Acta Pharmaceutica Turcica
45:31-35 (2003)

Synthesis and Antimicrobial Activities of Some New Flavone Derivatives

Yeni bazı Flavon Türevlerinin Sentezi ve Antimikrobiyal Aktiviteleri

Oya Bozdağ-Dündar[1], Meltem Ceylan[1], Nurten Altanlar[2] and Rahmiye Ertan[1*]

Ankara University, Faculty of Pharmacy, Departments of, [1]Pharmaceutical Chemistry, [2]Microbiology, 06100-Tandoğan, Ankara, Turkey

Abstract

A new series of amino substituted flavone derivatives (**IVa-d**) was prepared by reacting bromomethylflavone (**IIa-b**) with amine derivatives (**IIIa-b**). The synthesized compounds were tested for their antifungal and antibacterial activities in vitro. All of the tested compounds was found active against used microorganisms.

Key words: Flavone derivatives, antifungal and antimicrobial activities.

Introduction

A flavone ring system is present in many naturally occuring products (Mabry *et al.*, 1970) with diverse pharmacological activities such as antibacterial (Mori *et al.*, 1987), antifungal (Perry and Foster, 1994), antiviral (Perry and Foster, 1994; Conti *et al.*, 1990), antitumor (Zaharko *et al.*, 1986), antioxidant (Das and Ray, 1988), spasmolytic and antihepatotoxic (Gabor, 1981; Nardi *et al.*, 1993). Synthesized hydrazinic derivatives have also been to show potent antibacterial activity (Omar *et al.*, 1984). These results encouraged us to synthesize some flavonyl compounds having hydrazinic side chain and to evaluate their antimicrobial activities.

Material and Methods

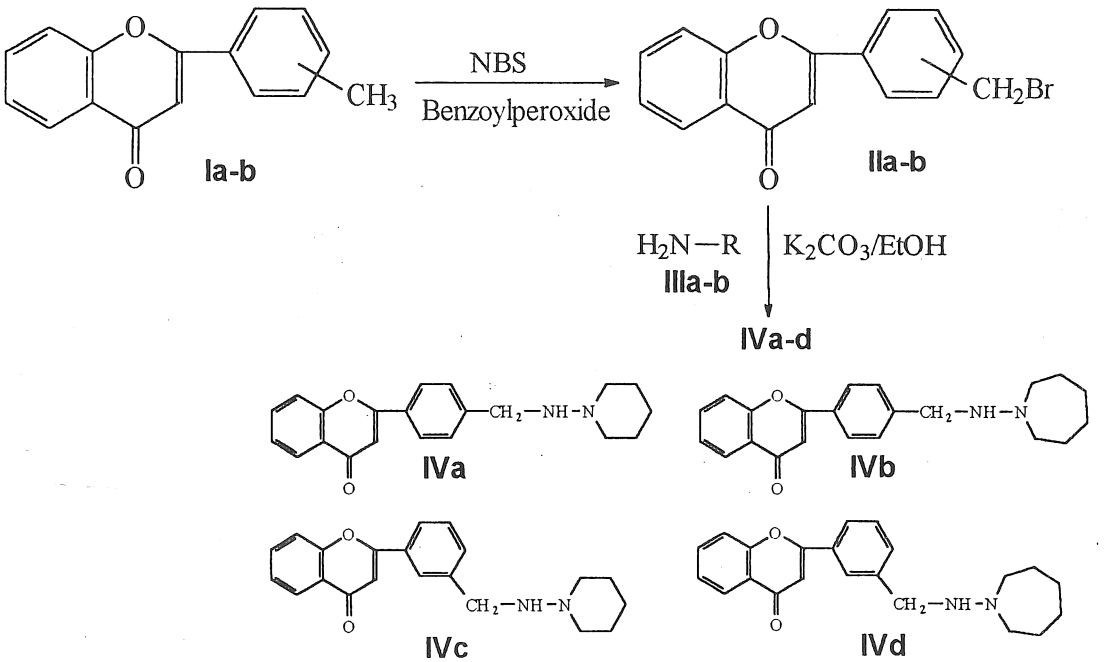
Chemistry: Melting points were determined with a Büchi SMP-20 melting point apparatus (Büchi, Flawil, Switzerland) and were uncorrected. All of the instrumental analyses were performed by the Instrumental Analysis Lab. of Scientific and Technical Research Council of Turkey (TUBITAK, Ankara, Turkey), using a Jasco FT/IR 420 spectrophotometer (Jasco Corp., Tokyo, Japan) (IR spectra were recorded as potassium bromide discs), a Bruker GmbH DPX-400, 400 MHz NMR spectrophotometer (Bruker, Rheinstetten, Germany) (the ^{1}H NMR spectra were measured in $CDCl_3$, all chemical shifts were reported as δ (ppm) values) and VG Platform II Micromass spectrometer (Micromass, Manchester, England). Elementary analyses were performed by a Leco CHNS 932 analyzer (Leco, St. Joseph, USA) and satisfactory results (±0.4%) of the calculated values (C, H, N, S) were obtained. For the column chromatographic

* *Corresponding author*

analysis Merck Silica Gel 60 (230-400 mesh ASTM) was used. The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, MI, USA). 3'or 4'-Metil flavone (Cramer and Elsching, 1956), 3' or 4'-Bromometil flavone (IIa-b) (Tunçbilek and Ertan, 1999) were synthesized according to the literature.

Synthesis of 3' (IIa)-4' (**IIb**)*-Bromomethyl flavone:* A mixture of N-bromosuccinimide (1.2g, 0.00672mol) and 3' or 4'-methyl flavone (**Ia-b**) was dissolved in 70 ml of carbon tetrachloride and benzoyl peroxide (0.1g) was added. The reaction mixture was refluxed for 7 hours and filtered while still hot. The crude product was crystallized from toluene [**IIa** m.p:137°C (Tunçbilek and Ertan, 1999:), **IIb** m.p:139°C (Tunçbilek and Ertan, 1999)].

Synthesis of compounds **IVa-d**: 0.01Mol of 3' or 4'-bromomethyl flavone (**IIa-b**) and 0.01 mol substituted amine derivatives (**IIIa-b**) were stirred in 5 ml of abs. EtOH under nitrogene atmosphere at room temperature for 2 days (Scheme). The mixture was filtered and the filtrate was evaporated to dryness in vacuo and the residue was purified by column chromatography using silica gel 60 (230-400 mesh ASTM) as the adsorbent and $CHCl_3$ as the eluent. Some physicochemical properties of the compounds so obtained (**IVa-d**) are given in Table 1.

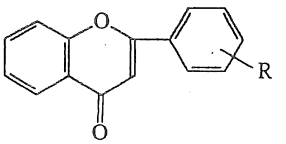


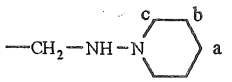
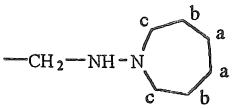
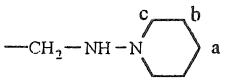
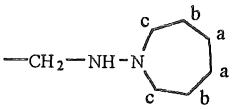
Scheme. General synthesis of **IVa-d**.

Antimicrobial activity: Disk diffusion method was used for assessing antibacterial activity against *Staphylococcus aureus* ATCC 250, *Escherichia coli* RSKK 313 and antifungal activity against *Candida albicans* RSKK 628. Cultures of each bacteria and yeast strain, kept in Mueller-Hinton broth (DIFCO), at 37°C for 18-24 h and diluted with the same broth to 10^5 cfu/ml, were pipetted into the Mueller-Hinton agar plates prepared according to the procedure. Paper disks (8 mm in diameter) embedded into 3000 $\mu g.ml^{-1}$ compound solution were placed on to the surface of the inoculated plates and were placed into an incubator at 37°C for 18-24 h.

and were then examined. All the compounds were found to be effective against the tested microorganisms by measuring the diameter of the growth inhibition zone according to Bauer *et al* (Bauer *et al.*, 1966).

Table 1. Some physicochemical properties of the compounds **IVa-d**.



Comp.	R	Yield (%)	Melting point (°C)	Formula	Analysis
IVa		55.8	225-226	$C_{21}H_{22}N_2O_2$ 2-[4-(Piperidine-1-yl-aminomethyl)-phenyl]-4H-benzopyrane-4-one	C, H, N
IVb		46.9	238	$C_{22}H_{24}N_2O_2$ 2-[4-(Azepan-1-yl-aminomethyl)- phenyl]-4H-benzopyrane-4-one	C, H, N
IVc		51.2	231	$C_{21}H_{22}N_2O_2$ 2-[3-(Piperidine-1-yl-aminomethyl)- phenyl]-4H-benzopyrane-4-one	C, H, N
IVd		42.5	223	$C_{22}H_{24}N_2O_2$ 2-[3-(Azepan-1-yl-aminomethyl)- phenyl]-4H-benzopyrane-4-one	C, H, N

Results and Discussion

The general method which is known as Baker-Venkataraman (Cramer and Elsching, 1956) was used to prepare 3'- and 4'-methyl flavone (**Ia-b**). The methyl group of the flavone was converted to bromomethyl with N-bromosuccinimide and catalytic amount of benzoyl peroxide. Derivatives **IVa-d** were synthesized starting with bromomethylflavone (**IIa-b**) and treating with the appropriate amine derivatives (**IIIa-b**) in the presence of Na_2CO_3/abs.EtOH (Scheme, Table 1). IR spectra of the compounds showed γ-pyrone C=O stretching bands at 1632-1648 cm^{-1}. In the ^1H NMR spectra, characteristic flavone protons were observed between 6.79-8.66 ppm. In the mass spectra, the compounds **IVb-c** have molecular (M^+) ion peaks and the compounds **IVa** and **IVd** have M-1, M-15 ion peak respectively. Some spectral data of the compounds are given in Table 2.

Table 2. Spectral data of **IVa-d**.

No	^{1}H NMR (δ ppm)	MS (70 eV) m/z	IR (cm^{-1}) (γ pyrone CO)
IVa	1.15-1.90 (m, 6H, a,b), 3.30-3.50 (m, 4H, c), 5.25 (s, 2H, CH_2), 5.85 (s, 1H, NH), 6.83 (s, 1H, 3-H), 7.43 (ddd, 1H, 6-H), 7.57 (d, 1H, $j_{8,7}$=7.88 Hz, 8-H), 7.72 (ddd, 1H, 7-H), 7.91 (d, 2H, $j_{3',2'}=j_{5',6'}$=8.36 Hz, 3',5'-H), 8.01 (d, 2H, $j_{2',3'}=j_{6',5'}$=8.36 Hz, 2',6'-H), 8.21 (dd, 1H, $j_{5,6}$=7.94 Hz, $j_{5,7}$=1.60 Hz, 5-H).	333 (M-1), 332 (M-2), 235, 220, 121, 120, 102, 101, 92, 64, 63, 41(100%)	1648
IVb	1.67 (t, 4H, a), 1.78-2.07 (m, 4H, b), 3.53-3.73 (m, 4H, c), 4.96 (s, 2H, CH_2), 5.89 (s, 1H, NH), 6.80 (s, 1H, 3-H), 7.39 (ddd, 1H, 6-H), 7.55 (d, 1H, $j_{8,7}$=8.28 Hz, 8-H), 7.69 (ddd, 1H, $j_{7,8}=j_{7,6}$=8.58 Hz, 7-H), 7.83 (d, 2H, $j_{3',2'}=j_{5',6'}$=8.28 Hz, 3',5'-H), 7.97 (d, 2H, $j_{2',3'}=j_{6',5'}$=8.28 Hz, 2',6'-H), 8.08 (dd, 1H, $j_{5,6}$=7.92 Hz, $j_{5,7}$=1.45 Hz, 5-H).	348 (M^+), 236, 221, 121, 120, 93, 92, 63, 42 (100%)	1633
IVc	1.15-1.77 (m, 6H, a,b), 3.51-3.57 (m, 4H, c), 4.91 (s, 2H, CH_2), 5.63 (s, 1H, NH), 6.87 (s, 1H, 3-H), 7.39 (ddd, 1H, 6-H), 7.56-7.62 (m, 2H, 4',6'-H), 7.69 (ddd, 1H, 7-H), 7.77 (d, 1H, $j_{8,7}$=7.68 Hz, 8-H), 8.03 (d, 1H, j_o=7.98 Hz, 5'-H), 8.08 (dd, 1H, $j_{5,6}$=7.94 Hz, $j_{5,7}$=1.42 Hz, 5-H), 8.29 (s, 1H, 2'-H).	334 (M^+), 236, 221, 220, 121, 92, 63, 41(100%)	1632
IVd	1.81 (t, 4H, a), 1.96-2.29 (m, 4H, b), 3.91-4.01 (m, 4H, c), 5.28 (s, 2H, CH_2), 6.27 (s, 1H, NH), 6.79 (s, 1H, 3-H), 7.36 (ddd, 1H, 6-H), 7.47-7.53 (m, 2H, 4',6'-H), 7.65 (ddd, 1H, 7-H), 7.81 (d, 1H, $j_{8,7}$=8.00 Hz, 8-H), 7.92 (d, 1H, j_o=7.92 Hz, 5'-H), 7.97 (dd, 1H, $j_{5,6}$=7.93 Hz, $j_{5,7}$=1.42 Hz, 5-H), 8.66 (s, 1H, 2'-H).	333 (M-15), 236, 234, 221, 121, 120, 92, 63, 41(100%)	1638

All of the new compounds were tested for their antimicrobial activity by the agar diffusion method (Bauer *et al*, 1966), using *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* and comparing with miconazole and ceftriaxone (Table 3). The resulting inhibition zones against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* were 10-17 mm, 8-12 mm and 8-12 mm respectively. Only compound **IVa** showed high activity against *Candida albicans* (17 mm). Compounds **IVc**, **IVd** were inactive against *Candida albicans*, while **IVa** was inactive against *Staphylococcus aureus*.

Table 3. Antimicrobial activities [a)] of the compounds **IVa-d**.

Compound	*C. albicans*	*S. aureus*	*E. coli*
IVa	17	*	11
IVb	10	8	9
IVc	*	11	12
IVd	*	12	8
Miconazole	30	-	-
Ceftriaxone	-	18	20

[a)]Growth inhibition diameter (mm). No activity. -:Not tested

Özet

Bromometilflavon (**IIa-b**) ve amin türevlerinden (**IIIa-b**) hareketle yeni bir seri amin sübstitüe flavon bileşikleri (**IVa-d**) sentez edilmiştir. Elde edilen bu bileşiklerin in vitro ortamda antifungal ve antibakteriyal aktiviteleri denenmiş ve hepsi test edilen mikroorganizmalara karşı aktif bulunmuştur.

Acknowledgement: This work was supported by TUBITAK (Project No: SBAG-AYD-360)

References

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. (1966). Antibiotics susceptibility testing by a standardized single disk method. *Am. Clin. Pathol.* 45: 493-496.

Conti, C., Genovese, D., Santoro, R., Stein, M. L., Orsi, N. and Fiore, L. (1990). Activities and mechanism of action of halogen-substituted flavonoids against poliovirus type 2 infection in vitro. *Antimic. Agents Chemother.* 34 (3): 460-466.

Cramer, F. and Elsching, G. H. (1956). Über Einsschluss-verbindungen, IX. Mitteil.:Die blauen jodverbundungen der flavone. *Chem. Ber.* 89: 1-12.

Das, M. and Ray, P. K. (1988). Lipid antioxidant properties of quercetin in vitro. *Biochemistry International* 17(2): 203-209.

Gabor, M. (1981). The Effect of O-(β-Hydroxyethyl)-rutosides (HR) on the skin capillary resistance of rats. *Arzneim.-Forsch./Drug, Res.* 31 (3): 442-445.

Mabry, T. J., Markham, K. R. And Thomas, M. B. (1970). *The Systematic Identification of Flavonoids, Springer Verlag, Berlin-Heidelberg, NY* 83.

Mori, A., Nishino, C., Enoki, N. And Tawata, S. (1987). Antibacterial activity and mode of action of plant flavonoids against *proteus vulgaris* and *staphylococcus aureus*. *Phytochemistry* 26: 2231-2234.

Nardi, D., Leonardi, A., Pennini, R., Tajana, A. and Cazzulani, R. (1993). New basics esters of 2-phenyl-3-methyl-4-oxo-4H-1-benzopyran-8-carboxylic acid endowed with spasmolytic properties. *Arzneim.-Forsch./Drug Res.* 43 (1): 28-34.

Omar, A. M. M. E., Salama, H. M. and Eshba, N. H. (1984). Novel thiazolidine-2,4-dione-4-thiosemicarbazone and 4-[(3,4-diaryl-3H-thiazolyl)azo]thiazolidine-2-one derivatives: synthesis and evaluation for antimicrobial and anticancer properties. *Il Farmaco-Ed.Sc.* 40(1): 49-57.

Perry, N. B. and Foster, L. M. (1994). Antiviral and antifungal flavonoids, plus a triterpene from Hebe cupressoides. *Planta Medica* 60: 491-492.

Tunçbilek, M. and Ertan, R. (1999). Synthesis of some 4-[3' or 4'-(4H-4-oxo-1-benzopyran-2-yl)phenyl]-1,4-dihydropyridine derivatives as potential calcium channel antagonists. *Pharmazie* 54: 255-259

Zaharko, D. S., Grieshaber, C. K., Plowman, J. and Cradock, J. C. (1986). Therapeutic and pharmacokinetic relationships of flavone acetic acid: an agent with activity against solid tumors. *Cancer Treatment Reports* 70 (12): 1415-1421.

Accepted: 03.05.2002