Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3387–3390

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Natural PTP1B Inhibitors from *Broussonetia papyrifera*

Rong Min Chen, Li Hong Hu,* Tian Ying An, Jia Li and Qiang Shen

Chinese National Center for Drug Screening, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

Received 11 June 2002; accepted 31 August 2002

Abstract—Two new compounds, 8-(1,1-dimethylallyl)-5′-(3-methylbut-2-enyl)-3′,4′,5,7-tetrahydroxyflanvonol (**1**), 3′-(3-methylbut-2-enyl)-3′,4′,7-trihydroxyflavane (**2**) and three known compounds 3,3′,4′,5,7-pentahydroxyflavone (**3**), uralenol (**4**), broussochalcone A (**5**) were isolated from the roots of *Broussonetia papyrifera,* and their structures determined by spectroscopic methods. Compounds **1**, **3**, **4** and **5** significantly show the inhibitory activities against the PTP1B enzyme.

Introduction

Non-insulin-dependent diabetes mellitus (type II) represents 80–90% of the human population with diabetes and, worldwide estimates approximately 215 million sufferers by 2010.[1] However, this is difficult since the current therapies for type II diabetes have inherent problems including compliance, ineffectiveness and hypoglycemic episodes with insulin and the soulfonylureas.[2] The glitazone-type therapeutic agents are not effective in all type II patients;[3] therefore, there still remains a great need for more effective, orally administered agents particularly ones that normalize both glucose and insulin levels.[4]

It is now appreciated that insulin resistance is the result of a defect in the insulin receptor signaling system, at a site post binding of insulin to its receptor. The interaction of insulin with its receptors leads to the phosphorylation of certain tyrosine molecules with the receptor protein, thus activating the receptor kinase. But PTPases dephsphorylate the activated insulin receptor, attenuating the tyrosine kinase activity. Therefore, it can be concluded that dephosphorylation of PTPases is one of reasons to the result of insulin resistance. The PTPases that appears most likely to closely associate with insulin receptors kinase activity, include PTP1B, LAR, PTPα and SH-PTP2.[5]

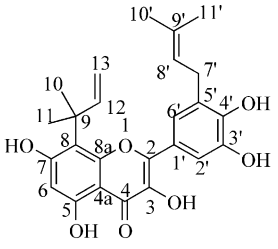
PTP1B have been shown to play a major role in the dephosphorylation of the insulin receptor in many cellular and biochemical studies. Therefore, orally active PTP1B inhibitors could be potential pharmacological agents for the treatment of Type-II diabetes and obesity.[6]

In our research work for natural products with antidiabetes activity, we screened our extract bank for inhibitors of PTP1B enzyme and found that a fraction from ethanol extract of the roots of *Broussonetia papyrifera* (L.) Vent. showed strong inhibitory bioactivity against PTP1B enzyme. Using the PTP1B enzyme bioassay as a guide, chromatography of the fraction afford two new compounds: 8-(1,1-dimethylallyl)-5′-(3-methylbut-2-enyl)-3′,4′,5,7-tetrahydroxy-flanvonol (**1**) and 3′-(3-methylbut-2-enyl)-3′,4′,7-dihydroxyflavane (**2**), and three known compounds: 3,3′,4′,5,7-pentahydroxyflavone (**3**), uralenol (**4**) and broussochalcone A (**5**).

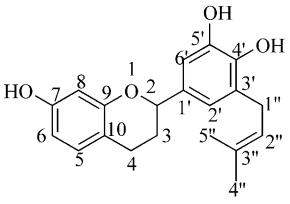
The roots of *B. papyrifera* were collected from Zhibo, Anhui of China, in August 2001. A voucher specimen (2001008) was deposited at the herbarium of Chinese National Center for Drug Screening, Shanghai, China. The dried and powdered roots of *B. papyrifera* (L.) Vent. (2 kg) was extracted with ethanol (8 L×3) at room temperature. The concentrated extract obtained under reduced pressure was partitioned with $CHCl_3$ and H_2O. The $CHCl_3$ layer (41 g) was subjected to D-101 macroporous resin, eluted with ethanol–H_2O (1:1) to yield fractions A (5 g), B (9 g), C (6 g). Fraction A was resubjected to MCI column, eluted with acetone-H_2O (1:1) and then applied to Sephadex LH-20 column ($CHCl_3/CH_3$ = 2:1), to afford compound **3** (18 mg).

*Corresponding author. Tel.: +86-21-5080-1313-212; fax: +86-21-5080-0792; e-mail: simmhulh@mail.shcnc.ac.cn

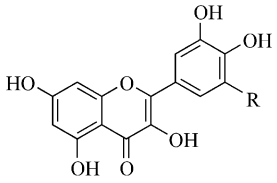
0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00757-6



1

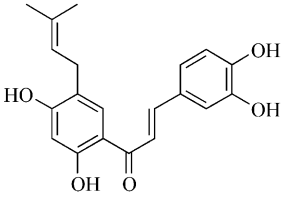


2

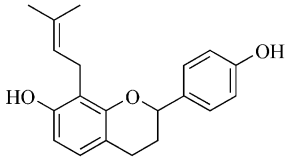


3 R=H

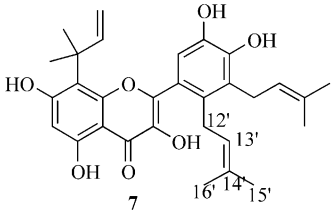
4 R=$CH_2CH=C(CH_3)_2$



5



6



7

Fraction B was resubjected to MCI column, eluted with acetone–H_2O (1:1) and then applied to TSK HW-40 column [acetone–H_2O (3:7)] and Sephadex LH-20 ($CHCl_3/CH_3$ = 2:1), to afford compounds **4** (30 mg), **5** (50 mg) and **2** (18 mg). Fraction C was resubjected to Si-gel column, eluted with petroleum ether–acetone (8:1–3:1) and then was purified with MCI column (acetone/water = 1:1) and Si-gel [$CHCl_3$–CH_3OH (10:1)], to afford compound **1** (16 mg).

Results and Discussion

Structural elucidation

Compound **1** was obtained as an amorphous powder, *m/z* 438.1669 ($[M]^+$), $C_{25}H_{26}O_7$, exhibiting a positive ferric chloride test. The infrared (IR) spectrum of **1** suggested the presence of hydroxyl groups (3407 cm^{-1}), aromatic rings (1593, 1547 cm^{-1}), and a conjugated carbonyl group (1653 cm^{-1}). The ultraviolet (UV)

Table 1. ^1H NMR (acetone-d_6, 400 Hz) and ^{13}C NMR (acetone-d_6, 100 Hz) spectra data for **1**, **7**,[8] 2 and **6**[12]

No.	Compd **1**		Compd **7**	No.	Compd **2**		Compd **6**
	δ_H	δ_C	δ_C		δ_H	δ_C	δ_C
2		144.7	148.7	2	5.04 (10.2, 2.3)	74.9	77.6
3		136.2	136.2	3	2.06 m	30.0	30.0
					1.88 m		
4		176.6	175.8	4	2.86 ddd (16.5, 5.2, 3.7)	24.6	24.9
4a		104.9	105.2	4	2.69 ddd (16.5, 10.8, 5.6)	24.6	24.9
5		159.7	159.5	5	6.86 d (8.7)	130.1	127.3
6	6.33 s	100.1	100.9	6	6.34 dd (8.7, 2.5)	108.1	108.1
7		163.6	161.8	7		156.8	153.0
8		111.7	110.1	8	6.26 d (2.5)	103.2	114.6
8a		156.2	155.7	9		156.6	153.6
9		41.8	40.5	10		112.8	113.9
10	1.64 s	30.6	27.5	1′		130.3	137.5
11	1.64 s	30.6	28.1	2′	6.78 d (2.3)	117.2	127.3
12	6.34 dd (17.6, 10.3)	150.9	149.1	3′		125.9	115.2
13	a 4.81 dd (17.6, 1.2)	109.4	113.6	4′		143.0	155.0
	b 4.80 dd (10.3, 1.2)						
1′		122.6	122.7	5′		144.0	115.2
2′	7.51 d (2.1)	114.3	114.2	6′	6.77 d (2.3)	112.7	127.3
3′		148.3	142.0	1″	3.46 d (7.1)	25.1	22.4
4′		146.0	144.8	2″	5.15 t (7.1)	123.9	122.2
5′		128.8	127.1	3″		131.9	134.0
6′	7.70 d (2.1)	122.1	132.7	4″	1.69 s	17.4	17.8
7′	3.38 d (7.3)	28.8	26.2	5″	1.63 s	25.2	25.8
8′	5.37 m	123.4	121.9				
9′		136.2	134.4				
10′	1.72	17.8	18.0				
11′	1.72 s	26.0	25.8				
12′			29.2				
13′			123.5				
14′			131.4				
15′			17.8				
16′			25.4				

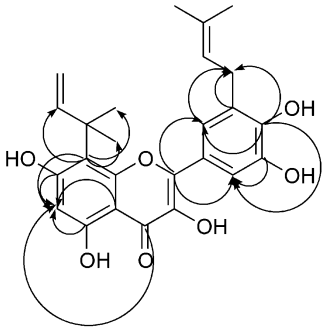


Figure 1. Key HMBC relationship in compound **1**.

spectrum of **1** showed absorptions maxima at 225, 256, 288, 308 and 355 nm. From above, **1** seems to be a flavonoloid derivative. The EI mass spectrum of **1** showed a significant peak at m/z 383 $[M-C_4H_7]^+$, 205 ($C_{12}H_{13}O_3$), suggesting that a prenyl group and two hydroxyl groups are located in the B-ring.[7] The ^1H NMR spectrum of **1** showed the presence of one 3,3-dimethylallyl group [δ 1.72 (6H, brs, Me×2), δ 3.38 (2H, d, $J=7.3$ Hz), δ 5.37 (1H, t, $J=5.9$ Hz) and one 1, 1-dimethylallyl group [δ 1.67 (6H, s, Me×2), δ 6.34 (1H, dd, $J=17.6$, 10.3 Hz), δ 4.81 (1H, dd, $J=17.6$, 1.2 Hz), δ 4.80 (1H, dd, $J=10.3$, 1.2 Hz). The spectrum also indicated the presence of AB type aromatic proton signals at δ 7.51 (1H, d, $J=2.1$ Hz), δ 7.70 (1H, d, $J=2.1$ Hz), a singlet of aromatic proton signal at δ 6.33 and a hydrogen bonded hydroxyl group signal at δ 12.6 (1H, brs). The ^{13}C NMR spectrum of **1** (Table 1) was assigned by H-decoupled spectra, DEPT pulse sequence, HMQC and HMBC correlations and comparison of chemical shifts with those of corresponding data for 2′,3′-bis-(3-methylbut-2-enyl)-8-(1,1-dimethylallyl)-4′, 5′-dihydroxyflavonol (**7**) (Table 1). This established that **1** has the proposed structure.[16] The characterization of **1** was also supported by HMBC correlations (Fig. 1).

Compound **2** gave a violet spot on TLC plate with cerric sulfate. It was assigned the molecular formula $C_{20}H_{22}O_4$ by its HREI–MS (m/z 326.1568, $[M^+]$, calcd, 326.1563). The IR spectrum of compound **2** suggested the presence of a hydroxyl group (3393 cm^{-1}) and an aromatic ring (1618 and 1457 cm^{-1}). Its ^1H NMR spectrum showed typical signals of a flavan skeleton [δ 5.04 (1H, dd, $J=10.2$ and 2.3 Hz, H-2), δ 1.88 and δ 2.06 (1H each, m, H_2-3), δ 2.69 (1H, ddd, $J=16.5$, 5.2 and 3.7 Hz, H-4a) and δ 2.89 (1H, ddd, $J=16.5$, 10.8 and 5.6 Hz, H-4b)].[9,10] Its ^1H NMR also suggested the presence of one 3,3-dimethylallyl group [δ 1.63 s (Me), δ 1.69s (Me), δ 3.46 d (2H, $J=7.1$ Hz), δ 5.15 m (1H, $J=7.1$)], one ABX type aromatic proton signals at δ 6.26d (1H, $J=2.5$ Hz), δ 6.34 dd (1H, $J=8.7$ and 2.5 Hz), δ 6.86d (1H, $J=8.7$ Hz) and one AB type aromatic proton signals at δ 6.77d (1H, $J=2.3$) and δ 6.78d (1H, $J=2.3$). The EI mass spectrum showed characteristic retro-Diels–Alder fragments at m/z 123 and 204, indicating the presence of 3,3-dimethylallyl and two hydroxyl groups in the B ring and two hydroxyl groups in the A-ring.[11] The ^{13}C NMR spectrum of **2** was assigned by H-decoupled spectra, DEPT pulse sequence, HMQC and HMBC correlations and comparison of chemical shifts with those of corresponding data for 4′,7-dihydroxy-8-prenylflavane (**6**). This established that **2** has the proposed structure.[16] The characterization of **2** was also supported by HMBC correlations (Fig. 2).

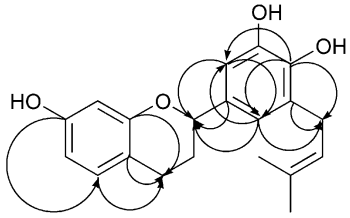


Figure 2. Key HMBC relationship in compound **2**.

Table 2. PTP1B inhibitory activity of compounds **1**–**5**[a]

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
1	4.3	**4**	21. 5
2	41.5	**5**	36. 8
3	23.3		

[a]PTP1B enzyme is human at 100 nm concentration. Substrate for hPTP1B is the *p*-nitro-phenyl phosphate (p-NPP) at 10 mM concentration. Three independent measurements were performed for IC_{50} determinations. Na_3VO_4 acts as positive control ($IC_{50}=2$ μM).

The known compounds 3,3′,4′,5,7-pentahydroxyflavone (**3**),[13] uralenol(**4**),[14] brousso-chalcone A (**5**),[15] were identified by comparison of their spectral and physical data with those reported in the literature.

Biological Activity Evaluation

The inhibitory activities of these compounds were tested toward PTP1B enzyme and their results were summarized in Table 2. As our knowledge, this is the first reported PTP1B inhibitors from natural plants. We found compounds **1**, **3**, and **4** have the same 3′,4′,5,7-tetrahydroxyflanvnol skeleton, and more nonpolar substituents at this skeleton increase their inhibitory activities [from **1** (two isoprenyl substituents) to **4** (one isoprenyl substituent), **3**].

Acknowledgements

This work was supported by the Natural Science Fund of China and Science and Technology Development Fund of Shanghai, China (01QB14051), and these supports are gratefully acknowledged.

References and Notes

1. Bennett, P. H. Prevention of NIDDM: A Practical Reality. *Diabet. Metab. Rev.* **1997**, *13*, 583.
2. Kumar, S.; Boulton, A. J. M.; Beck-Nielsen, H.; Berthezene, F.; Mugge, M.; Person, B.; Spinas, G. A.; Donoghue, S.; Lettis, S.; Stewart-Long, P.; F. T.T. Group. *Diabetologia* **1996**, *30*, 701.
3. Witcher, J. W. *Diabetes Rev.* **1994**, *2*, 272.
4. Malamas, M. S.; Redy, J. S.; Moxham, C.; Katz, A.; Xu,

W.; MaDevitt, R.; Adebayo, F. O.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Taylor, J. R *J. Med. Chem.* **2000**, *43*, 1293.
5. Goldstein, B. J. *J. Cell. Biochem.* **1992**, *31*, 33.
6. Byon, J. C. H.; Kusari, A. B.; Kusari, J. *Mol. Cell. Biochem.* **1998**, *182*, 101.
7. Mabry, T. J.; Markham, K. R. In *The Flavonoids Part 1*. Harborne, J. B., Mabry, T. J., Mabry, H. (Eds.); Academic: New York, 1975; p 79.
8. Lin, C. N.; Chiu, P. H.; Fang, S. C.; Shieh, B. J.; Wu, R. R. *Phytochemistry* **1996**, *41*, 1215.
9. Saini, K. S.; Ghosal, S. *Phytochemistry* **1984**, *23*, 2415.
10. Torres, S. L.; Monteiro, J. C.; Arruda, M. S.; Muller, A. H.; Arruda, A. C. *Phytochemistry* **1997**, *44*, 347.
11. Gottlieb, O. R. *Israel J. Chem.* **1977**, *16*, 45.
12. Torres, S. L.; Arruda, M. S. P.; Arruda, A. C.; Muèller, A. H.; Silva, S. C. *Phytochemistry* **2000**, *53*, 1047.
13. Batterham, T. J. *Aust. J. Chem.* **1964**, *17*, 428.
14. Jia, S. S.; Ma, C. M.; Wang, J. M. *Yaoxue Xuebao* **1991**, *25*, 758.
15. Matsumoto, J.; Fujimoto, T.; Takino, C.; Saitoh, M.; Hano, Y.; Fukai, T.; Nomura, T. *Chem. Pharm. Bull.* **1985**, *33*, 3250.
16. Physical and spectroscopic data: compd **1**: Yellow prism (Me_2CO–petroleum ether), mp 181–182 °C, UV (MeOH) λ_{max} (logε): 225 (4.56), 256 (4.26), 288 (3.67), 308 (3.66), 355 (4.01) nm; IR (KBr) ν_{max}: 3407, 1653, 1622, 1593, 1547, 1323 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopy data, see Table 1; EIMS *m*/*z* (rel. int): 438 [M^+] (7), 423 (5), 383 (2), 207 (14), 189 (4), 165 (6), 149 (12), 129 (15), 105 (28), 91 (52), 60 (100), HREIMS *m*/*z* 438.1669 for C_{25} H_{26} O_7 (calcd 438.1675).

Compd **2**: colorless amorphous power, $[\alpha]_D^{25}$ −5.88 (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (logε): 225 (4.25), 269 (3.78), 278 (4.01), 267 (3.89), 317 (3.30); IR (film) λ_{max}: 3393, 1618, 1596, 1503, 1457, 1108, 1066 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopy data, see Table 1; EIMS *m*/*z* (rel. int): 326 [M^+] (100), 271 (12), 204 (81), 123 (58), HREIMS *m*/*z* 326.1568 for C_{20} H_{22} O_4 (calcd 326.1563).