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Flavone and xanthone derivatives related to fluoroquinolones

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Abstract

A number of flavone and xanthone derivatives bearing some characteristic features of fluoroquinolones such as the fluorine atom and an *ortho* piperazine ring are described. The new compounds have been tested for possible cytotoxic and antimicrobial activities. Cytotoxicity of both groups of compounds is rather poor, while the antibacterial activity is restricted to xanthones.

Keywords: Flavone; Xanthen-9-one; Fluoroquinolones; Cytotoxicity; Antimicrobial activity

1. Introduction

The O-heterocycles flavone and xanthone, parent compounds of a large number of naturally occurring as well as synthetic derivatives, occupy a prominent position in medicinal chemistry [1–6]. The outstanding variety of their biological properties (of flavonoids especially) has made these two prototypes noteworthy substrates in pharmaceutical research.

As the medicinal chemistry of these two heterocycles has interested researchers for some time [7], we report an attempt to associate their structure with some characteristic features of antimicrobial fluoroquinolones **1** [8,9], such as the fluorine atom and the *ortho* basic group, especially of the piperazine type, as shown in Scheme 1.

The purpose of this research was to provide more information about the relationships between flavone and quinolone antibacterial activity as pointed out by Hilliard et al. [10].

A very valid reference on this topic is the paper of Radl [11], describing two new interesting tricyclic fluoroquinolone-type derivatives (i.e. the *N*-cyclopropylmethyl-1-hydroxy-6-(4-methyl-1-piperazinyl)-7-fluoroacridin-9*H*-(10*H*)-one and the 1-hydroxy-6-(4-methyl-1-piperazinyl)-7-fluoro-9*H*-xanthen-9-one), in which the carboxylic function is present in a sort of vinylog form.

Furthermore, greater interest in the antimitotic properties of flavones [6] and some types of fluoroquinolones [9] prompted us to test the new synthesized derivatives as possible cytotoxic agents.

2. Chemistry

The 2-hydroxy-4-chloro-5-fluoroacetophenone (**5**) as well as its immediate precursor, 4-chloro-5-fluorophenol (**4**), are the two basic intermediates as illustrated in Scheme 2. The key intermediates **9** and the regioisomers **7** and **8** were prepared according to known methods: the former by Baker–Venkataraman acylation [12,13] of **5** and the latter two via diarylether **6** [14], which through intramolecular acylation gives rise to the regioisomers **7** and **8** in a 1:2 ratio. The final compounds were obtained by reacting the previous intermediates with the selected base.

3. Biological assay

Cytotoxic activity of the new compounds was tested at the Oncology Division of Boehringer-Mannheim Italy.

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Cytotoxicity has been examined in the human colon adenocarcinoma HT29 cell line according to the Mosmann method (MTT colorimetric assay) [15,16]. All compounds showed poor cytotoxicity: the IC_{50} values are on average 10^2–10^3 lower than the reference compounds mitoxantrone and daunorubicine (8.6×10^{-4} and 9×10^{-3} μg/ml, respectively).

4. Antimicrobial activity

The in vitro antibacterial activity of synthesized compounds **2a**–**e**, **3a**–**e** and **4a**–**e** was investigated against representative Gram positive and Gram negative bacteria such as *Escherichia coli* ATCC11105, *Proteus mirabilis* M81, *Streptococcus faecalis* SF 8043, and *Staphylococcus aureus* ATCC 6538/P. Minimum inhibitory concentrations (MIC μg/ml) were determined by the agar dilution method culturing all strains on Mueller–Hinton agar (Difco, Detroit, MI, USA).

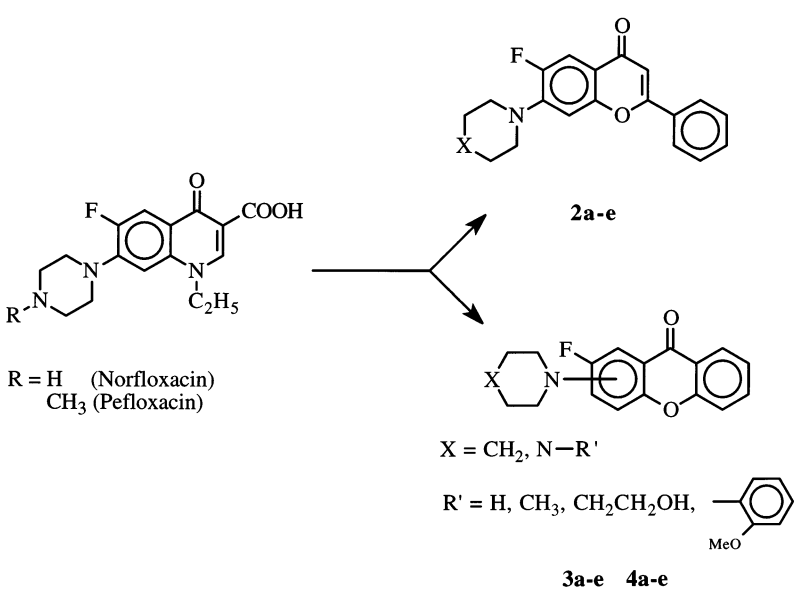
5. Results

The results obtained failed to meet our expectations. Cytotoxicity is poor in both flavone and xanthone derivatives. Antimicrobial activity, however, is more discriminative: flavone derivatives (**2a**–**e**) were inactive on both selected representative Gram positive and Gram negative pathogen strains (MIC > 50 μg/ml against all the strains), while the xanthone derivatives showed some activity. In the **3a**–**e** series, compounds **3a** and **3b** exhibited activity against *S. aureus* (MIC = 50 μg/ml), while they were ineffective against the Gram positive *S. faecalis* (MIC > 50 μg/ml) and the Gram negative strains. Concerning the action of **4a**–**e** compounds, **4b** exhibited the highest activity (MIC = 50 μg/ml) against *E. coli*, *S. faecalis* and *S. aureus* but was inactive against *P. mirabilis* (MIC > 50 μg/ml). Compound **4c** showed some activity only against *S. aureus* (MIC = 50 μg/ml).

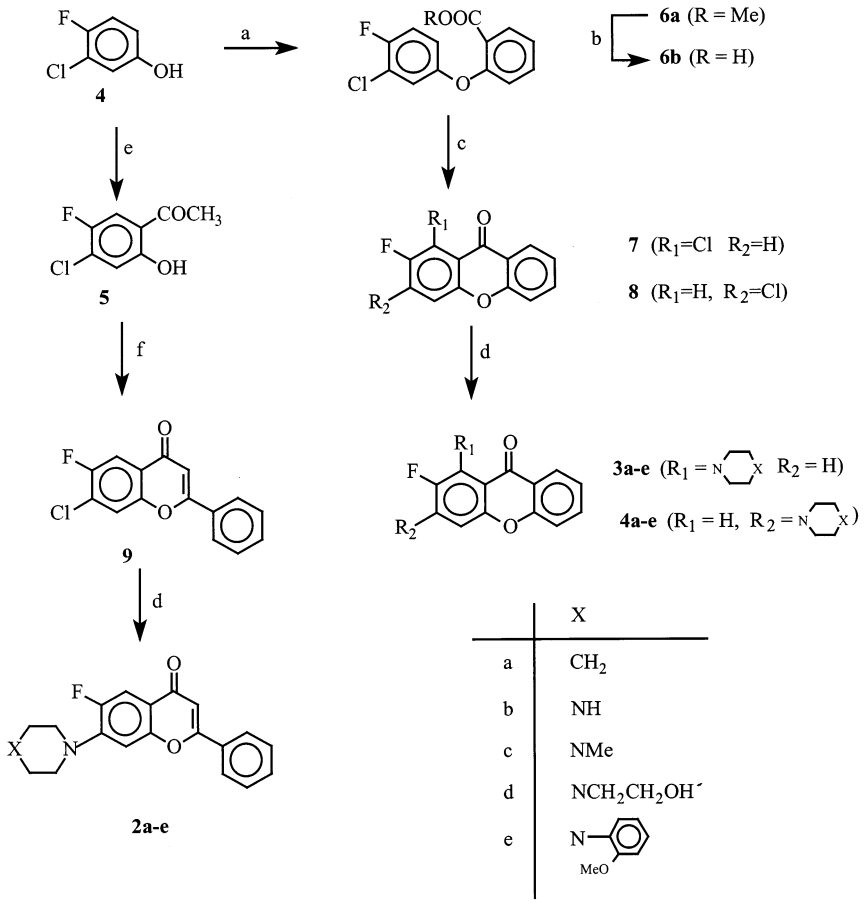
On the whole, these results (in particular the lack of antibacterial activity of flavone derivatives) have been rather disappointing. It may be baffling that two qualifying features of quinolones, the fluorine atom and the piperazine ring, work negatively against potential antimicrobial activity of flavones. The effectiveness of the above substituents seems to be linked to the structure of the heteroring: it is utmost in fluoroquinolones, but is also remarkable in default of the 3-carboxy group [9] as well as in derivatives in which the ring nitrogen has been substituted by sulfur [17]. These replacements only partly violate the prerequisites of the heteroring of the parent compounds and are not detrimental. On the contrary, the contemporary lack of these three features, as in **2a**–**e** compounds causes the observed loss of antibacterial activity.

6. Experimental

A Büchi apparatus and open glass capillaries were used to determine all melting points (which are not corrected). Wherever analyses are only indicated with element symbols, analytical results obtained for those elements are within ±0.4% of the theoretical values. The NMR spectra were recorded in the stated solvent $CDCl_3$ on a Varian Gemini 300 spectrometer, with TMS as the internal standard.



Scheme 1.



Scheme 2. Reagents: (a) methyl 2-iodobenzoate, Cu_2O, DMA, reflux 18 h; (b) AcOH–HBr (1:1) reflux 1 h; (c) PPA 100°C 1 h; (d) HNR_2 100°C overnight; (e) $AlCl_3$ 150°C 1 h; (f) i. PhCOCl, K_2CO_3, reflux, 20 h; ii. AcOH, H_2SO_4 reflux 15 h.

*6.1. 2-Hydroxy-4-chloro-5-fluoroacetophenone (**5**)*

A mixture of 20 g (0.11 mol) of 1-acetoxy-3-chloro-5-fluorobenzene (white solid m.p. 52–53°C from 3-chloro-4-fluorophenol and acetyl chloride) and 50 g $AlCl_3$ was heated for 1 h at 150°C. After decomposing with ice and conc. HCl, the product was extracted with diethyl ether. The organic layer was washed with water, 5% $NaHCO_3$ solution and then dried. Removing the solvent left the crude product which on crystallizing from petroleum ether, gave 18 g (90% yield) of white solid m.p. 82–85°C. ^{1}H NMR: δ 2.6 (s, 3H, CH_3), 7.1 (d, $J = 8.95$ Hz, 1H), 7.42 (d, $J = 6.27$ Hz, 1H), 12.4 (s, 1H). *Anal.* ($C_8H_6ClFO_2$): C, H.

*6.2. 2-Phenyl-6-fluoro-7-chloro-4H-1-benzopyran-4-one (**9**)*

To a mixture of **5** (10 g, 0.05 mol) and 30 g K_2CO_3 in 150 ml acetone, 7.5 g (0.053 mol) of benzoyl chloride were added dropwise. The mixture was refluxed under stirring for 20 h. After cooling, the mixture was filtered and the isolated yellow solid was washed with acetone and then stirred in water for 1 h. The crude diketone was isolated by filtration and dried. On crystallizing from ethanol 9.5 g (58%) of yellow crystalline 2-benzoyl-2′-hydroxy-4′-chloro-5′-fluoro-acetophenone, m.p. 160–162°C, were obtained.

The above β-diketone in 120 ml AcOH with 3 ml conc. H_2SO_4 was refluxed for 15 min. After cooling the reaction mixture was poured into water and the separated solid isolated by filtration. On crystallizing the crude product from ethanol 7.8 g (53%) of white crystalline solid m.p. 203–205°C were obtained. ^{1}H NMR: δ 6.8 (s, 1H, H-3), 7.4–7.94 (m, 7H, Ar). *Anal.* ($C_{15}H_8ClFO_2$): C, H.

*6.3. 3-Chloro-4-fluoro-2′-methoxycarbonyldiphenyl ether (**6a**)*

A mixture of 3.65 g (0.025 mol) of 3-chloro-4-fluorophenol (**4**), 6.78 g (0.025 mol) methyl *o*-iodobenzoate and 1.8 g copper(I) oxide in 40 ml DMA was refluxed for 18 h under N_2 and then hot filtered. Removal of the solvent left a residue which was treated with 50 ml 1 N HCl and extracted with methylene chloride. The organic layer was washed twice with 10% NaOH, water and dried. Evaporation of the solvent afforded 5.6 g of an oil which, without purification, was employed in the subsequent reaction.

*6.4. 3-Chloro-4-fluoro-2′-carboxyldiphenyl ether (**6b**)*

The above product (5.6 g, 0.02 mol) was refluxed in 30 ml of a 1:1 mixture of acetic acid and 48% hydrobromic acid for 1 h. The cooled solution was poured into water and the separated solid collected by filtration. The crude product (4.5 g) on crystallizing from cyclohexane gave 4.2 g (80%) of white solid m.p. 113–114°C. ^{1}H NMR: δ 6.9–8.1 (m, 7H), 11.6 (s, 1H). *Anal.* ($C_{13}H_8ClFO_3$): C, H.

*6.5. 1-Chloro-2-fluoroxanthen-9-one (**7**) and 2-fluoro-3-chloroxanthen-9-one (**8**)*

Compound **6b** (2.66 g, 0.01 mol) in 50 g PPA was stirred at 100–110°C for 1 h. On pouring the reaction mixture into water the separated solid was isolated by filtration. The collected solid was digested for 1 h in 5% $NaHCO_3$ solution and then recovered by filtration. The crude product is a mixture in 1:2 ratio of the two regioisomers **7** and **8** which were separated by column chromatography using a 9:1 mixture of petroleum ether and ethyl acetate: the first fraction contains the 2-fluoro-3-chloroxanthen-9-one (**8**) 1.66 g (67%) as white crystalline product m.p. 170–172°C (ethanol). ^{1}H NMR: δ 7.3–7.82 (m, 4H), 8.02 (d, $J = 8.51$ Hz, 1H), 8.28 (d, $J = 7.77$ Hz, 1H). *Anal.* ($C_{13}H_6ClFO_2$): C, H.

The second fraction consisted of the 1-chloro-2-fluoroxanthen-9-one (**7**), 0.8 g (33%) as a white crystalline product m.p. 140–142°C (ethanol). ^{1}H NMR: δ 7.3–7.56 (m, 4H), 7.7 (m, 1H), 8.24 (d, $J = 8.69$ Hz, 1H). *Anal.* ($C_{13}H_6ClFO_2$): C, H.

*6.6. 2-Phenyl-6-fluoro-7-piperidinyl-4H-1-benzopyran-4-one (**2a**)*

A mixture of 0.5 g (0.0018 mol) of **9** and 3 ml piperidine was stirred at 100°C overnight. On pouring the reaction mixture into water the separated solid was filtered, washed with water and dried. The crude product on crystallizing from ethanol gave 0.4 g (68%) of yellow crystalline solid m.p. 128–131°C. ^{1}H NMR: δ 1.85 (m, 6H), 3.22 (m, 4H), 6.75 (s, 1H, H-3), 6.94–7.95 (m, 7H, Ar). *Anal.* ($C_{20}H_{18}FNO_2$): C, H, N.

With the same procedure the following compounds were prepared.

*6.7. 2-Phenyl-6-fluoro-7-piperazinyl-4H-1-benzopyran-4-one (**2b**)*

60% yield of yellow crystalline product m.p. 158–160°C (ethyl acetate). ^{1}H NMR: δ 3.1 (m, 4H), 3.2 (m, 4H), 6.75 (s, 1H, H-3), 6.9–8 (m, 7H, Ar). *Anal.* ($C_{19}H_{17}FN_2O_2$): C, H, N.

*6.8. 2-Phenyl-6-fluoro-7-(4-methyl-1-piperazinyl)-4H-1-benzopyran-4-one (**2c**)*

65% yield of yellow crystalline product m.p. 155–158°C (ethanol). ^{1}H NMR: δ 2.4 (s, 3H), 2.7 (m, 4H), 3.35 (m, 4H), 6.75 (s, 1H, H-3), 6.95–7.95 (m, 7H, Ar). *Anal.* ($C_{20}H_{19}FN_2O_2$): C, H, N.

*6.9. 2-Phenyl-6-fluoro-7-(4-hydroxyethyl-1-piperazinyl)-4H-1-benzopyran-4-one (**2d**)*

72% yield of yellow crystalline product m.p. 220°C (dec.) (ethanol). ^{1}H NMR: δ 2.62 (m, 2H), 2.7 (m, 4H), 3.3 (m, 4H), 3.7 (m, 2H), 6.74 (s, 1H, H-3), 6.94–7.95 (m, 7H, Ar). *Anal.* ($C_{21}H_{21}FN_2O_3$): C, H, N.

*6.10. 2-Phenyl-6-fluoro-7-(4-o-methoxyphenyl-1-piperazinyl)-4H-1-benzopyran-4-one (**2e**)*

70% yield of yellow crystalline product m.p. 204–206°C (ethyl acetate). ^{1}H NMR: δ 3.26 (m, 4H), 3.48 (m, 4H), 3.9 (s, 3H), 6.78 (s, 1H, H-3), 6.92–7.95 (m, 7H, Ar). *Anal.* ($C_{26}H_{23}FN_2O_3$): C, H, N.

*6.11. 1-Piperidinyl-2-fluoroxanthen-9-one (**3a**)*

85% yield of yellow solid m.p. 156–157°C (ethanol). ^{1}H NMR: δ 1.76 (m, 6H), 3.22 (m, 4H), 6.84–8.32 (m, 6H, Ar). *Anal.* ($C_{18}H_{16}FNO_2$): C, H, N.

*6.12. 1-Piperazinyl-2-fluoroxanthen-9-one (**3b**)*

40% yield of yellow solid m.p. 145–146°C (ethanol). ^{1}H NMR: δ 3.12 (m, 4H), 3.30 (m, 4H), 6.94–8.3 (m, 6H, Ar). *Anal.* ($C_{17}H_{15}FN_2O_2$): C, H, N.

*6.13. 1-(4-Methyl-1-piperazinyl)-2-fluoroxanthen-9-one (**3c**)*

50% yield of yellow solid m.p. 162–165°C (ethanol). ^{1}H NMR: δ 2.4 (s, 3H, CH_3), 2.68 (m, 4H), 3.38 (m, 4H), 6.96–8.36 (m, 6H, Ar). *Anal.* ($C_{18}H_{17}FN_2O_2$): C, H, N.

*6.14. 1-(4-Hydroxyethyl-1-piperazinyl)-2-fluoroxanthen-9-one (**3d**)*

65% yield of yellow solid m.p. 118–120°C (ethanol). ^{1}H NMR: δ 2.64 (m, 2H), 2.76 (m, 4H), 3.36 (m, 4H), 3.66 (m, 2H), 6.96–8.36 (m, 6H, Ar). *Anal.* ($C_{19}H_{19}FN_2O_3$): C, H, N.

*6.15. 1-(4-o-Methoxyphenyl-1-piperazinyl)-2-fluoroxanthen-9-one (**3e**)*

65% yield of yellow solid m.p. 132–135°C (ethanol).

^{1}H NMR: δ 3.36 (m, 4H), 3.58 (m, 4H), 3.9 (s, 3H, OCH_3), 6.92–8.38 (m, 6H, Ar). *Anal.* ($C_{24}H_{21}FN_2O_3$): C, H, N.

*6.16. 2-Fluoro-3-piperidinylxanthen-9-one (**4a**)*

70% yield of yellow solid m.p. 138–141°C (ethanol). ^{1}H NMR: δ 1.8 (m, 6H), 3.3 (m, 4H), 6.9–8.36 (m, 6H, Ar). *Anal.* ($C_{18}H_{16}FNO_2$): C, H, N.

*6.17. 2-Fluoro-3-piperazinylxanthen-9-one (**4b**)*

60% yield of yellow solid m.p. 182–185°C (ethanol). ^{1}H NMR: δ 3.05 (m, 4H), 3.3 (m, 4H), 6.8–8.36 (m, 6H, Ar). *Anal.* ($C_{17}H_{15}FN_2O_2$): C, H, N.

*6.18. 2-Fluoro-3-(4-methyl-1-piperazinyl)xanthen-9-one (**4c**)*

65% yield of yellow solid m.p. 110–113°C (aqueous ethanol). ^{1}H NMR: δ 2.38 (s, 3H, CH_3), 2.62 (m, 4H), 3.32 (m, 4H), 6.8–8.38 (m, 6H, Ar). *Anal.* ($C_{18}H_{17}FN_2O_2$): C, H, N.

*6.19. 2-Fluoro-3-(4-hydroxyethyl-1-piperazinyl)-xanthen-9-one (**4d**)*

75% yield of yellow solid m.p. 132–135°C (ethanol). ^{1}H NMR: δ 2.68 (m, 2H), 2.76 (m, 4H), 3.36 (m, 4H), 3.68 (m, 2H), 6.8–8.36 (m, 6H, Ar). *Anal.* ($C_{19}H_{19}FN_2O_3$): C, H, N.

*6.20. 2-Fluoro-3-(4-o-methoxyphenyl-1-piperazinyl)-xanthen-9-one (**4e**)*

70% yield of yellow solid m.p. 132–133°C (ethanol). ^{1}H NMR: δ 3.24 (m, 4H), 3.52 (m, 4H), 3.9 (s, 3H, OCH_3), 6.85–8.36 (m, 6H, Ar). *Anal.* ($C_{24}H_{21}FN_2O_3$): C, H, N.

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