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Phenylpropanoids and flavonoids from *Phlomis kurdica* as inhibitors of human lactate dehydrogenase

Ammar Bader [a], Tiziano Tuccinardi [b,c], Carlotta Granchi [b], Adriano Martinelli [b,c], Marco Macchia [b,c], Filippo Minutolo [b,c], Nunziatina De Tommasi [d,*], Alessandra Braca [b,c]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, P.O. Box 13174, 21955 Makkah, Saudi Arabia
[b] Dipartimento di Farmacia, Università di Pisa, via Bonanno 6 and 33, 56126 Pisa, Italy
[c] Centro Interdipartimentale di Ricerca "Nutraceutica e Alimentazione per la Salute", Università di Pisa, via del Borghetto 80, 56124 Pisa, Italy
[d] Dipartimento di Farmacia, Università degli Studi di Salerno, via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy

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ABSTRACT

Two flavonoids, jaceosidin 7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**) and hispidulin 7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**2**), and one phenylpropanoid, 3,3′-dimethyl-lunariifolioside (**3**), along with 11 known compounds (**4**–**14**), were isolated from the aerial parts of *Phlomis kurdica* growing in Jordan. Structures of **1**–**3** were elucidated on the basis of spectroscopic data. These isolated compounds were assayed for their inhibitory activity against isoform 5 of human lactate dehydrogenase. Compound **4**, luteolin 7-*O*-β-D-glucopyranoside, showed an IC_{50} value comparable to that of galloflavin, used as reference compound. Docking studies were carried out to hypothesize the interaction mode of compound **4** in the enzyme active site.

1. Introduction

Phlomis genus (Lamiaceae family) comprises more than 100 species mainly distributed in the Mediterranean region, central Asia, and China (Limem-Ben Amor et al., 2009). Ten *Phlomis* species are found in the flora of Jordan, which is considered one of most attractive countries in the Middle East due to its biodiversity (Dal Piaz et al., 2009; Malafronte et al., 2012). Among them, *Phlomis kurdica* Rech.f. is a herb up to 60 cm with leaves ± densely (often whitish) stellate-lanate-tomentose, especially below, oblong-ovate, obtuse, cordate at base, crenate, 5–12 × 38 cm, petiole to 11 cm; the yellow flowers arranged in verticillasters 3–6, lower remote, 8–10-flowered (Al-Eisawi, 1998). Many species of *Phlomis* have been used for decades in folk medicine as pain relievers, tonics, wound healers and stimulants (Limem-Ben Amor et al., 2009). Their flowered parts are generally used as herbal tea to treat gastrointestinal disorders and to protect the liver, kidneys, bones and the cardiovascular system (Limem-Ben Amor et al., 2009; Li et al., 2010). Iridoids, flavonoids, phenylpropanoids, phenylethanoids, lignans, neolignans, diterpenoids, alkaloids, and essential oils are typical metabolites of the *Phlomis* genus (Li et al., 2010). Among phenylethanoids, forsythoside B, verbascoside, alyssonoside, and leucosceptosides A and B, have been identified from *P. kurdica* (Kirmizibekmez et al., 2005).

Since extracts of *Phlomis* species showed antiproliferative activity, the crude polar residues of *P. kurdica* aerial parts were assayed for their lactate dehydrogenase (LDH) inhibitory activity, an enzyme whose isoform 5 (*h*LDH5) is up-regulated in human tumor tissues (Granchi and Minutolo, 2012). In fact, cancer cells depend mainly on anaerobic respiration and their glycolytic rate is up to 200 times higher than that of the normal tissue. This fermentative glycolysis is promoted by an overexpression of several enzymes and transporters, which may offer rather safe therapeutic windows for anticancer agents that target them (Granchi and Minutolo, 2012). In particular, the A-subunit of LDH (LDH-A), which exclusively generates *h*LDH5 in its fully functional tetrameric form, catalyzes a crucial step in glycolysis, the reduction of pyruvate to lactate. Therefore, inhibition of LDH may be considered a promising strategy in cancer treatment, since it should cause a starvation of cancerous cells by reducing their conversion of glucose to lactate (Fiume et al., 2014). So far, several small molecules displaying efficient LDH inhibitory potencies have been reported, and they are generally characterized by the presence of carboxylates and/or phenolic OH groups in their structures (Granchi et al., 2013a). These observations prompted the present investigation of *P. kurdica* aerial parts. Hence, two new flavonoids (**1**–**2**) and one new phenylethanoid (**3**), together with eleven known phenolic

* Corresponding author. Tel.: +39 089 969754; fax: +39 089 969602.
E-mail address: detommasi@unisa.it (N. De Tommasi).

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compounds (**4**–**14**) (Fig. 1), including flavonoids and phenylethanoids, were isolated and assayed for their LDH inhibitory activity.

2. Results and discussion

During a wide screening for LDH inhibitory activity, the $CHCl_3$–MeOH, the MeOH and the *n*-BuOH extracts of *P. kurdica* aerial parts were assayed. The $CHCl_3$–MeOH and the *n*-BuOH extracts showed a promising inhibitory activity and therefore were subjected to different chromatographies to afford three new (**1**–**3**) and 11 known compounds (**4**–**14**).

Compound **1**, a yellow amorphous solid, showed a quasi-molecular ion peak at *m*/*z* 653.1725 for $[M-H]^-$ in the HRESIMS; this result, together with ^{13}C NMR, allowed the assignment of molecular formula $C_{29}H_{34}O_{17}$ to **1**. In the ESIMS spectrum fragments at *m*/*z* 491 $[M-H-162]^-$ and 329 $[M-H-162-162]^-$ revealed the presence of two hexose residues in the molecule. The UV spectrum of **1** showed two absorption maxima at 340 and 277 nm, indicating its flavone skeleton. Compound **1** aglycone was deduced to be jaceosidin (Martinez et al., 1987) on the basis of the following observation in its ^1H NMR spectrum (Table 1): a 5,6,7-trisubstituted pattern for ring A (one singlet at δ 7.04, one methoxy group at δ 3.93) and a 3′,4′-disubstitution for ring B (ABX system signals at δ 6.96, d, *J* = 8.0 Hz; 7.58, d, *J* = 2.0 Hz; 7.60, dd, *J* = 8.0, 2.0 Hz; one methoxy signal at δ 4.00). Two anomeric protons arising from the sugar moieties appeared at δ 4.85 and 5.38 each (d, *J* = 7.5 Hz), which correlated respectively with signals at δ 104.0 and 99.4 ppm in the HSQC spectrum. Assignments of compound **1** NMR chemical shifts were accomplished by 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments. DQF-COSY and 1D-TOCSY experiments led to the identification of the sugars as two β-glucopyranosyl units. Hydrolysis of **1** with 1 N HCl followed by GC analysis through a chiral column of the monosaccharides

Table 1
^1H and ^{13}C NMR data of compounds **1**–**2**.[a]

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
2		167.1		167.4
3	6.78 s (2.1)	103.5	6.71 s (2.1)	103.0
4		183.6		184.0
5		154.3		154.1
6		134.2		134.4
7		158.0		157.0
8	7.04 s	94.6	6.98 s	95.0
9		157.0		157.2
10		107.3		107.1
1′		123.8		123.2
2′	7.58 d (2.0)	110.4	7.91 d (8.5)	129.4
3′		149.3	6.95 d (8.5)	117.0
4′		152.5		163.8
5′	6.96 d (8.0)	116.4	6.95 d (8.5)	117.0
6′	7.60 dd (8.0, 2.0)	121.8	7.91 d (8.5)	129.4
6-OCH_3	3.93 s	60.9	3.89 s	61.0
3′-OCH_3	4.00 s	56.2		
Glc I 1	5.38 d (7.5)	99.4	5.40 d (7.8)	100.0
2	3.90 dd (9.0, 7.5)	82.0	3.88 dd (9.5, 7.8)	82.0
3	3.76 t (9.0)	77.4	3.76 t (9.5)	77.0
4	3.48 t (9.0)	70.6	3.54 t (9.5)	70.0
5	3.62 m	77.8	3.61 m	78.0
6a	3.98 dd (12.0, 3.0)	61.9	3.96 dd (12.0, 2.5)	62.5
6b	3.74 dd (12.0, 4.5)		3.73 dd (12.0, 4.5)	
Glc II 1	4.85 d (7.5)	104.0	4.79 d (7.5)	104.3
2	3.25 dd (9.0, 7.5)	75.2	3.26 dd (9.0, 7.5)	75.8
3	3.42 t (9.0)	77.2	3.40 t (9.0)	78.0
4	3.40 t (9.0)	70.6	3.48 t (9.0)	70.0
5	3.31 m	77.1	3.30 m	77.8
6a	3.62 dd (12.0, 3.0)	61.3	3.66 dd (12.0, 3.0)	62.0
6b	3.48 dd (12.0, 4.5)		3.46 dd (12.0, 4.5)	

[a] Spectra were run in methanol-d_4 at 600 MHz (^1H) and 150 MHz (^{13}C). *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by COSY, 1D-TOCSY, HSQC, and HMBC experiments.

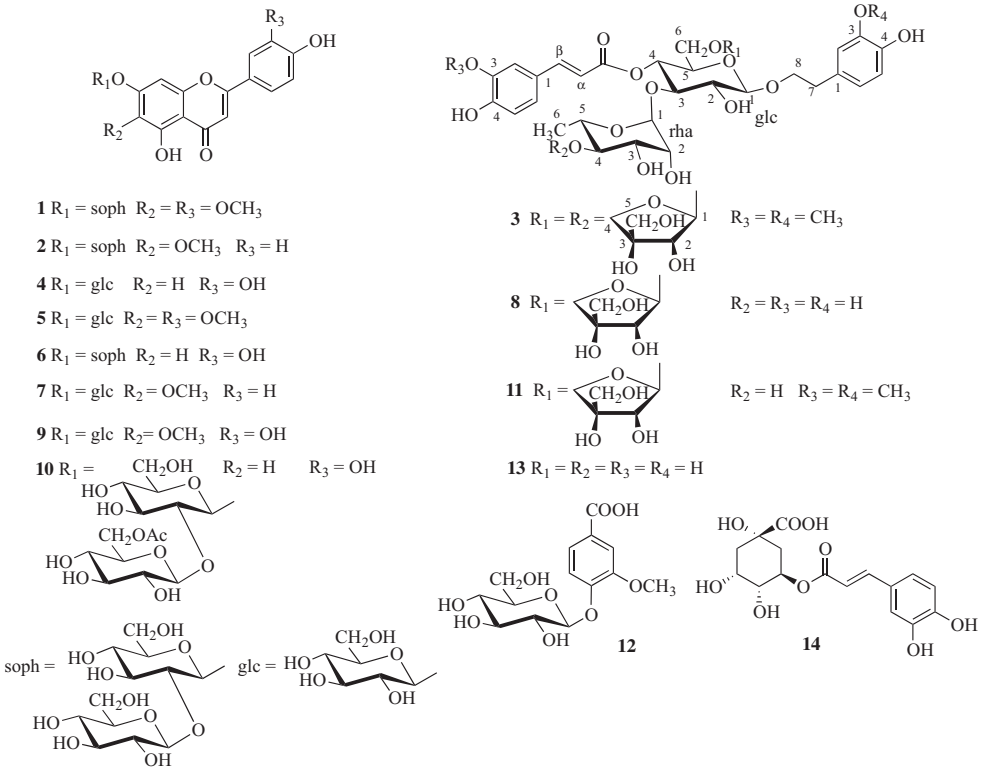

Fig. 1. Structures of compounds **1**–**14** isolated from *P. kurdica*.