Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4753–4755
Bioorganic & Medicinal Chemistry Letters

Pomiferin, histone deacetylase inhibitor isolated from the fruits of *Maclura pomifera*

Il Hong Son,[a] Ill-Min Chung,[b] Sung Ik Lee,[a] Hyun Duk Yang[a] and Hyung-In Moon[a,c,*]

[a]*Department of Neuroscience, Inam Neuroscience Research Center, Sanbon Medical Center, Wonkwang University, Gunpo-city, Kyunggido 435-040, South Korea*
[b]*Department of Applied Life science, Kon Kuk University, Seoul 143-701, South Korea*
[c]*National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677-1848, USA*

Received 1 May 2007; revised 19 June 2007; accepted 21 June 2007
Available online 26 June 2007

Abstract—The major constituents from the fruits of *Maclura pomifera* are the prenylated isoflavones, osajin (**1**) and pomiferin (**2**). Their structures were elucidated using NMR spectroscopic techniques and mass spectrometric analysis. Compound **2** showed potential inhibitory activity in histone deacetylase (HDAC) enzyme assay. It also exhibited growth inhibitory activity on five human tumor cell lines and more sensitive inhibitory activity on the HCT-15 colon tumor cell line. Further structure–activity relationships of position 3 on ring B from aromatic ring will be reported in due course.

The turnover of histone acetylation is regulated by the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs).[1] Histone deacetylase (HDAC) inhibitors have been identified that inhibit proliferation and induce differentiation and/or apoptosis of tumor cells in culture and in animal models. A number of structurally diverse histone deacetylase inhibitors have shown potent antitumor efficacy with little toxicity in vivo in animal models.[2] Recently, HDAC inhibitors are emerging as an exciting new class of potential anticancer agents for the treatment of solid and hematological malignancies.[3] A number of natural and synthetic HDAC inhibitors have shown an anti-proliferative activity on tumor cells. Among them, trichostatin A (TSA),[4] apicidin,[5] trapoxin B (TPX),[6] and FK-228[7] were classified as natural substances, while suberoylanilide hydroxamic acid (SAHA)[8] and other TSA or SAHA-like analogues were reported as synthetic HDAC inhibitors.[9]

The flavonoids are a heterogeneous group of phenolic compounds in the plant kingdom. Many positive as well as negative effects on plant and animal cells of flavonoids have been documented.[10] Many of their effects are significant.[11] In oncology the flavonoids are used as compounds, which reduce the side effects of cytostatics and on the other hand, they enhance the therapeutic effects. This field is documented very well.[12] In the context of our natural product chemistry program dealing with the development of new potent anticancer agents, we have examined the isolation of flavonoid compounds as leads for novel HDAC inhibitors. Herein, we describe our results on the isolation, enzyme inhibition, and cancer cell growth inhibition of one such flavonoid-based HDAC inhibitor.

Ground fruit of *Maclura pomifera*, Moraceae, was subjected to consecutive Soxhlet extraction using methanol, yielding yellow crystals of osajin–pomiferin mixture after cooling. Pomiferin was separated from osajin by the addition of lead acetate which reacts with the two

Table 1. HDAC enzyme and growth inhibition by osajin (**1**), pomiferin (**2**), and SAHA

Compound	IC_{50} (μM) Enzyme	GI_{50} (μM) PC-3
1	6.53 ± 0.11	12.32 ± 0.12
2	1.05 ± 0.03	3.78 ± 0.08
SAHA	0.14 ± 0.02	0.71 ± 0.02

*Values are means of a minimum of five experiments.

Keywords: Pomiferin; HDAC; *Maclura pomifera*; Colon tumor cell line.
* Corresponding author. Fax: +82 313902414; e-mail: himun68@nate.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.06.060

Table 2. GI_{50} and origin type for cells treated with osajin (**1**), pomiferin (**2**), and SAHA

Human tumor cell lines and primary cells	Origin	Growth inhibition (μM)		
		1	**2**	SAHA
ACHN	Kidney	12.13 ± 1.03	3.18 ± 0.05	0.65 ± 0.03
NCI-H23	Lung	11.97 ± 0.92	5.14 ± 0.06	1.34 ± 0.05
PC-3	Prostate	13.32 ± 0.11	3.95 ± 0.05	0.76 ± 0.04
MDA-MB-231	Breast	11.34 ± 0.13	2.92 ± 0.09	0.89 ± 0.03
LOX-IMVI	Melanoma	10.98 ± 0.15	3.34 ± 0.11	1.60 ± 0.05
HCT-15	Colon	9.65 ± 0.13	1.32 ± 0.02	0.95 ± 0.08
Hepatocyte	Hepatocytes[a]	>500 μM	123 μM	>500 μM

*Values are means of a minimum of five experiments.
[a] Human primary cells.

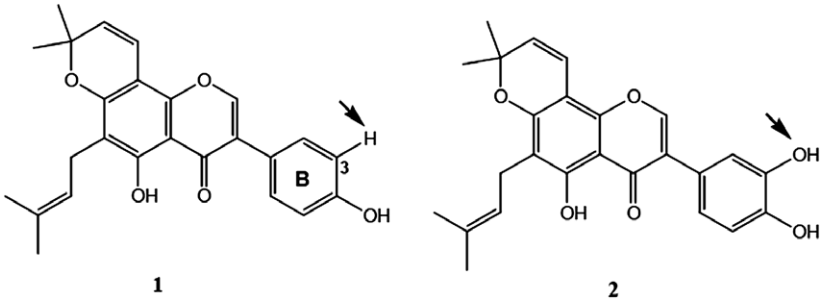

Figure 1. Structure of osajin (**1**) and pomiferin (**2**).

hydroxyl groups at position 3 and 4 on ring B of pomiferin to form a light yellow insoluble coagulate while osajin remains in solution since it has only one hydroxyl group on ring B. Osajin was then further re-crystallized from the methanolic solution after concentration under vacuum. Compounds were identified as osajin (**1**) and pomiferin (**2**) by comparing its spectral data with those previously reported.[13] We have evaluated the HDAC inhibitory activities of the newly isolated flavonoid compounds on partially purified HDAC enzyme obtained from HeLa cell lysate and their anti-proliferative effects using PC-3 cells as well.[14] Compound **2** was active in the HDAC enzyme assay. The compound showed potential growth inhibitory activities on the PC-3 cell line (Table 1).

Growth inhibitory activities of osajin (**1**), pomiferin (**2**), and SAHA were evaluated in six human tumor cell lines. Cell proliferation assays were also performed on six human tumor cell lines and one type of human primary cells (hepatocytes) (Table 2). Osajin (**1**) and pomiferin (**2**) did exhibit cytotoxicity effects in the cancer cells tested, but with a two- to tenfold lower activity than SAHA. This result is consistent with the enzymatic and histone acetylation assay results. The toxicity of osajin (**1**) against hepatocytes is comparable to that of SAHA, while pomiferin (**2**) has increased cytotoxicity against hepatocytes (GI_{50} = 123 μM). Growth inhibition (GI_{50}) measured by the MTT assay of these HDAC inhibitors and the tumor cell line types are listed in Table 2. With a similar pattern to the enzyme inhibition, compound **2** exhibited growth inhibitory activity on six human tumor cell lines. Among them, **2** showed the potential inhibitory activity on the HCT-15 colon tumor cell line (Fig. 1).

Pomiferin (**2**) thus has potential enzyme inhibitory and cell growth inhibitory activities and showed the potential growth inhibitory activity to HCT-15 among the six human tumor cell lines tested. We have found that pomiferin showed improving growth inhibiting potency in vitro. Further study for mode of action of position 3 on ring B is under progress.[15,16]

Taken together, our findings provide important information of the structural features that influence the functional activities of this class of compounds and offer new possibilities for further explorations to improve potency.

Acknowledgments

This work has been supported by Wonkwang University (2007). Dr. Moon is thankful to INRC for start-up funds. INRC is also acknowledged for partial support of this work.

References and notes

1. Vigushin, D. M.; Coombes, R. C. *Anti-Cancer Drugs* **2002**, *13*, 1.
2. Mai, A.; Massa, S.; Rotili, D.; Cerbara, L.; Valente, S.; Pezzi, R.; Simeoni, S.; Ragno, R. *Med. Res. Rev.* **2005**, *25*, 261.
3. Imre, G.; Gekeler, V.; Leja, A.; Beckers, T.; Boehm, M. *Cancer Res.* **2006**, *66*, 5409.
4. Vigushin, D. M.; Coombes, R. C. *Curr. Cancer Drug Targets* **2004**, *4*, 205.
5. Han, J. W.; Ahn, S. H.; Park, S. H.; Wang, S. Y.; Bae, G. U.; Seo, D. W.; Kwon, H. K.; Hong, S.; Lee, H. Y.; Lee, Y. W.; Lee, H. W. *Cancer Res.* **2000**, *60*, 6068.

6. Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S.; Beppu, T. *J. Biol. Chem.* **1993**, *268*, 22429.
7. Ueda, H.; Manda, T.; Matsumoto, S.; Mukumoto, S.; Nishigaki, F.; Kawamura, I.; Shimomura, K. *J. Antibiot.* **1994**, *47*, 315.
8. Butler, L. M.; Agus, D. B.; Scher, H. I.; Higgins, B.; Rose, A.; Cordon-Cardo, C.; Thaler, H. T.; Rifkind, R. A.; Marks, P. A.; Richon, V. M. *Cancer Res.* **2000**, *60*, 5165.
9. Mai, A.; Massa, S.; Ragno, R.; Cerbara, I.; Jesacher, F.; Loidl, P.; Brosch, G. *J. Med. Chem.* **2003**, *46*, 512.
10. Van Hoorn, D. E. C.; Nijveldt, R. J.; Van Leeuwen, P. A. M.; Hofman, Z.; M'rabet, L.; De Bont, D. B. A.; Norren, K. V. *Eur. J. Pharmacol.* **2002**, *451*, 111.
11. (a) Lee, S. J.; Wood, A. R.; Maier, C. G. A.; Dixon, R. A.; Mabry, T. J. *Phytochemistry* **1998**, *49*, 2573; (b) Havsteen, B. H. *Pharmacol. Therapeut.* **2002**, *96*, 67.
12. (a) Kandaswami, C.; Perkins, E.; Soloniuk, D. S.; Drzewiecki, G.; Middleton, E., Jr. *Cancer Lett.* **1991**, *56*, 147; (b) Nishino, H.; Tokuda, H.; Satomi, Y.; Masuda, M.; Osaka, Y.; Yogosawa, S.; Wada, S.; Mou, X. Y.; Takayasu, J.; Murakoshi, M.; Jinnno, K.; Yano, M. *Biofactors* **2004**, *22*, 57; (c) Scambia, G.; Ranelletti, F. O.; Panici, P. B.; Piantelli, M.; Bonanno, G.; De Vincenzo, R.; Ferrandina, G.; Maggiano, N.; Capelli, A.; Mancuso, S. *Gynecol. Oncol.* **1992**, *45*, 13.
13. (a) Vesela, D.; Kubinova, R.; Muselik, J.; Zemlicka, M.; Suchy, V. *Fitoterapia* **2004**, *75*, 209; (b) Giuliano, D.; Monache, R. S.; Alberto, V.; Bruno, B.; Barbara, M.; Gabriella, P.; Cleofe, P.; Enrico, C. *Phytochemistry* **1994**, *37*, 893.
14. Cell lines purchased from ATCC (Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% horse serum and 5% fetal bovine serum and incubated in a CO_2 incubator (5%) at 37 °C. Cells were serum-deprived by three washes of PBS and resuspended in DMEM. The suspended cells were plated on 96-well plates (1×104 cells/well) and treated with the indicated reagent(s). After treatment for 21 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to the medium (0.5 mg/mL), and the mixture was incubated at 37 °C for another 3 h. After discarding the medium, DMSO (100 mL) was then applied to the well to dissolve the formazan crystals, and the absorbances at 570 and 630 nm in each well were measured on a micro-ELISA reader. HDAC fluorescent activity assays using a Fluror de Lys™ Substrate (Biomol, Plymouth Meeting, PA, USA), which contains an acetylated lysine side chain, were performed according to manufacturer's instructions. In brief, HeLa nuclear extracts, which were used as an HDAC enzyme source, were incubated at 25 C with 250 mM of Fluror de Lys™ Substrate and various concentrations of each sample. Reactions were stopped after 20 min with Fluror de Lys™ Developer and fluorescence was measured using a microplate spectrofluorometer (Bio-Rad) with excitation at 360 nm and emission at 460 nm.
15. (a) The fruits of *Maclura pomifera* were purchased in a local market for Oriental medicine in Kyung-Dong, Seoul, Korea, and Voucher specimens (KKU-0892) have been deposited in the Herbarium of the College of Medicine, Kon Kuk University (Seoul, South Korea). The botanical identification was made by Dr. Kim Tae-Jin, KRIBB (Dae-Jeon, South Korea). The purchased fruits of *M. pomifera* (1 kg) were re-dried under freeze-drying for 7 days and then were extracted at room temperature with MeOH (two times, 5 L each) for 2 weeks. Ten grams of osajin/pomiferin crystals was dissolved in 200 mL of hot methanol and then mixed with 15 g of lead acetate trihydrate (dissolved in 20 mL methanol). The light yellow coagulate created overnight was filtered and then rinsed with hot acetone. Osajin and pomiferin were obtained by re-crystallization from the thickened methanol and acetone solutions. (b) Wolfrom, M. L.; John, M., *J. Am. Chem. Soc*, **1942**, *64*, 308.
16. For the purpose of identification and purity ascertainment of osajin and pomiferin during all the extraction process, reversed phase high performance liquid chromatography was performed. The HPLC (HP 1100) system consists of quaternary pump, autosampler, thermostatic column compartment, and diode array detector. The analytical column was Supelcosil ABZ + Plus and LC-8, 15 cm × 4.6 mm, 3 μm. The mobile phase consisted of two eluents, (A) acetonitrile and (B) 40 mM formic acid. Separation of compounds was carried out with gradient elution profile: 1st min, 70:30 (v:v), during 15 min, 100:0 (v:v). Chromatography was performed at 40 °C with a flow-rate of 1.0 mL/min, and detection was at 280 and 350 nm. The 50% inhibitory concentration (IC_{50}) values for **1**, **2**, and SAHA were obtained from the dose–response curves, using non-linear dose–response curve fitting analysis with Sigma pro software. Statistical significance was determined using the Student *t* tests. Results are presented as means ± standard error of mean (SEM). All p values quoted are two-tailed and were accepted as significantly different when p was $\leqslant 0.05$.