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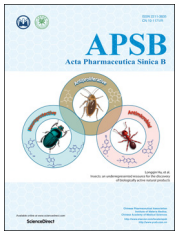

SHORT COMMUNICATION

Isocartormin, a novel quinochalcone *C*-glycoside from *Carthamus tinctorius*

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KEY WORDS

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Abstract A new semi-quinonechalcone *C*-glycoside isocartormin along with cartormin and safflomin C were isolated from the water extract of *Carthamus tinctorius* L. The structure of isocartormin was determined by extensive analysis of HR-MS, 1D- and 2D NMR data, and by comparison with those of cartormin reported previously by our group. Isocartormin was identified as a diastereoisomer of cartormin with a reverse configuration at C-18.

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1. Introduction

The florets of *Carthamus tinctorius* L. (Compositae) are widely used for the treatment of stroke, coronary heart disease through promoting blood circulation by removal of blood stasis[1]. Phytochemical and pharmacological studies suggested that water-soluble components of *C. tinctorius*, especially quinochalcone *C*-glycosides, should contribute to its therapeutic effects. In China, the water extract has been extensively used in hospitals for cardiovascular diseases as an intravenous injection[2]. To date, more than 200 compounds from *C. tinctorius* have been identified, including flavonoids[3,4], alkaloids[5,6], lignans[7–9], alkane diols[10,11], riboflavin[12], steroids[7], and quinochalcone *C*-glycosides[13–18]. Among these ingredients, quinochalcone *C*-glycosides are unique components in this species, which are regarded as the characteristic and active components in its water extract. So far, 20 quinochalcone *C*-glycosides have been isolated from the title plant, including one semi-quinonechalcone sharing a pyrrole ring *C*-glycoside, cartormin, reported by our group[16]. Recently, Jin et al.[14] characterized a hydroxycartormin and predicted the existence of cartormin isomer using ultraperformance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UPLC–QTOF-MS). In our continuing effort in the study of the chemical constituents from *C. tinctorius*, the cartormin isomer named isocartormin, a novel semi-quinone chalcone was characterized (Fig. 1). The structure was elucidated on the basis of HR-MS, MS/MS, 1D- and 2D- NMR data, as well as by comparison with those of its diastereoisomer cartormin. In this study, we describe the isolation and structural elucidation of the new compound isocartormin.

2. Results and discussion

Isocartormin was obtained as yellow crystals. The positive ESI-MS ion peaks at *m/z* 576 $[M+H]^+$ and 414 $[M–Glc+H]^+$ and the negative one at *m/z* 574 $[M–H]^-$ suggest a molecular weight of 575. The corresponding molecular formula was established as $C_{27}H_{29}NO_{13}$ by HR-ESI-MS measurement. The IR spectrum showed the presence of a keto-enol carboxyl system (1600–1640 cm^{-1}) and hydroxyl groups belonging to sugar moieties (3376, 1062 br cm^{-1}). The $\nu_{C\text{-}N}$ absorption[16] was also observed at 1276 cm^{-1}. In the UV spectrum, the strong absorption band at 405 nm and a small absorption peak at 221 nm suggested that a semi-quinone chalcone moiety is present in this structure. In the ^1H NMR spectrum, four aromatic protons at δ_H 6.72 (2H, d, $J=8.6$ Hz, H-12, H-14), 7.43 (2H, d, $J=8.6$ Hz, H-11, H-15) and two *E*-olefinic protons at δ_H 7.43 (1H, d, $J=15.8$ Hz) and 7.67 (1H, d, $J=15.8$ Hz) revealed the existence of a *p*-hydroxycinnamic acid group (Fig. 1). Additionally, two anomeric-proton peaks for *C*-glycoside at δ_H 4.82 (1H, d, $J=5.1$ Hz, H-18) and 3.31 (1H, *d*, $J=9.5$ Hz, H-22), along with two anomeric carbons at δ_C 78.5 (C-18) and 86.1 (C-22), confirmed that two sugar moieties were attached to the structure (ring A: erythrosyl; ring E: glucosyl)[16].

Table 1 ^1H NMR and ^{13}C NMR data of isocartormin in CD_3OD (δ in ppm, J in Hz).

Position	δ_H (J Hz)[a]	δ_C	HMBC (C→H)
1		198.4 s	H-22
2		79.6 s	
3		142.2 s	H-16, H-22
4		117.5 s	H-16
5		188.2 s	H-16
6		110.4 s	
7		183.0 s	H-8, H-9
8	7.43 d (15.8)	119.9 d	H-9
9	7.67 d (15.8)	144.3 d	H-8, H-11, H-15
10		128.7 s	H-8, H-9, H-11, H-12 H-14, H-15
11	7.43 d (8.6)	132.0 d	H-9, H-15, H-12
12	6.72 d (8.6)	117.2 d	H-14, H-11
13		161.6 s	H-11, H-12, H-14, H-15
14	6.72 d (8.6)	117.2 d	H-12, H-15
15	7.43 d (8.6)	132.0 d	H-9, H-11, H-14
16	6.42 s	106.5 d	H-18
17		134.3 s	H-16
18	4.82 d (5.1)	78.5 d	H-19, H-20, H-21
19	4.18 t (4.8)	74.5 d	H-21
20	4.33 m	73.5 d	H-18, H-21
21	3.87 dd (9.1, 4.8) 3.78 dd (9.1, 4.8)	73.1 t	H-19, H-20
22	3.31 d (9.5)	86.1 d	H-23, H-24
23	3.47 m	71.4 d	H-22
24	3.19 (overlap)	80.3 d	H-22, H-26, H-23, H-25
25	3.22 (overlap)	71.2 d	H-24, H-27
26	2.95 m	81.7 d	H-22, H-27
27	3.62 dd (12.1, 2.2) 3.55 dd (11.9, 4.8)	63.0 t	H-25

[a]Data was measured at 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR.

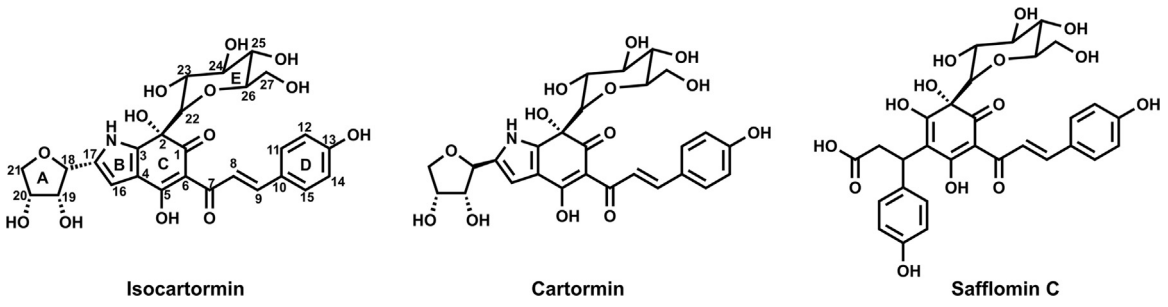

Figure 1 Structures of isocartormin, cartormin and safflomin C.

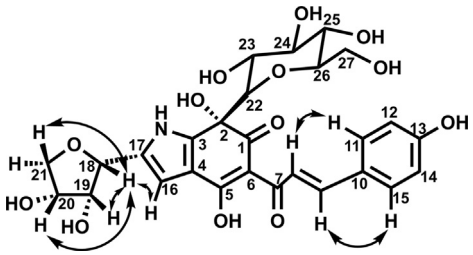

Figure 2 The selected NOSEY correlations of isocartormin.

The HMBC spectrum (Table 1) showed the correlations from H-18 to C-16, which suggested ring A was linked to the ring B *via* the C_{17}–C_{18} bond, and the correlation from H-22 to C-1, suggesting that ring E was attached to C-2 of ring C *via* C_{22}–C_2. The signals generated from H-9 to C-7 and C-11 further implied the existence of a *p*-hydroxyl cinnamoyl moiety. In the NOESY spectrum, the cross-peaks of H-18/H-19, H-18/H-21, and H-19/H-20 provided the solid evidence for H-18, H-19 and H-20 being in the same face of ring A, namely, these three protons being in *cis* form (Fig. 2). Although the IR, ^{1}H NMR and ^{13}C NMR spectroscopic data of isocartormin showed high similarities to those of cartormin, the anomeric proton of isocartormin at δ_H 4.82 (H-18) and its coupling constant (J=5.1 Hz) was different from those of cartormin (δ_H 4.50, J=7.7 Hz). As shown in Table 1, the chemical shifts of C-19, 20, 21 in the ring A (δ_C 74.5, 73.5 and 73.1) also have obvious changes when compared with those in cartormin (δ_C 77.8, 72.6 and 74.6). As a matter of fact, isocartormin and cartormin have different retention time in the UPLC–QTOF-MS analysis (Supplementary Fig. S1). We confirmed the difference between these two compounds by employing alternative mobile phases and detector (UV) (Supplementary Fig. S2). MS/MS of cartormin and isocartormin also displayed the similar fragmental pattern (Fig. 3), in which the fragmental ions are interpreted in the inlaid structural diagram. Based on the above evidences and comparison with the NMR data of cartormin, the novel compound was identified as isocartormin, a diastereoisomer of cartormin. The recent research on *C. tinctorius* likewise observed the existence of the isomer of cartormin. The authors predicted the possible formation of cartormin isomer results from various linking position of the erythrosyl moiety to the pyrrole ring[14]. Unfortunately, no information concerning the isomer is available in the previous study. In this study, we isolated and characterized the isomer of cartormin for the first time.

3. Conclusions

Although the chemical components of *C. tinctorius* L. have been extensively investigated, we successfully isolated and characterized isocartormin, a diastereoisomer of cartormin guided by UPLC–QTOF-MS. Our study further confirmed the existence of the cartormin isomer predicted in the previous study by the isolation and characterization of isocartormin. In addition, it is well known that a compound and its stereoisomer may exhibit different bioactivities. This study allows us to test the bioactivities of cartormin and its isomer in future study.

4. Experimental

4.1. General experimental procedures

The melting points (uncorr.) were determined on a Buechi 510 melting point apparatus. The optical rotation values were obtained on a DIP-181 digital polarimeter. The UV spectra were taken on a Varian Cary 300 Bio spectrophotometer. The IR spectra were recorded on a Nicolet 750 instrument. The NMR spectra were collected on a Bruker AM-400 spectrometer, with tetramethylsilane (TMS) as internal standard in CD_3OD. The HR-ESI-MS and MS/MS were obtained on UPLC–QTOF-MS (Waters, Milford, MA, USA). The mixture of isocartormin and cartormin was monitored by UPLC–QTOF-MS. In brief, a 100 mm × 2.1 mm (Acquity 1.7 μm) BEH C18 column (Waters, Milford, MA, USA) was used for their separation. The flow rate of the mobile phase was set as 0.3 mL/min. The gradient ranged from 2% to 98% acetonitrile containing 0.1% formic acid in a 10-min run. QTOF-MS was operated in a positive mode with electrospray ionization. The source and desolvation temperature were set at 120 and 350 °C, respectively. Nitrogen was applied as the cone gas (10 L/h) and desolvation gas (700 L/h). Argon was applied as the collision gas. QTOF-MS was calibrated with sodium formate and monitored by the intermittent injection of lock mass leucine enkephalin in real time, generating a reference ion at *m/z* 556.2771. The capillary voltage and the cone voltage were set at 3.5 kV and 35 V, respectively. Tandem mass spectrometry fragmentation was conducted with collision energy ramp ranging from 10 to 40 V.

4.2. Plant material

The florets of *C. tinctorius* were collected in Sichuan province, China, and authenticated by Prof. Lan Xu. A voucher specimen (No. 77) has been deposited at Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

4.3. Extraction and isolation

The air-dried florets (10 kg) were extracted with water, and then with EtOAC, followed by *n*-BuOH. The *n*-BuOH fraction (1.0 kg) was chromatographed on macro resin (DA 201) using gradient elution with EtOH–H_2O (0%, 30%, 70% EtOH, *v/v*). The 30% fraction was subjected to a polyamide column with H_2O, H_2O–CH_3OH (1:1, *v/v*) and CH_3OH successively. The fraction (H_2O–CH_3OH) was chromatographed on a silica gel column eluted with $CHCl_3$–MeOH–H_2O (3:1:0.1, *v/v*) repeatedly to afford isocartormin (18 mg), and cartormin (150 mg). Safflomin C (15 mg) was isolated by preparative TLC using $CHCl_3$–MeOH–H_2O (2.5:1:0.02, *v/v/v*) as a developing solution.

4.3.1. Isocartormin

Yellow needle crystals, mp 240 °C (dec.); $[\alpha]_D^{25}$ − 196 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (logε): 405 (4.59), 221 (4.34) nm; IR (KBr) ν_{max} (film): 3376, 1604, 1488, 1394, 1168, 1276, 1062 and 827 cm^{-1}; for ^{1}H NMR and ^{13}C NMR (Table 1); ESI-MS (positive): *m/z* 598 $[M+Na]^+$ (100), 576 $[M+H]^+$ (50), 414 $[M–Glc+H]^+$ (69); ESI-MS (negative): *m/z* 574.2 $[M–H]^-$ (100); HR-ESI-MS *m/z* 576.1722 $[M]^+$ (Calcd. for $C_{27}H_{29}NO_{13}$ 576.1717).

4.3.2. Cartormin

Yellow crystals, mp 230 °C (dec.); $[\alpha]_D^{25}$ − 155 (*c* 0.20, MeOH); IR (KBr) ν_{max} (film): 3400, 1600, 1490, 1388, 1269, 1170, 1078 and 831 cm^{-1}; ESI-MS (negative): *m/z* 574 $[M–H]^-$; ^{1}H NMR (CD_3OD, 400 MHz) δ_H: 7.60 (1H, *d*, J=15.8 Hz), 7.38 (2H, *d*, J=8.8 Hz), 7.33 (1H, *d*, J=15.8 Hz), 6.52 (2H, *d*, J=8.8 Hz), 6.34 (1H, s), 4.51 (1H, *d*, J=7.7 Hz), 3.23 (1H, *d*, J=9.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ_C: 198.5 (s), 188.3 (s), 182.6 (s),

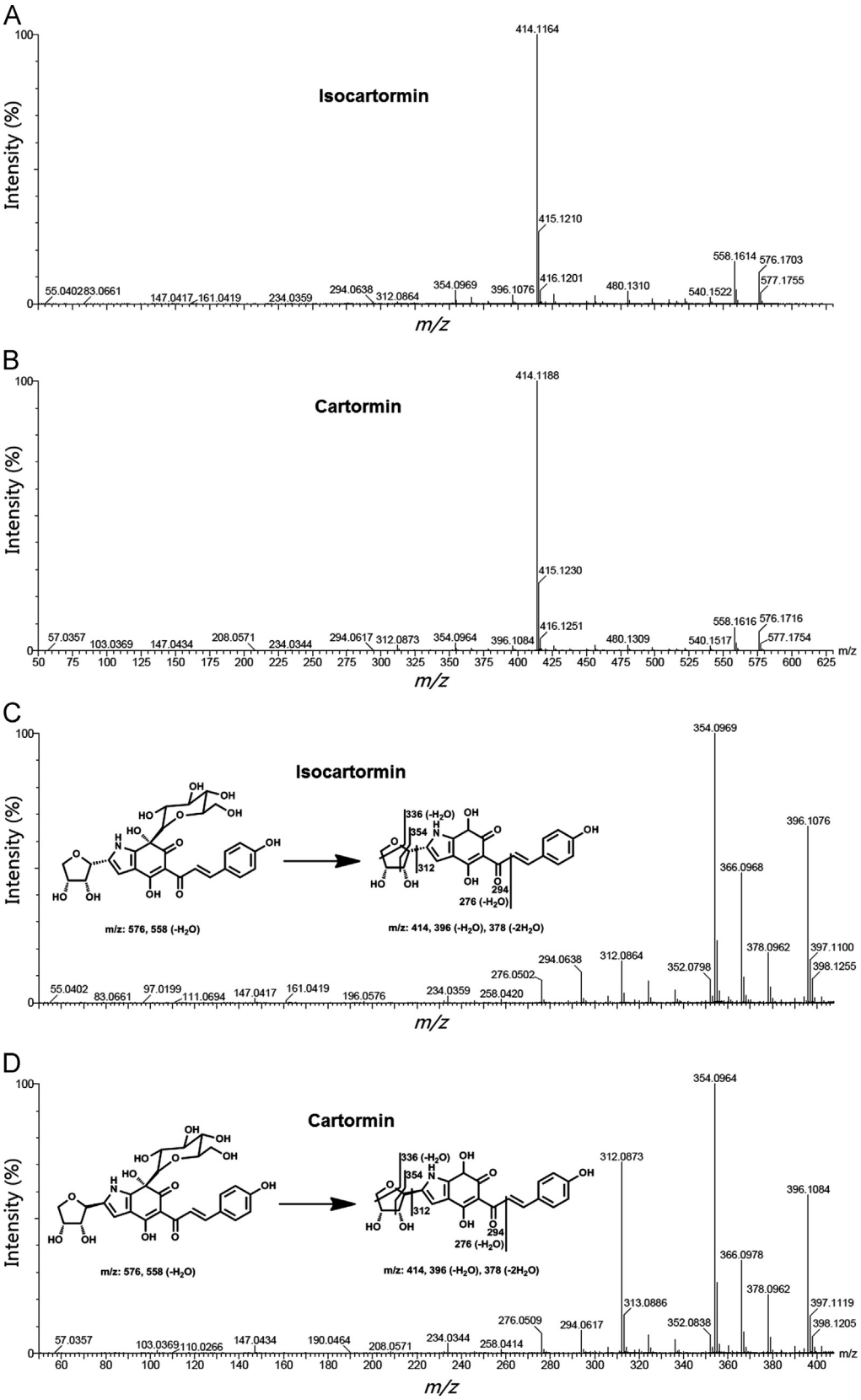

Figure 3 Comparison of MS/MS patterns between isocartormin and cartormin.

161.7 (s), 144.2 (d), 142.8 (s), 136.0 (s), 132.1 (d), 128.7 (s), 119.8 (d), 117.8 (s), 117.2 (d), 110.5 (s), 104.7 (d), 86.2 (d), 81.4 (d), 80.3 (d), 79.7 (s), 78.6 (d), 77.8 (d), 74.6 (t), 72.6 (d), 71.2 (d), 70.9 (d), 62.6 (t).

4.3.3. Safflomin C

Yellow powder, mp 300 °C (dec.); IR (KBr) ν_{max} (film): 3400, 1700, 1613, 1595, 1510, 1400, 1230, 1162, 1068, 920 and 825 cm^{-1}; ESI-MS (negative): *m/z* 613 $[M–H]^-$ (100); ^1H NMR and ^{13}C NMR were consistent with those in literature[19].

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2017.04.005.

References

1. Jiangsu New Medica Institute. The Dictionary of Chinese Medicine. Shanghai: Shanghai Science and Technology Press; 1985.
2. Fan L, Zhao HY, Xu M, Zhou L, Guo H, Han J, et al. Qualitative evaluation and quantitative determination of 10 major active components in *Carthamus tinctorius* L. by high-performance liquid chromatography coupled with diode array detector. *J Chromatogr A* 2009;**1216**:2063–70.
3. Kim MN, Scao-Bogaert FL, Paris M. Flavonoids from *Carthamus tinctorius* flowers. *Planta Med* 1992;**58**:285–6.
4. Li F, He ZS, Ye Y. Flavonoids from *Carthamus tinctorius*. *Chin J Chem* 2002;**20**:699–702.
5. Yin HB, He ZS, Ye Y. Cartorimine, a new cycloheptenone oxide derivative from *Carthamus tinctorius*. *J Nat Prod* 2000;**63**:1164–5.
6. Roh JS, Han JY, Kim JH, Hwang JK. Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis. *Biol Pharm Bull* 2004;**27**:1976–8.
7. Nagatsu A, Zhang HL, Watanabe T, Taniguchi N, Hatano K, Mizukami H, et al. New steroid and matairesinol glycosides from safflower (*Carthamus tinctorius* L.) oil cake. *Chem Pharm Bull* 1998;**46**:1044–7.
8. Nishibe S, Sakushima A, Hisada S, Inagaki I. Tracheloside from the seeds of *Carthamus tinctorius*. *Phytochemistry* 1972;**11**:2629.
9. Palter R, Lundin RE, Haddon WF. A cathartic lignan glycoside isolated from *Carthamus tinctorius*. *Phytochemistry* 1972;**11**:2871–4.
10. Akihisa T, Nozaki A, Inoue Y, Yasukawa K, Kasahara Y, Motohashi S, et al. Alkane diols from flower petals of *Carthamus tinctorius*. *Phytochemistry* 1997;**45**:725–8.
11. Akihisa T, Oinuma H, Tamura T, Kasahara Y, Kumaki K, Yasukawa K, et al. Erythro-hentriacontane-6,8-diol and 11 other alkane-6,8-diols from *Carthamus tinctorius*. *Phytochemistry* 1994;**36**:105–8.
12. Li F, He ZS, Ye Y. Riboflavin in and its degradation product from *Carthamus tinctorius*. *Chin Trad Herbal Drugs* 2004;**35**:3.
13. Jiang JS, He J, Feng ZM, Zhang PC. Two new quinochalcones from the florets of *Carthamus tinctorius*. *Org Lett* 2010;**12**:1196–9.
14. Jin Y, Zhang XL, Shi H, Xiao YS, Ke YX, Xue XY, et al. Characterization of *C*-glycosyl quinochalcones in *Carthamus tinctorius* L. by ultraperformance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom* 2008;**22**:1275–87.
15. Kazuma K, Takahashi T, Sato K, Takeuchi H, Matsumoto T, Okuno T. Quinochalcones and flavonoids from fresh florets in different cultivars of *Carthamus tinctorius* L. *Biosci Biotechnol Biochem* 2000;**64**:1588–99.
16. Yin HB, He ZS. A novel semi-quinone chalcone sharing a pyrrole ring *C*-glycoside from *Carthamus tinctorius*. *Tetrahedron Lett* 2000;**41**:1955–8.
17. Yue S, Tang Y, Li S, Duan JA. Chemical and biological properties of quinochalcone *C*-glycosides from the florets of *Carthamus tinctorius*. *Molecules* 2013;**18**:15220–54.
18. Yue S, Tang Y, Xu C, Li S, Zhu Y, Duan JA. Two new quinochalcone *C*-glycosides from the florets of *Carthamus tinctorius*. *Int J Mol Sci* 2014;**15**:16760–71.
19. Onodera J, Obara H, Hirose R, Matsuba S, Sato N, Sato S, Suzuki M. The structure of safflomin C, a constituent of safflower. *Chem Lett* 1989;**18**:1571–4.