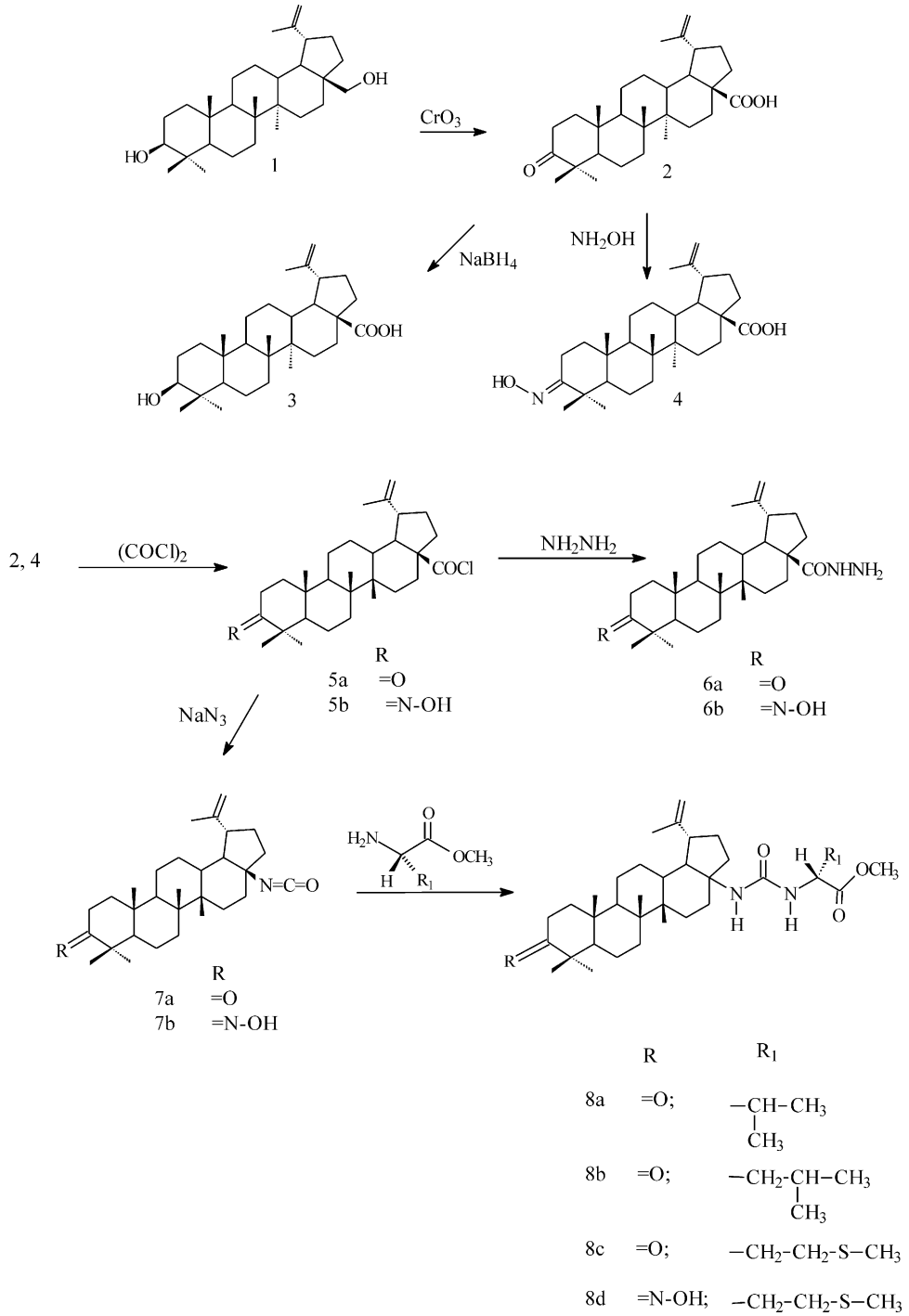
doi:10.1016/S0960-894X(03)00714-5

MNTC of compounds were determined in non-infected cell cultures after 72 h incubation at 37 °C. The results of experiments are given in Table 1.

Chemical Modification of Lupane Triterpenes

Betulonic acid **2** was converted into 3-oxime **4** (90%) by refluxing for 2 h in a solution of hydroxylamine hydrochloride in pyridine. Mp 223–225 °C. Acid chlorides **5a** and **5b** were obtained in 95 and 92% yields, respectively, by treatment of **2** and **4** in dry benzene with oxalyl chloride according to refs 10 and 11. Hydrazides **6a** and **6b** were prepared by the reaction of acid chlorides **5a** and **5b** with NH_2NH_2 in ether solution at 0±5 °C with 80 and 90% yields, respectively.

Isocyanates **7a** and **7b** were obtained from acid chlorides **5a** and **5b** (1 mmol) and NaN_3 (125 mg) by mixing in acetone solution for 6 h at 20–22 °C. The reaction of isocyanates **7a** and **7b** with primary amines and L-amino acids methyl esters gave good yields (84–88%) of 28-*N*′-alkylureides **8a**–**8d** (Scheme 1). Products were purified by chromatography on Al_2O_3. Structures of all synthesized



Scheme 1.

Table 1. Antiviral activity of betulinic acid and related compounds

Compd	Influenza A		HSV-1	
	EC_{50} (μM)	MNTC/EC_{50}	EC_{50}	MNTC/EC_{50}
2	5.7	9.6	2.5	43.9
3	>219.0	<1	8.2	53.3
4	2.2	392.2	81.6	1.3
6a	NT	NT	10.0	21.3
6b	2.0	25.2	>51.6	<1
7a	>221.4	<1	>221.4	<1
8a	>85.7	<1	0.2	454.0
8b	5.0	>67.0	14.6	11.5
8c	27.1	3.0	2.2	298.5
8d	2.3	35.2	0.6	67.6
9b	55.5	4.6	1.4	177.0
9c	>754.7	<1	>377.4	<1
10a	>234.4	<1	662.8	1.4
10b	13.3	3.4	141.8	2.5
10c	72.3	1.2	1373.2	<1
10d	14.2	11.9	>666.0	<1
11	0.7	1333.3	220.4	<1

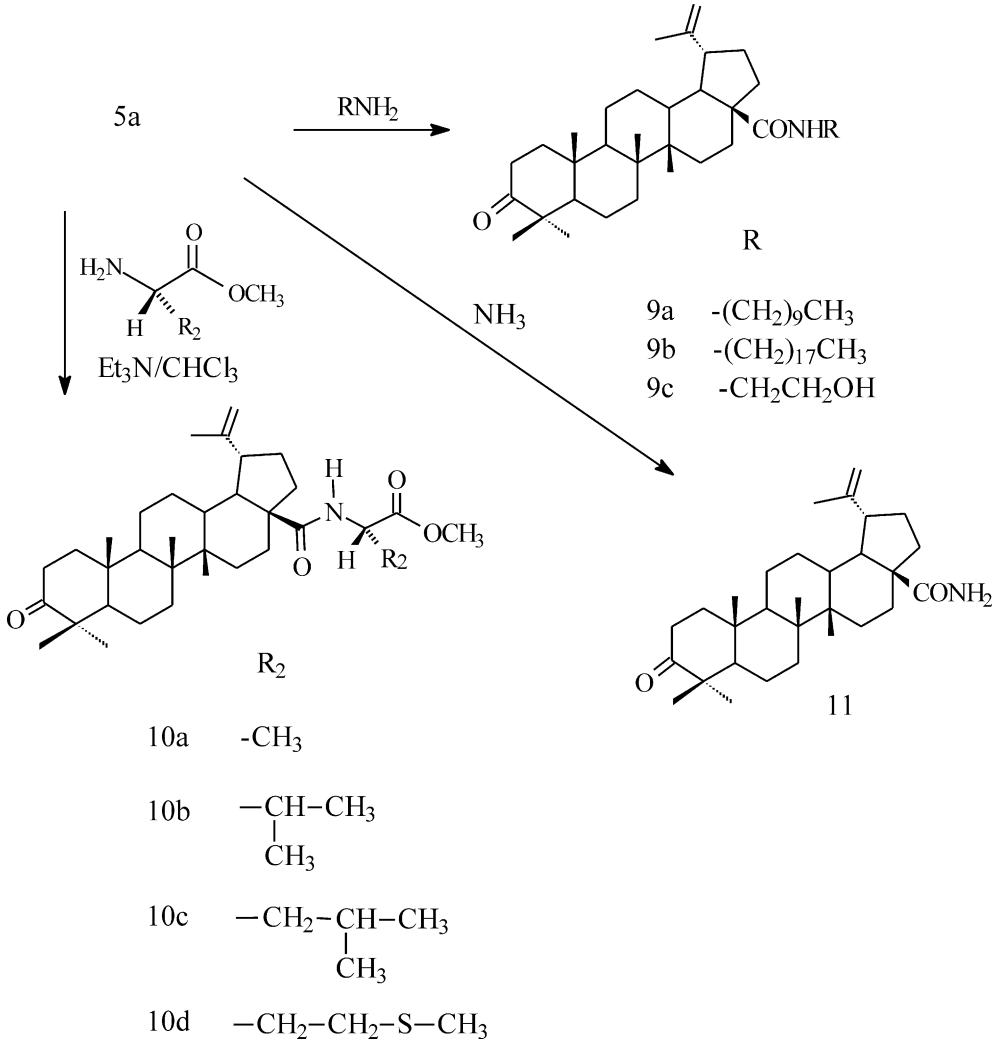
NT, not tested.

compounds were confirmed by IR, ^{1}H and ^{13}C NMR spectra.

Acid chloride **5a** (1 mmol) was reacted with amines (hexylamine, octadecylamine, colamine) (1.6 mmol) in dry refluxing benzene or dioxane giving amides **9a–9c** (Scheme 2) (67–74% yields). Amino acids conjugates **10a–10d** were synthesized by the reaction of acid chloride **5a** (1 mmol) with L-amino acids (valine, leucine, methionine) methyl esters hydrochlorides (1.3 mmol) in chloroform. Yields of conjugates were 79–85%. The reaction of acid chloride **5a** with liquid ammonia in chloroform resulted in amide **11** with 89% yield (Scheme 2).

Biological Activity

It was shown by different groups that the introduction of the CONH function in the molecule of BA resulted in increase of anti-HIV activity.[10,11] Lupane triterpenes and derivatives were evaluated against influenza A and



Scheme 2.

herpes simplex type 1 viruses in vitro (Table 1). Compounds **4**, **11** were active against influenza A virus. 3-Oxime **4** showed a significantly enhanced activity (EC_{50} 2.2 μM) and a remarkably high $MNTC/EC_{50}$ ratio of 392.2. When the C-28 carboxyl group was substituted by a $CONH_2$-group the activity was further improved. 28-amide **11** showed on EC_{50} of 0.7 μM and was characterized by the widest range of active nontoxic concentrations ($MNTC/EC_{50} = 1333.3$). Ureide **8d** was effective as influenza A virus inhibitor but its activity was less expressed. BA was not active in such experiments.

Compounds **8a**, **8c**, **8d** and **9b** were highly effective against HSV-1: EC_{50} 0.2, 2.2, 0.6 and 1.4, respectively (Table 1). Their activity was more pronounced than the activity of lupane acids **2** and **3**. Compounds **8a** and **8c**, which both contain the NHCONH group, exhibited the most potent anti-HSV-1 activity and had the widest range of $MNTC/EC_{50}$ ratio (454.0 and 298.5). Compound **9b** containing a long 28-amide chain showed a high inhibition of HSV-1 (EC_{50} 1.4, $MNTC/EC_{50} = 177.0$). Betulonic and betulinic acids were less active as compared with derivatives **8a**, **8c**, **8d** and **9b** as HSV-1 inhibitors. It is obvious that the addition of CONH or NHCONH groups at position 28 of lupane acids enhances their antiviral activity against influenza A and HSV-1 viruses.

Acknowledgements

This research was supported by the Russian Fund for Basic Research (grants No. 00-03-81174, No. 01-03-33131, No. 02-03-81007) and Belarussian Fund for Basic Research (grants No. B99P-031, No. B02R-016).

References and Notes

1. Jaaskelainen, P. *Paperi ja Puu* **1981**, *10*, 599.
2. Fujioka, T.; Kashiwada, Y.; Kilkuskie, R. E.; Cosentino, I. M.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Chen, I. S.; Lee, K. H. *J. Nat. Prod.* **1994**, *57*, 243.
3. El-Seedi, R.; Hesham, A.-M.; Sobaih, S. *Rev. Latinoam. Quim.* **1999**, *27*, 17.
4. Kim, D. S.; Chen, Z.; Van Tuyen, N.; Pezzuto, J. M.; Qiu, S.; Lu, Z. Z. *Synthetic Commun.* **1997**, *27*, 1607.
5. Pisha, E.; Chai, H.; Lee, I.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hicken, T. J.; Das Gupta, T. K.; Pezzuto, J. M. *Nature Med.* **1995**, *1*, 1046.
6. Zuco, V.; Supino, R.; Righetti, S. C.; Cleris, L.; Marchesi, E.; Gambacorti-Passerini, C.; Formelli, F. *Cancer Lett.* **2002**, *175*, 17.
7. Schmidt, M. L.; Kuzmanoff, K. L.; Ling-Indeck, L.; Pezzuto, J. M. *Eur. J. Cancer* **1997**, *33*, 2007.
8. Vlientinck, A. J.; De Bruyne, T.; Apers, S.; Pieters, L. A. *Planta Med.* **1998**, *64*, 97.
9. Kashiwada, Y.; Hashimoto, F.; Cosentino, L. M.; Chen, Ch.-H.; Garrett, P. E.; Lee, K.-H. *J. Med. Chem.* **1996**, *39*, 1016.
10. Evers, M.; Poujade, F. S.; Ribeill, Y.; James, C.; Lelievre, Y.; Gueguen, J.Ch.; Reisdorf, D.; Morize, I.; Pauwels, R.; De Clercq, E.; Henin, Y.; Bousseau, A.; Mayaux, J.-F.; Le Pecq, J.-B.; Dereu, N. *J. Med. Chem.* **1996**, *39*, 1056.
11. Soler, F.; Poujade, Ch.; Evers, M.; Carry, J.-Ch.; Henin, Y.; Bousseau, A.; Huet, Th.; Pauwels, R.; De Clercq, E.; Mayaux, J. F.; Le Pecq, J.-B.; Dereu, N. *J. Med. Chem.* **1996**, *39*, 1069.
12. Sun, I.-Ch.; Shen, J.-K.; Wang, H.-K.; Cosentino, L. M.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1267.
13. Kashiwada, Y.; Chiyo, J.; Ikeshiro, Y.; Nagao, T.; Okabe, H.; Cosentino, L. M.; Fowke, K.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 183.
14. Jeong, H.-J.; Chai, H.-B.; Park, S.-Y.; Kim, D. S. H. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1201.
15. Carlson, R.M.; Krasutsky, P.A.; Karim, R.-U. US Patent 5,750,578, 12.05, 1998.
16. Flekhter, O. B.; Nigmatullina, L. R.; Baltina, L. A.; Karachurina, L. T.; Galin, F. Z.; Zarudyi, F. S.; Tolstikov, G. A.; Boreko, E. I.; Pavlova, N. I.; Nikolaeva, S. N.; Savinova, O. V. *Chim.-Pharm. J.* **2002**, *36*, 26.
17. Pezutto, J. M.; Kim, D. S. H. L. US Patent 5,804,575, 8.09, 1998.
18. Boreko, E. I.; Pavlova, N. I.; Votiakov, V. I. *Voprosy Virusol.* **1999**, *44*, 115.
19. Zaitseva, G. V.; Zinchenko, A. I.; Barai, V. N.; Pavlova, N. I.; Boreko, E. I.; Nikhailopulo, I. A. *Nucleosides Nucleotides* **1999**, *18*, 687.