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Article

2''-Ethyl-furanoflavone Derivatives from the Stems of *Cassia fistula* and their Cytotoxicity

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Duas novas 2"-etil-furanoflavonas chamadas fistulaflavonas A e B juntamente com seis furanoflavonas conhecidas foram isoladas das hastes da *Cassia fistula*. As estruturas foram elucidadas por métodos espectroscópicos, incluindo as técnicas de NMR 1D e 2D e espectrometria de massa com ionização por *electrospray* de alta resolução (HRESIMS), e comparação com dados da literatura. Todos os compostos foram avaliados com relação a citotoxicidade para cinco linhas de células tumorais humanas. Um dos compostos mostrou potente citotoxicidade contra células SHSY5Y e MCF7, com valores de IC_{50} de 2,6 e 2,7 μmol L^{-1}, respectivamente.

Two new 2"-ethyl-furanoflavones named fistulaflavones A and B together with six known furanoflavones were isolated from the stems of *Cassia fistula*. The structures were elucidated by spectroscopic methods including extensive 1D, 2D NMR and high resolution electrospray ionization mass spectrometry (HRESIMS) techniques, and comparison with literature data. All the compounds were evaluated for their cytotoxicity against five human tumor cell lines. One of the compounds showed potent cytotoxicity against SHSY5Y and MCF7 cells with IC_{50} values of 2.7 and 2.6 μmol L^{-1}, respectively.

Keywords: *Cassia fistula*, 2"-ethyl-furanoflavones, furanoflavones, cytotoxicity

Introduction

Cassia fistula L., (Leguminosae) is an ornamental tree with beautiful yellow flowers. This plant can be found in various countries in Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil.[1] In China, it has been widely used as traditional Chinese medicine for treatment of diarrhea, gastritis, ringworm and fungal skin infections.[2,3] Previous phytochemical studies of *C. fistula* have shown the presence of anthraquinones,[4,5] steroids,[6] chromones[7,8] and flavonol derivatives.[9] With the aim at searching for new natural compounds from medicinal plants, the stems of *C. fistula* were investigated and two new 2"-ethyl-furanoflavones (**1**-**2**) and six known furanoflavones (**3**-**8**) were isolated. The structures of the isolated compounds were established by spectroscopic methods including extensive 1D (^{1}H, ^{13}C and DEPT) and 2D (one-bond HSQC and long-range HMBC) NMR techniques and high resolution electrospray ionization mass spectrometry (HRESIMS), and by comparison with literature data. This work deals with the isolation, structural characterization of these compounds and their cytotoxicity against five human tumor cell lines.

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Results and Discussion

The stems of *C. fistula* were extracted with 70% aqueous acetone. The extract was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and semi-preparative RP-HPLC (reverse-phase high performance liquid chromatography) separation to afford two new furanoflavones named fistulaflavones A and B (**1**-**2**), together with six known furanoflavones (**3**-**8**). Structures of compounds **1**-**8** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of the compounds **1** and **2** were

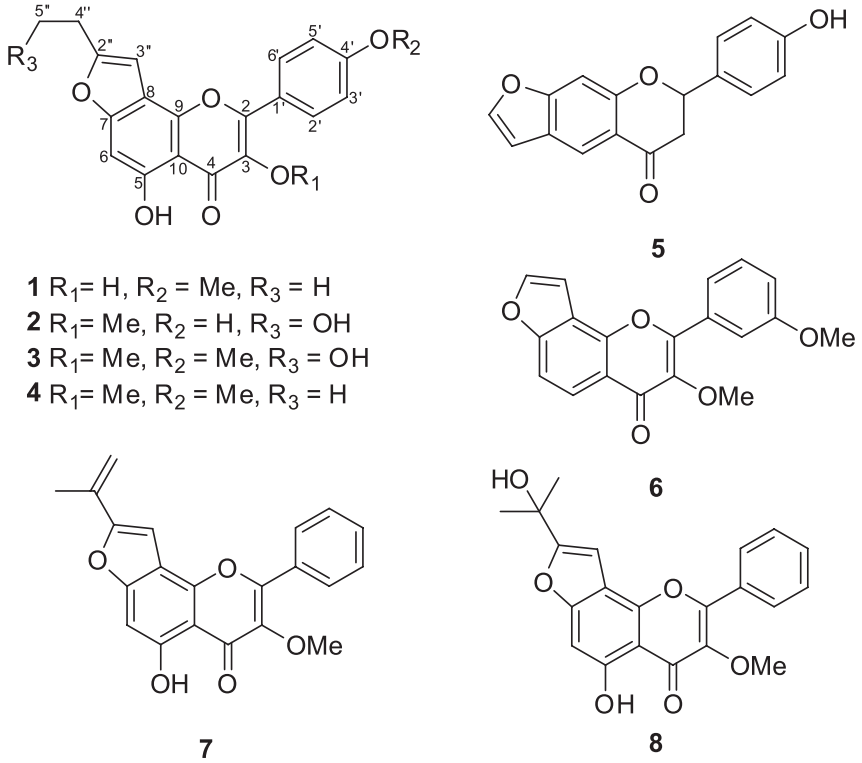

Figure 1. Structures of compounds **1**-**8**.

listed in Table 1. The known compounds were identified as 3,4'-dimethoxy-5-hydroxy-7,8-[2"-(2-hydroxyethyl) furan]-flavone (**3**),[10] 3,4'-dimethoxy-5-hydroxy-7,8-(2"-ethyl furan)-flavone (**4**),[11] furano-(2",3":7,6)-4'-hydroxyflavanone (**5**),[12] pachycarin D (**6**),[13] 5-hydroxy-2"-isopropenyl-3-methoxyfurano-(2",3":7,8)-flavone (**7**)[14] and 5-hydroxy-2"-(1-hydroxy-1-methylethyl)-3-methoxyfurano-(2",3":7,8)-flavone (**8**)[14] by comparison with literature data.

Compound **1** was obtained as an orange gum. Its HRESIMS spectrum in the positive mode revealed a quasi molecular ion at *m/z* 375.0843 $[M + Na]^+$, and assigned the molecular formula of $C_{20}H_{16}O_6$, corresponding to thirteen degrees of unsaturation. Its IR spectrum exhibited the presence of hydroxy group (3420 cm^{-1}), carbonyl group (1662 cm^{-1}) and aromatic ring (1615, 1557, 1450 cm^{-1}). Its ^1H, ^{13}C and DEPT NMR spectra showed signals for 20 carbon and 16 hydrogen atoms (Table 1). The ^{13}C NMR signals [δ_C 155.7 (s), 136.2 (s), 179.9 (s), 158.3 (s), 96.9 (d), 159.3 (s), 111.5 (s), 148.4 (s), 109.9 (s), 122.9 (s), 133.9 (d, 2C), 113.4 (d, 2C), 160.7 (s), 157.4 (s), 101.5 (d)], the ^1H NMR signals [δ_H 6.93 (s, 1H), 6.84 (s, 1H), 7.90 (d, 2H, *J* 8.8 Hz), 7.05 (d, 2H, *J* 8.8 Hz)] for six aromatic protons, one methoxy group at δ_C 55.9 (q), δ_H 3.85 (s) and two chelated hydroxyl group at δ_H 10.83 (brs) and 11.11 (brs) indicated the presence of a 2"-substituted furanoflavone.[12,14] The signals at δ_C [21.4 (t), 14.8 (q)], δ_H [2.33 (m) and 0.94 (t, *J* 6.9 Hz)] suggested an ethyl group at C-2", whose carbons were numbered as 4" and 5". The HMBC correlations of H-4" (δ_H 2.33, m) with C-2" (157.4, s) and C-3" (δ_C 101.5, d), of H-3" (6.84, s) with C-4" (δ_C 21.4, t) confirmed the ethyl group located at C-2". Cross peaks (Figure 2) of H-3" (δ_H 6.84, s) to C-7 (δ_C 159.3, s), C-8 (δ_C 111.5, s) and C-9 (δ_C 148.4, s) were also observed in compound **1**. This allowed us to conclude that the 2"-ethylfurano moiety was fused in an angular manner on the aromatic ring at positions C-7 and C-8. The HMBC correlations of the methoxy protons (δ_H 3.85, s) with C-4' (160.7, s) revealed that the methoxy group should be located at C-4'. Two chelated hydroxy groups were assigned to C-3 and C-5 on the basis of HMBC correlations between the hydroxy proton (δ_H 10.83, brs) and C-2 (δ_C 155.7, s), C-3 (δ_C 136.2, s), and C-4 (δ_C 179.9, s), as well as those between the other hydroxy proton (δ_H 11.11, brs) and C-5 (δ_C 158.3, s), C-6 (δ_C 96.9, d) and C-10 (δ_C 109.9, s). Two doublets [7.90 (d, *J* 8.8, 2H) and 7.05 (d, *J* 8.8, 2H)] and two singlets [(δ_H 6.93 (s), 1H and 6.84 (s), 1H)] in the ^1H NMR spectrum also supported the substituent positions in compound **1**. Thus, the structure of compound **1** was established as 3,5-dihydroxy-4'-methoxy-7,8-(2"-ethyl furan)-flavone and named as fistulaflavone A.

Compound **2** was obtained as an orange gum, and showed a quasi molecular ion at *m/z* 391.0790 in the HRESIMS data, corresponding to the molecular formula $C_{20}H_{16}O_7$. Comparison of 1D NMR spectra of compound **2** with those

Table 1. ^{1}H and ^{13}C NMR data (500 and 125 MHz, respectively) of compounds **1** and **2** in C_5D_5N[a]

No.	**1**		**2**	
	δ_C	δ_H (m, *J* / Hz)	δ_C	δ_H (m, *J* / Hz)
2	155.7 (s)		155.8 (s)	
3	136.2 (s)		138.6 (s)	
4	179.9 (s)		179.8 (s)	
5	158.3 (s)		158.3 (s)	
6	96.9 (d)	6.93 (s)	96.7 (d)	6.91 (s)
7	159.3 (s)		158.9 (s)	
8	111.5 (s)		111.9 (s)	
9	148.4 (s)		148.2 (s)	
10	109.9 (s)		110.0 (s)	
1'	122.9 (s)		122.8 (s)	
2', 6'	133.9 (d)	7.90 (d, 8.8)	134.9 (d)	7.84 (d, 8.8)
3', 5'	113.4 (d)	7.05 (d, 8.8)	114.9 (d)	7.02 (d, 8.8)
4'	160.7 (s)		158.1 (s)	
2"	157.4 (s)		157.1 (s)	
3"	101.5 (d)	6.84 (s)	101.2 (d)	6.81 (s)
4"	21.4 (t)	2.33 (m)	34.5 (t)	2.75 (t, 7.1)
5"	14.8 (q)	0.94 (t, 6.9)	62.7 (t)	3.55 (t, 7.1)
OMe-3			60.7 (q)	3.83 (s)
OMe-4'	55.9 (q)	3.85 (s)		
OH-3		10.83 (s)		
OH-4'				10.81 (s)
OH-5		11.11 (s)		11.11 (s)

[a]Hydrogenation pattern of the carbons determined by DEPT in comparison with the HSQC spectrum.

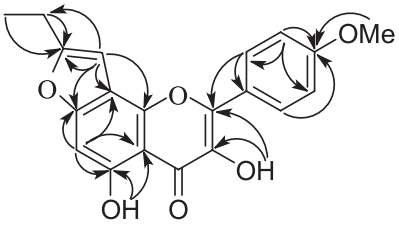

Figure 2. Selected HMBC (H → C) correlations of compound **1**.

of compound **1** showed the presence of signals for a primary alcohol at δ_H 3.55 (t, *J* 7.1 Hz) and δ_C 62.7 (t), and the absence of the signal for an aliphatic methyl group. Other differences were the downfield-shift of the C-3 resonance from δ_C 136.2 (s) to δ_C 138.6 (s), and the upfield-shift of C-4' from δ_C 160.7 (s) to δ_C 158.1 (s), thus indicating a different pattern of substitution at these two carbons for compounds **1** and **2**. Analysis of the HMBC spectrum of compound **2** showed a methoxy group located at C-3, and two hydroxy groups located at C-4' (δ_H 10.83) and C-5 (δ_H 11.11). Accordingly, the structure of fistulaflavone B was determined as 3-methoxy-4',5-dihydroxy-7,8-[2"-(2-hydroxyethyl) furan]-flavone.

Since several flavone derivatives exhibited potential cytotoxicity,[15-17] compounds **1**-**8** were tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) using the MTT (yellow dye 3-(4,5-dimethyl- 2-thiazolyl)-2,5-diphenyl-2*H*-tetrazoliumbromide) method as previously reported[18] and Taxol® (paclitaxel) as positive control. The results were shown in Table 2. Compounds **2**, **5**, **6** and **8** showed no activity (IC_{50} values > 10 μmol L^{-1}, concentrations that induce 50% inhibition of cell growth) for all tested tumor cell lines. Compound **3** showed potent cytotoxicity against SHSY5Y and MCF7 cells with IC_{50} values of 2.7 and 2.6 μmol L^{-1}, respectively. Compounds **1**, **4** and **7** showed modest cytotoxicity with IC_{50} below 10 μmol L^{-1} for some selected cell lines.

Table 2. The IC_{50} values for the compounds from the *C. fistula*

Compound	IC_{50} / (μmol L^{-1})				
	NB4	A549	SHSY5Y	PC3	MCF7
1	8.7	> 10	9.47	> 10	9.6
2	> 10	> 10	> 10	> 10	> 10
3	8.9	5.9	2.7	8.3	2.6
4	> 10	5.5	> 10	6.9	8.2
5	> 10	> 10	> 10	> 10	> 10
6	> 10	> 10	> 10	> 10	> 10
7	> 10	4.9	8.6	> 10	5.9
8	> 10	> 10	> 10	> 10	> 10
Taxol	0.03	0.02	0.2	0.2	0.1

NB4: human leukemia cell; A549: carcinomic human alveolar basal epithelial cell; SHSY5Y: human neuroblastoma cell; PC3: human prostate cancer cell; MCF7: human breast adenocarcinoma cell.

Experimental

General experimental procedures

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS spectra were performed on a VG Autospec-3000 spectrometer. Semi-preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 × 250 mm) or Venusil MP C_{18}

(20 × 250 mm) columns. Column chromatography (CC) was performed on silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by thin layer chromatography (TLC), and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant material

The stems of *Cassia fistula* L. (Leguminosae) were collected at Xishuangbangna Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Dr. Yuan N., Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 10-9-25) was deposited in our Laboratory.

Extraction and isolation

The air-dried and powdered stems of *C. fistula* (8 kg) were extracted four times with 70% aqueous acetone (4 × 8 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (522 g) was applied to CC on silica gel (150-200 mesh), eluting with $CHCl_3$-MeOH gradient systems (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to give six fractions A-F. Further separation of fraction B ($CHCl_3$-MeOH 9:1, 22.8 g) using CC on silica gel, eluted with petroleum ether-acetone (9:1-1:2) yielded mixtures B1-B6. Fraction B2 (5.3 g) was subjected to CC on silica gel using petroleum ether-acetone and semi-preparative HPLC, eluting with an isocratic system (62% MeOH-H_2O, flow rate 12 mL min^{-1}) to give **4** (11.2 mg), **6** (21.9 mg) and **7** (13.4 mg). Fraction B3 (4.6 g) was subjected to CC on silica gel using petroleum ether-acetone and semi-preparative HPLC, eluting with an isocratic system (55% MeOH-H_2O, flow rate 12 mL min^{-1}) to give **2** (7.6 mg), **3** (12.2 mg), **5** (12.8 mg) and **8** (24.6 mg). Further separation of fraction C (8:2, 31.5 g) by CC on silica gel, eluted with petroleum ether-acetone (9:1-1:2) and yielded mixtures C1-C6. Fraction C4 (5.7 g) was subjected to CC on silica gel using petroleum ether-acetone and semi-preparative HPLC, eluting with an isocratic system (46% MeOH-H_2O, flow rate 12 mL min^{-1}) to give **1** (11.5 mg).

Cytotoxicity assay

The cytotoxicity tests were performed against NB4, A549, SHSY5Y, PC3 and MCF7 tumor cells by MTT (yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide) assay using Taxol® as the positive control.[18] Firstly, 2500 cells suspended in 100 μL MEM medium were seeded, in a 96-well plate. After 24 h incubation, fresh medium containing various concentrations of each compound were added into the 96-well plate to replace the old medium. The OD_{595} values of the control groups at 0 and 72 h together with the compound treated groups at 72 h from the MTT assay were measured using a plate reader.

Fistulaflavone A (**1**)

Orange gum; UV (MeOH) λ_{max}/nm (log ε) 370 (3.90), 258 (4.15), 210 (4.54); IR (KBr) ν_{max}/cm^{-1} 3420, 2925, 1662, 1615, 1557, 1450, 1358, 1224, 1155, 1005, 765, 688; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), see Table 1. Positive ESIMS *m/z* 375 $[M + Na]^+$; HRESIMS *m/z* 375.0843 $[M + Na]^+$ (calcd. for $C_{20}H_{16}O_6Na$, 375.0845).

Fistulaflavone B (**2**)

Orange gum; UV (MeOH) λ_{max}/nm (log ε) 370 (3.94), 255 (4.18), 210 (4.59); IR (KBr) ν_{max}/cm^{-1} 3416, 2922, 1662, 1618, 1558, 1453, 1355, 1220, 1156, 1002, 768, 682; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz), see Table 1. Positive ESIMS *m/z* 391 $[M + Na]^+$; HRESIMS *m/z* 391.0790 $[M + Na]^+$ (calcd. for $C_{20}H_{16}O_7Na$, 391.0794).

Supplementary Information

The ^1H and ^{13}C NMR spectra of **1** and **2** are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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References

1. Duraipandiyan, V.; Ignacimuthu, S.; *J. Ethnopharmacol.* **2007**, *112*, 590.
2. Rajan, S.; Baburaj, D. S.; Sethuraman, M.; Parimala, S.; *Ethnobotany* **2001**, *6*, 19.
3. Ma, J.; Zhang, L. X.; Guan, Y .H.; *Chin. J. Ethnomed. Ethnopharm.* **2004**, *5*, 178.

4. Abo, K. A.; Adeyemi, A. A.; Sobowale, A. O.; *Afr. J. Med. Med. Sci*. **2001**, *30*, 9.
5. Aurapa, S.; Wandee, G.; *Int. J. Biomed. Pharm. Sci*. **2009**, *3*, 42.
6. Sartorelli, P.; Andrade, S. P.; Melhem, M. S. C.; Prado, F. O.; Tempone, A. G.; *Phytother. Res*. **2007**, *21*, 644.
7. Kuo, Y. H.; Lee, P. H.; Wein, Y. S.; *J. Nat. Prod*. **2002**, *65*, 1165.
8. Jothy, S. L.; Zakaria, Z.; Chen, Y.; Lau, Y. L.; Latha, L. Y.; Shin, L. N.; Sasidharan, S.; *Molecules* **2011**, *16*, 7583.
9. Bahorun, T.; Neergheen, V. S.; Aruoma, O. I.; *Afr. J. Biotechnol*. **2005**, *4*, 1530.
10. Hu, Q. F.; Li, Y. K.; Yang, L. Y.; Shen, Y. Q.; Shu, L. D.; *Chinese pat*. CN 102675330A20120919 **2012**.
11. Tan, J. L., Chen, Y. K.; Su, Z. B.; Wang, L.; Yang, G. Y.; Zhang, C. M.; Miu, M. M.; *Chinese pat*. CN 102584845A20120718 **2012**.
12. Liu, H.; Bai, Y. J.; Chen, Y. Y.; Zhao, Y. Y.; *China J. Chin. Mat. Med*. **2008**, *33*, 1410.
13. Shao, W. Y.; Huang, X. F.; Zhou, Y. F.; Guan, S. Y.; Chen, F. T.; Zhao, S. Z.; *Chin. J. Instrum. Anal*. **2001**, *20*, 8.
14. Cavalcante, M. G. B.; Silva, R. M.; Bandeira, P. N.; dos Santos, H. S.; Pessoa, O. D. L.; Braz-Filho, R.; Albuquerque, M. R. J. R.; *J. Braz. Chem. Soc*. **2012**, *23*, 301.
15. Norat, T.; Riboli, E.; *Eur. J. Clin. Nutr*. **2003**, *57*, 1.
16. Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P.; *Nat. Rev. Drug Discovovery* **2009**, *8*, 69.
17. Amado, N. G.; Fonseca, B. F.; Cerqueira, D. M.; Neto, V. M.; Abreu, J. G.; *Life Sci*. **2011**, *89*, 545.
18. Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.

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