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Synthesis and antimicrobial activity of flavone-6-carboxaldehyde oxime ether derivatives

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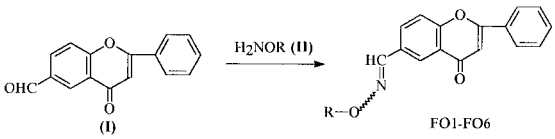
Flavone derivatives represent a class of chemical products with interesting pharmacological activities such as antibacterial [1], antifungal [2], antiviral [3], antitumor [4], antioxidant [5], spasmolytic [6]. In recent years, oxime ether derivatives of some known antibacterial compounds like erythromycin and cephalosporins have been prepared and exerted good antibacterial activity [7, 8]. These results encouraged us to synthesize some oxime ethers of flavone-6-carboxaldehyde (Table 1) and to evaluate their antimicrobial activities. Derivatives **FO1–FO6** were synthesized starting with flavone-6-carboxaldehyde (I) by treatment with the appropriate O-substituted hydroxyl amine derivatives (II) in the presence of pyridine/absolute ethanol (Scheme in Table 1). Compounds **FO1**, **FO2** and **FO6** showed *Z* configuration. Compounds **FO3**, **FO4** and **FO5** were obtained as a mixture of *E* and *Z* isomers in 1:11, 1:12 and 1:9 isomeric ratio, respectively according to ^{1}H NMR data. In the ^{1}H NMR spectra, the characteristic protons belonging to the flavone and oxime ether moiety can be seen. The H-5 proton of flavone was observed at 8.20–8.32 ppm with the deshielding effect of the CO group of the γ-pyron ring. −CH=N− protons were seen at 8.14–8.21 ppm with the effect of conjugation. The oxime ethers are theoretically able to exist as *E* and *Z* isomers. C=N−O−CH_2− protons were observed at 4.35–4.45 ppm for the *E* isomer and at 4.20–4.30 ppm for the *Z*-isomer [9–12]. In our findings, *Z* and *E* isomers were observed at 4.22–4.36 and 4.35–4.42 ppm, respectively.

All the new compounds were tested for their antimicrobial activity by the agar diffusion method [13], using *S. aureus, E. coli* and *C. albicans* as test organisms. Ketoconazole, fluconazole and ampicillin were used for comparison. Only the compounds which showed inhibition zones ≥ 10 mm diameter are recorded in Table 2. Compounds **FO3–FO6** exhibited better activity than fluconazole, but all of the compounds were less active than ketoconazole against *C. albicans*. According to this results, it is supposed that the effect of the size of side chain on the activity may be important. The compounds which carry a bulky side chain (dimethylamino-, pyrolidino-, piperidino- and morpholino) showed better activity than the others.

Table 1: Some physical properties of the compounds FO1–FO6



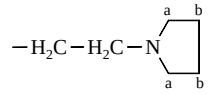
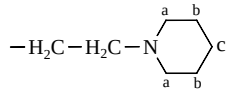
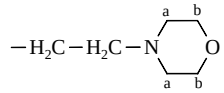
Compd.	R	Yield (%)	M.p. (°C)	Molecular formula
FO1	$-CH_3$	77.7	163	$C_{17}H_{13}NO_3$
FO2	$-CH_2CH_3$	47.2	102	$C_{18}H_{15}NO_3$
FO3	$-CH_2CH_2N(CH_3)_2$	69.5	78	$C_{20}H_{20}N_2O_3$
FO4		57.6	95	$C_{22}H_{22}N_2O_3$
FO5		62.1	77	$C_{23}H_{24}N_2O_3$
FO6		33.1	120	$C_{22}H_{22}N_2O_4.0.85\,HCl$

Table 2: Antimicrobial activities[a] of the compounds

Compd.	*C. albicans*	*S. aureus*	*E. coli*
FO1	12	*	*
FO2	11	*	*
FO3	18	20	*
FO4	17	17	12
FO5	22	14	11
FO6	20	21	11
Ketoconazole	30	–	–
Fluconazole	16	–	–
Ampicillin	–	29	22

[a] growth inhibition zone diameter (mm) * no activity – not tested

Experimental

1. Apparatus

Melting points were determined with a Büchi SMP-20 apparatus and are uncorrected. IR spectra were recorded on a Jasco FT/IR 420 spectrometer as potassium bromide discs. All the instrumental analyses were performed by TUBITAK (Instrumental analyse Lab. Ankara/Turkey). ^{1}H NMR spectra were recorded with a Bruker GmbH DPX-400,400 MHz instrument in $CDCl_3$, δ scale from internal standard TMS. The MS were obtained with a VG Platform II Spectrometer by using Electron Ionisation (EI): Ionisation Energy 70 eV. Elemental analyses (C, H, N) were determined on a Leco CHNS 932 instrument. All values of C, H, N were within ±0.4% of the calculated data. Column chromatography was performed using Merck Silica Gel (230–400 mesh ASTM). Flavone-6-carboxaldehyde [14] and O-substituted hydroxyl amine derivatives were synthesized according to the literature [15].

2. General procedure for preparing compounds FO1–FO6

Flavone-6-carboxaldehyde (0.001 mol) and O-substituted hydroxyl amine derivatives (0.001 mol) were heated in pyridine (1 ml)/abs. EtOH (10 ml) for 10 h. The mixture was evaporated to dryness in vacuo and the residue was dissolved in H_2O and treated with NaOH 10% solution. The aqueous solution was extracted with $CHCl_3$ and the organic layer was dried over anh. Na_2SO_4 and evaporated to dryness. The residue was purified with CC using $CHCl_3$/n-hexane (1:1) as eluant (Compound **FO6** was purified without neutralization). Some physical properties of the compounds **FO1–FO6** are given in Table 1. We obtained only one isomer for compounds **FO1**, **FO2** and **FO6** and mixtures of isomers for compounds **FO3–FO5** in the isomers rate. The general formula represents the *Z* isomer (O−CH_2−R group *cis* to H).

2.1. Flavone-6-carboxaldehyde-O-methyloxime (***FO1***)

IR (KBr) cm^{-1}: 1655 (γ-pyron CO). ^{1}H NMR ($CDCl_3$): δ = 4.00 (s, 3 H, OCH_3), 6.84 (s, 1 H, 3-H), 7.53–7.59 (m, 4 H, 8,3′,4′,5′-H), 7.92 (dd, 2 H, J_o = 7.22 Hz, J_m = 1.60 Hz, 2′,6′-H), 8.09 (dd, 1 H, $J_{7,8}$ = 8.79, $J_{7,5}$ = 2.16 Hz, 7-H), 8.15 (s, 1 H, −CH=N), 8.27 (d, 1 H, $J_{5,7}$ = 2.07 Hz, 5-H). MS (EI): m/z (%) = 279 (100) $[M]^{+\cdot}$, 264 (3.20), 248 (8.31), 221 (21.68), 177 (85.44), 163 (14.56), 102 (68.99), 76 (48.73), 63 (85.44).

2.2. Flavone-6-carboxaldehyde-O-ethyloxime (***FO2***)

IR (KBr) cm^{-1}: 1661 (γ-pyron CO). ^{1}H NMR ($CDCl_3$): δ = 1.35 (t, 3 H, $OCH_2\underline{CH_3}$), 4.26 (q, 2 H, $OC\underline{H_2}CH_3$), 6.84 (s, 1 H, 3-H), 7.53–7.58 (m, 4 H, 8,3′,4′,5′-H), 7.93 (dd, 2 H, J_o = 7.32 Hz, J_m = 1.69 Hz, 2′,6′-H), 8.08 (dd, 1 H, $J_{7,8}$ = 8.78, $J_{7,5}$ = 2.15 Hz, 7-H), 8.16 (s, 1 H, −CH=N), 8.28 (d, 1 H, $J_{5,7}$ = 2.11 Hz, 5-H). MS (EI): m/z (%) = 293.2 (16.90) $[M]^{+\cdot}$, 264.2 (11.81), 248.2 (1.39), 221.2 (2.99), 193.2 (1.76), 149.1 (3.05), 101.98 (100), 76.2 (77.16), 63.2 (70.37).

2.3. Flavone-6-carboxaldehyde-O-(2-dimethylaminoethyl)oxime (***FO3***)

IR (KBr) cm^{-1}: 1654 (γ-pyron CO). ^{1}H NMR ($CDCl_3$): δ = 2.30 (s, 6 H, $N(CH_3)_2$), 2.65 (t, 1.83 H, CH_2N, *Z* isomer), 2.72 (t, 0.17 H, CH_2N, *E* iso-

mer), 4.27 (t, 1.83 H, OCH_2, *Z* isomer), 4.35 (t, 0.17 H, OCH_2, *E* isomer), 6.76 (s, 1 H, 3-H), 7.28–7.41 (m, 4 H, 8, 3′, 4′, 5′-H), 7.84 (dd, 2 H, $J_o = 8.07$ Hz, $J_m = 1.60$ Hz, 2′,6′-H), 7.99 (dd, 1 H, $J_{7,8} = 8.71$ Hz, $J_{7,5} = 1.71$ Hz, 7-H), 8.14 (s, 1 H, −CH=N), 8.20 (d, 1 H, $J_{5,7} = 1.64$ Hz, 5-H). MS (EI): m/z (%) 337 (1.04) $[M + 1]^{+\cdot}$, 101.95 (5.00), 70.84 (6.83), 58.15 (100).

2.4. Flavone-6-carboxaldehyde-O-[2-(1-pyrolidino)ethyl]oxime (***FO4***)

IR (KBr) cm^{-1}: 1650 (γ-pyron CO). ^1H NMR ($CDCl_3$): δ = 1.82 (m, 4 H, b), 2.64 (m, 4 H, a), 2.87 (t, 1.85 H, CH_2N, *Z* isomer), 2.94 (t, 0.15 H, CH_2N, *E* isomer), 4.36 (t, 1.85 H, OCH_2, *Z* isomer), 4.42 (t, 0.15 H, OCH_2, *E* isomer), 6.84 (s, 1 H, 3-H), 7.53–7.58 (m, 4 H, 8,3′,4′,5′-H), 7.93 (dd, 2 H, $J_o = 7.28$ Hz, $J_m = 1.62$ Hz, 2′, 6′-H), 8.02 (dd, 1 H, $J_{7,8} = 8.77$ Hz, $J_{7,5} = 2.11$ Hz, 7-H), 8.21 (s, 1 H, −CH=N), 8.28 (d, 1 H, $J_{5,7} = 2.07$ Hz, 5-H), MS (EI): m/z (%) = 363 (0.63) $[M + 1]^{+\cdot}$, 221.2 (1.59), 101.97 (9.55), 84 (100).

2.5. Flavone-6-carboxaldehyde-O-[2-(1-piperidino)ethyl]oxime (***FO5***)

IR (KBr) cm^{-1}: 1649 (γ-pyron CO). ^1H NMR ($CDCl_3$): δ = 1.46 (m, 2 H, c), 1.61–1.64 (m, 4 H, b), 2.45–2.53 (m, 4 H, a), 2.74 (t, 1.8 H, CH_2N, *Z* isomer), 2.79 (t, 0.2 H, CH_2N, *E* isomer), 4.36 (t, 1.8 H, OCH_2, *Z* isomer), 4.40 (t, 0.2 H, OCH_2, *E* isomer); 6.84 (s, 1 H, 3-H), 7.53–7.58 (m, 4 H, 8,3′,4′,5′-H), 7.92 (dd, 2 H, $J_o = 7.68$ Hz, $J_m = 1.97$ Hz, 2′,6′-H), 8.07 (dd, 1 H, $J_{7,8} = 8.75$ Hz, $J_{7,5} = 1.89$ Hz, 7-H), 8.19 (s, 1 H, −CH=N), 8.28 (d, 1 H, $J_{5,7} = 1.77$ Hz, 5-H). MS (EI): m/z (%) = 376 (0.23) $[M]^{+\cdot}$, 249.1 (1.84), 221.2 (2.66), 127.2 (1.72), 98.05 (100), 64.2 (1.41).

2.6. Flavone-6-carboxaldehyde-O-[2-(4-morpholino)ethyl]oxime (***FO6***)

IR (KBr) cm^{-1}: 1645 (γ-pyron CO). ^1H NMR ($CDCl_3$): δ = 2.92 (t, 4 H, a), 3.05 (t, 2 H, CH_2N), 3.92 (t, 4 H, b), 4.52 (t, 2 H, OCH_2), 6.82 (s, 1 H, 3-H), 7.54–7.61 (m, 4 H, 8,3′,4′,5′-H), 7.93 (dd, 2 H, $J_o = 7.48$ Hz, $J_m = 1.57$ Hz, 2′,6′-H), 8.04 (dd, 1 H, $J_{7,8} = 8.77$ Hz, $J_{7,5} = 2.11$ Hz, 7-H), 8.20 (s, 1 H, −CH=N), 8.32 (d, 1 H, $J_{5,7} = 2.06$ Hz, 5-H). MS (EI): m/z (%) = 379.2 (8.28) $[M + 1]^{+\cdot}$, 221 (6.37), 100.08 (100), 69.99 (13.01).

3. Antimicrobial activity

A paper disc (8 mm in diameter) was soaked in a 3000 μg/ml solution of the test compound in propylene glycol (propylene glycol as a blank has not any inhibition zone), and placed on an agar plate containing fungi or bacteria cells, which was incubated at 37 °C for 24 h. The diameter of the growth inhibition zone around the paper disc was measured [13]. Antimicrobial activity results are shown in Table 2.

Acknowledgement: This work was supported by the Research Organisation of the Ankara University, Turkey (No: 97030002) and TUBITAK (No: SBAG-AYD-158).

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Received August 10, 1998
Accepted October 7, 1998

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Some azolylthioacetamides and their analgesic and antiinflammatory activities

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As part of our continuing effort to prepare nonsteroidal anti-inflammatory drugs (NSAIDs), the aim of the present study was to synthesize compounds having 1*R,2S-N*-(2-phenyl-2-hydroxyl-1-methyl)ethyl-2-(2-benzoxazolyl and/or benzothiazolyl)thioacetamide structure and to determine their analgesic and anti-inflammatory activities.

Four benzoxazolyl- and benzothiazolylthioacetamide derivatives **1b**–**4b** were synthesized by the reaction of the acid derivatives **1a**–**4a** with 1*R,2S*-2-methylamino-1-phenylpropanol (Scheme).

Since the m.p.'s of the compounds are very low, they could not been determined. All spectral data are in accordance with the assumed structures. In the IR spectra, OH and NH and amid-I and amid-II bands were seen at expected values. The ^1H NMR spectra of all compounds showed a doublet at 4.90 ppm, a multiplet at 4.35–4.60 ppm, a singlet at 3.90–4.15 pp, attributable to $C\underline{H}-OH$, $C\underline{H}-CH_3$ and $C\underline{H}_2$ protons respectively. Due to deutorium exchange $O-\underline{H}$ and $N-\underline{H}$ protons were not seen. Aromatic and methyl protons were seen at the expexted chemical shift and integral values. ^{13}C NMR spectra of all compounds supported their structures. In MS molecular ion peaks did not appear for all compounds.

The analgesic activity of the compounds was screened by a "modified Koster's test" using aspirin as a reference analgesic. As seen in the Table, the compounds synthesized did not show analgesic activity.

The anti-inflammatory activity of these compounds was measured in the carrageenan paw edema test. The mechanism of the antiinflammatory action was further investigated by the mice air pouch test. For this purpose, air was injected to the back of mice for three days, thus forming bilateral invagination. The exudate formed within this invagination after carrageenan injection enabled detailed investigation. NSAIDs reduced vasodilatation, edema and hyperalgesia by inhibiting cyclooxygenase activity [1–5]. An ideal NSAID should reduce exudate volume and PMNL accumulation. In our work, compounds **1b**, **2b** and **4b** inhibited carrageenan-induced paw edema and reduced the number of PMNL in mice air-pouch. While compound **3b** was ineffective on carrageenan-induced paw edema, compounds **1b**, **2b** and **4b** inhibit the exudation of plasma proteins, the formation of edema within the tissues, and the emigration of leucocytes from the blood into the tis-

Table: Results of pharmacological studies of the compounds 1b–4b

Compd. (100 mg/kg)	% Analgesic activity (n = 6)	%Anti-inflammatory activity (n = 6)	Number of PMNLs* (PMN × 10^5)
1b	no activity	40	117.33 ± 7.84
2b	no activity	40	84.67 ± 2.81
3b	no activity	6	–
4b	no activity	25	87.33 ± 7.26
Aspirin	56.7	45	102.37 ± 6.30
Control	–	–	157.00 ± 4.88