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Original article

Synthesis and in vitro inhibitory activity on human platelet aggregation of novel properly substituted 4-(1-piperazinyl)coumarins

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Abstract

Pursuing our chemical and biological studies in this field, we described the multistep preparation of the new 5-, 6-, or 7-alkoxy and 7-alkoxy-8-methyl substituted 4-(1-piperazinyl)coumarins **5d-v**, as well as the in vitro evaluation of their inhibitory activity on human platelet aggregation induced in platelet-rich plasma by ADP, collagen or the Ca^{2+} ionophore A23187. Compounds **5h-j,p,r-u** showed notably high activity towards all the platelet aggregation inducers used, and the most active one, 8-methyl-4-(1-piperazinyl)-7-(3-pyridylmethoxy)coumarin (**5t**), proved to be a potent in vitro antiplatelet agent.

Keywords: Substituted 4-(1-piperazinyl)coumarins; Synthesis; Antiplatelet activity; Structure-activity relationships

1. Introduction

In a number of previous papers we described the syntheses and human platelet aggregation in vitro inhibitory properties of substituted 2-aminochromones **1** [1–3], their benzo-fused derivatives **2-4** [3,4], and the corresponding 4-amino-coumarin derivatives **5-8** [3,4], as well as of the 1,2-fused pyrimidine derivatives **9-11** [4–7] and **12-14** [6], isosteric analogues of compounds **1-3** and **5-7**, respectively. Also several compounds **15**, whose structures were derived from that of the very active compound **9a** [6] (Table 1) by properly modifying its pyridine ring and/or 2-substituent, were synthesized and tested for their antiplatelet activity [4,5,7] (Fig. 1).

Considering the antiplatelet activity data afforded by the compounds **1-15** tested in the above studies, towards all the platelet aggregation inducers employed [adenosine diphosphate (ADP), collagen, and the Ca^{2+} ionophore A23187], the following remarks can be made.

- In each structural class 1-piperazinyl was the most effective amino substituent among all those used. In the case of compounds **12-14** the (1-piperazinyl)derivatives were not obtainable, and the few derivatives prepared showed low or very low activity [6].
- The impressive activity difference between compounds **9a** [6] and **15b** [7] (Table 1) seems to confirm the great importance for the activity, in this structural field, of the characteristic β-enaminonic moiety.
- Nevertheless, the activity of the β-enaminonic (1-piperazinyl)derivatives **1-11, 15** is appreciably influenced by the structure of the supporting cyclic system (Table 1).
- On the whole, the 7,8-disubstituted 4-(1-piperazinyl)coumarin **5a** [4] is clearly the most active of all compounds **1-15** previously studied by us, and the presence of proper substituents in the benzene ring proved to be very effective (see Table 1, compounds **5a, 5b** and **5c**).

In this connection, the antiplatelet activity data previously shown by the most active compounds **1-11, 15** [i.e. the (1-piperazinyl) substituted derivatives **1a-11a, 15a**] and by compounds **5b,c** and **15b** are summarized in Table 1.

Regarding the expected bioisosterism of the 2,3-fused pyran derivatives **1-3** and 1,2-fused pyrimidine derivatives **9-11**, it is interesting to point out that both 2-(diethylamino)-7-hydroxychromone [8,9], chosen as an example of compounds **1**, and 2-(1-piperazinyl)-4*H*-pyrido[1,2-*a*]pyrimidin-

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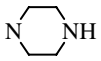
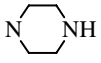
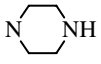
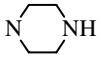
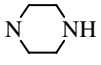
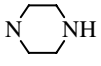
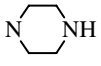
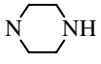
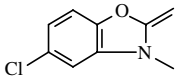
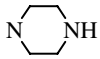
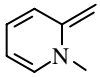
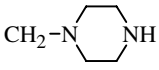
Table 1
In vitro inhibitory activity of some significant previously described[a] compounds **1-11, 15** (Figure 1) on human platelet aggregation induced in PRP[b] by ADP, collagen and A23187 (see Introduction)

Compd	NRR[1]	R[2]	R[3]	X		Y	IC_{50} (μM)±SD		
							ADP (5.0 μM)	Collagen (5.0 μg mL^{-1})[c]	A23187 (20.0 μM)
1a		H	H	-	-	-	56±13	60±12	240±90
2a		-	-	-	-	-	39±3	56±16	201±61
3a		-	-	-	-	-	119±40	117±32	278±15
4a		H	-	-	-	-	105±30	159±31	180±18
5a		7-OCH_2 	8-CH_3	-	-	-	1.9±0.2	1.8±0.4	1.1±0.2
5b		7-$OCH(CH_3)_2$	H	-	-	-	5.6±1.4	4.4±2.0	3.8±1.0
5c		H	H	-	-	-	49±14	36±10	250±38
6a		-	-	-	-	-	53±15	49±17	245±65
7a		-	-	-	-	-	81±23	71±27	334±89
8a		CH_3	-	-	-	-	24±4	31±14	32±8
9a		H	H	O	-	-	6±1.8	3.6±1.2	19±9
10a		-	-	-	-	-	13±4	15±8	28±8
11a		-	-	-	-	-	38±10	21±9	54±17
15a	-	-	-	-			3.6±1.2	8.8±5.6	13.0±5
15b	-	-	-	-			>1000	>1000	>1000

[a] Compounds **1a, 2a, 3a, 5c, 6a, 7a** : ref 3; compounds **4a, 5a,b, 8a, 15a** : ref 4; compounds **9a, 10a, 11a** : ref 6; compound **15b** : ref 7.
[b] PRP = platelet-rich plasma.
[c] 10.0 μg/mL for compounds **1a, 2a, 3a, 5c, 6a, 7a**.

4-one **9a** [6,10] (the most active among all compounds **1-4, 9-11** studied by us) proved to exert their platelet antiaggregating activity by specifically inhibiting the activity of high affinity cAMP phosphodiesterase and, consequently, increasing the intracellular cAMP concentration.

Starting from the results outlined above, we have now regarded it interesting to synthesize and test in vitro for their human platelet antiaggregating properties several novel 4-(1-piperazinyl)coumarins **5** suitably substituted in the benzene ring, in order to increase our knowledge of the structure-activity relationships in this structural class and, if possible, obtain further significantly active compounds.

2. Chemistry

The intermediates **17d,e,h-v, 18d,e,h-v, 19d-h,l,m,p-t**, and the final 4-(1-piperazinyl)coumarin derivatives **5i-k,n,o,u,v** and **5d-h,l,m,p-t** were prepared as illustrated in Scheme 1.

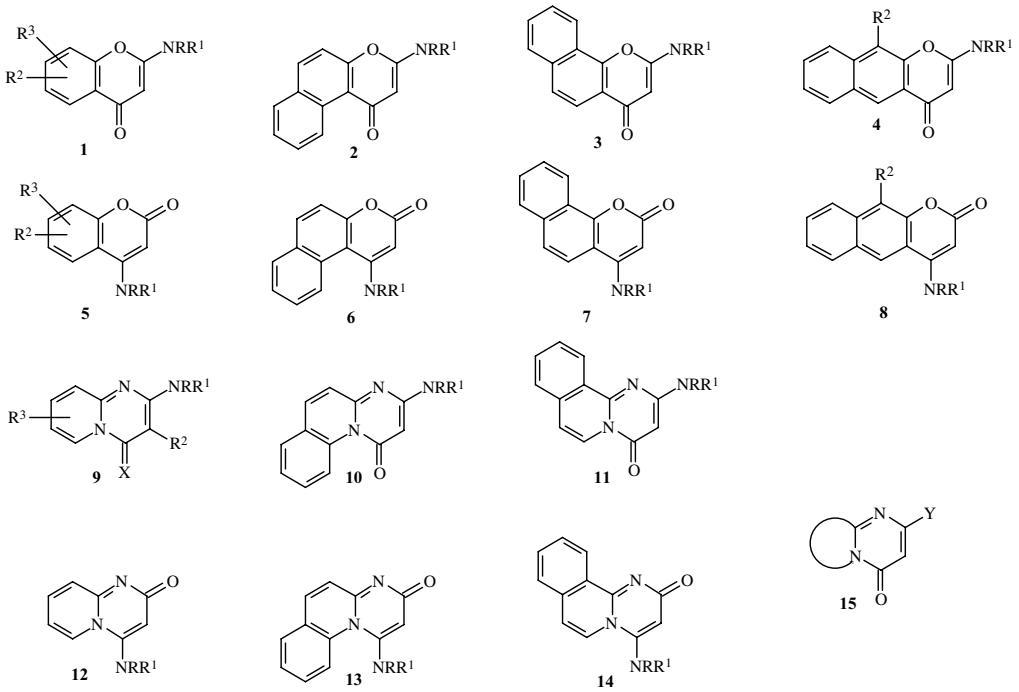


Fig. 1. Structures of 2-aminochromone and 4-aminocoumarin bicyclic and tricyclic derivatives **1-4** and **5-8**, pyrido[1,2-*a*]pyrimidine bicyclic and tricyclic derivatives **9-11** and **12-14**, and 1,2-fused pyrimidine derivatives **15**.

Thus, the treatment of the dihydroxyacetophenones **16a-c** with proper alkyl halides (anhydrous K_2CO_3 or KOH/anhydrous K_2CO_3, dry 2-butanone at reflux) afforded the corresponding alkoxyderivatives **17d,e,h-v**, whose cyclocondensation with diethyl carbonate in the presence of Na (Dowtherm A, 150°C) gave good yields of the substituted 4-hydroxycoumarins **18d,e,h-v**. Compounds **18i-k,n,o,u,v** were in turn heated (160°C) with a large excess of piperazine to give the corresponding 4-(1-piperazinyl)coumarin derivatives **5i-k,n,o,u,v**.

On the other hand, in the case of compounds **5d-h,l,m,p-t**, they were more conveniently prepared via the intermediate 4-chloroderivatives **19**. Actually, by heating (130°C) the 4-hydroxycoumarins **18d-h,l,m,p-t** with excess phosphorus oxychloride, in the presence of triethylamine, the 4-chloroderivatives **19d-h,l,m,p-t** were obtained, which were then treated with excess piperazine (ethanol, room temperature) to afford the above mentioned 4-(1-piperazinyl)coumarins **5**. The starting 4-hydroxycoumarin derivatives **18f** and **18g** were previously described in the literature [11].

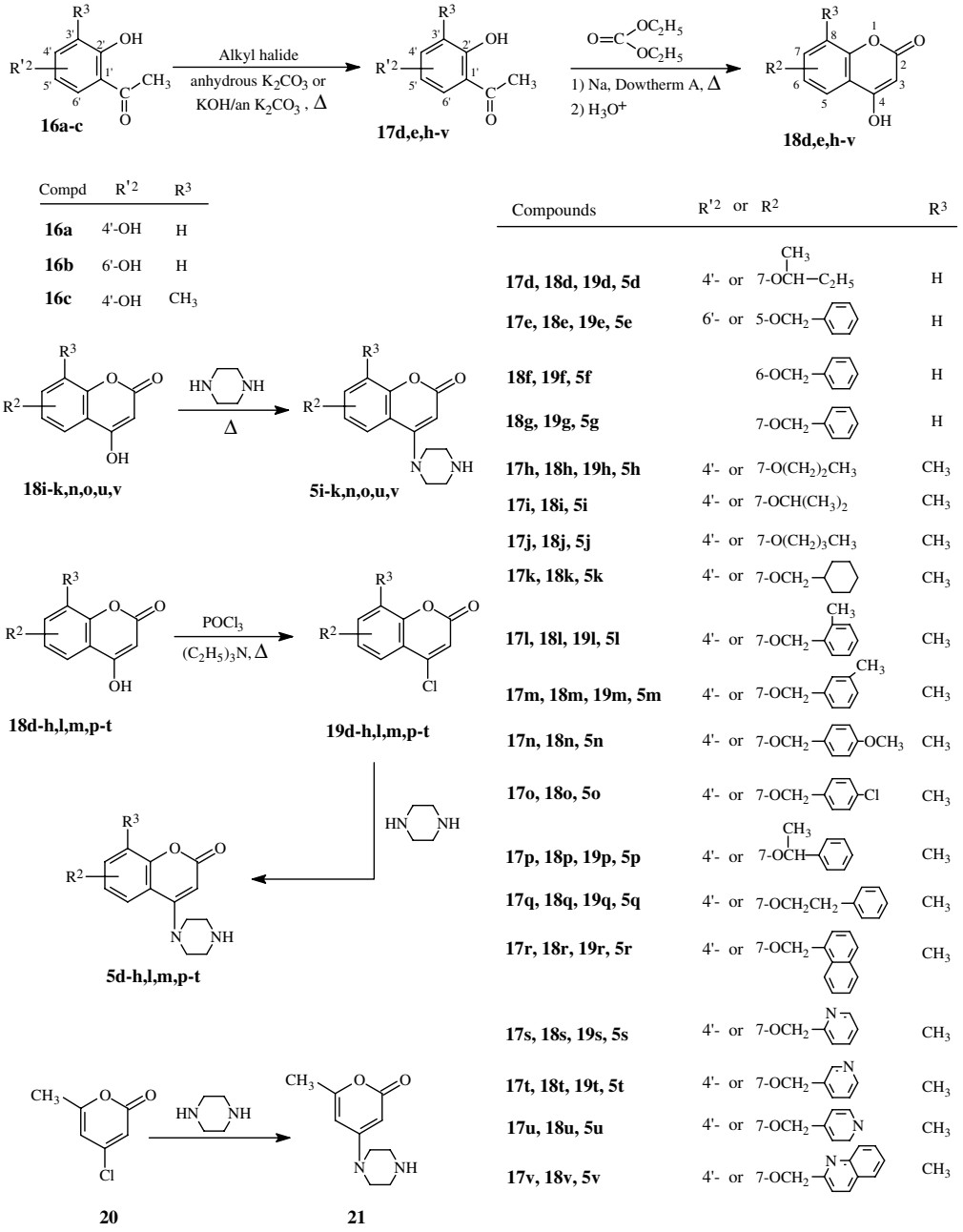
Finally, 6-methyl-4-(1-piperazinyl)-2*H*-pyran-2-one (**21**) was prepared by treating the corresponding 4-chloroderivative **20** [12] with an excess of piperazine (ethanol, room temperature) (see Scheme 1).

The structures attributed to the compounds described in this paper are supported by the results of elemental analyses, as well as by the IR and ^{1}H NMR spectral data (see Experimental) which agree with the ones previously reported by us for analogous compounds [3,4].

3. Biological results and discussion

The twenty novel compounds **5d-v** and **21** were tested in vitro for their inhibitory activity on the human platelet aggregation induced in platelet-rich plasma (PRP) by adenosine diphosphate (ADP), collagen, or the Ca^{2+} ionophore A23187 (calcimycin) (see Experimental). Acetylsalicylic acid (ASA), trifluoperazine and propranolol were also tested under the same conditions as reference compounds. The IC_{50} values obtained are reported in Table 2 and suggest the following observations on the structure-activity relationships (SAR) in this structural field, taking the 4-(1-piperazinyl)coumarin **5c** [3] as a lead (see Table 1).

- Concerning the 7-alkoxyderivatives of **5c**, it can be pointed out that, as we previously reported for the 7-ethoxy-4-(1-piperazinyl) [3] and 7-isopropoxy-4-(1-piperazinyl) [4] substituted coumarins **5** (see Table 1, compound **5b**), also the 7-(*sec*-butoxy) and 7-benzyloxy substituted 4-(1-piperazinyl)coumarins **5d** and **5g**, respectively, (Table 2) have now proved to be differently, but significantly more active than 4-(1-piperazinyl) coumarin **5c** (Table 1), towards all the platelet aggregation inducers used ($P< 0.0005$ for **5d** in the presence of all tested agonists ; $P< 0.0005$ in the presence of ADP and A23187, and $P< 0.0125$ in the presence of collagen, for **5g**). Compound **5b** [4] appears to be the most active one synthesized by us in this class of derivatives.
- In this connection, the antiplatelet activity data afforded by the 5-, 6-, and 7-benzyloxy substituted isomers **5e, 5f** and **5g**, respectively, seem to confirm that the 7-alkoxyderivative is the most effective one in order to increase

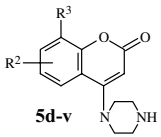


Scheme 1. Synthetic routes to the substituted 4-(1-piperazinyl)coumarins **5d-v** and 6-methyl-4-(1-piperazinyl)-2*H*-pyran-2-one **21**.

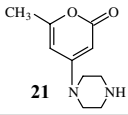
the activity of the lead compound **5c**, whose activity, on the contrary, can be strongly decreased by the presence of 5-alkoxy substituent (see **5c** and **5e**, Tables 1 and 2, respectively).

- On the other hand, the biological data until now obtained by us clearly indicate that the introduction of 8-methyl substituent usually increases, depending on the type of alkoxy substituent, the antiplatelet activity of 7-alkoxy derivatives of **5c**. For instance, the antiplatelet activity differences between compounds **5i** and **5b** [4] and between compounds **5a** [4] and **5g** (see Tables 1 and 2) correspond respectively, on the whole, to the lowest and the highest increase of activity observed by us between a 7-alkoxy-4-(1-piperazinyl)coumarin and its 8-methyl-derivative.
- Considering in detail the IC_{50} values now obtained for the 7-alkoxy-8-methyl derivatives of **5c** (**5h-v**) (Table 2), we can observe that both compounds **5h-k**, whose 7-substituents contain only alkyl or cycloalkyl moieties, and compounds **5l-q**, whose 7-substituents contain benzyl moieties differently substituted either in the phenyl ring or in the methylene group, generally show notable and in some cases (**5h-j,p**) very high antiplatelet activity, but always lower than that of the 7-benzyloxy-8-methyl-4-(1-piperazinyl)coumarin **5a** (Table 1). The isosteric replacement in compound **5a** of

Table 2
In vitro inhibitory activity of the new (1-piperazinyl) substituted compounds **5d-v, 21** on platelet aggregation induced in human PRP by ADP, collagen and A23187



5d-v



21

Compd	R2	R3	IC$_{50}$ (μM)±SD		
			ADP (5.0 μM)	Collagen (5.0 μg mL^{-1})	A23187 (20.0 μM)
5d	7-OCH(CH$_3$)–C$_2$H$_5$	H	9.3±5.4	18.0±3.1	29.5±5.1
5e	5-OCH$_2$–C$_6$H$_5$	H	187±42	144±40	429±105
5f	6-OCH$_2$–C$_6$H$_5$	H	23.6±4.7	34.2±3.3	116±12.3
5g	7-OCH$_2$–C$_6$H$_5$	H	16.3±4.1	24.2±5.1	78.8±10.3
5h	7-OCH$_2$CH$_2$CH$_3$	CH$_3$	2.95±0.78	2.58±0.46	7.77±1.36
5i	7-OCH(CH$_3$)$_2$	CH$_3$	2.4±0.5	3.2±0.7	4.4±1.8
5j	7-OCH$_2$CH$_2$CH$_2$CH$_3$	CH$_3$	2.79±1.30	1.92±0.96	5.03±1.02
5k	7-OCH$_2$–C$_6$H$_{11}$	CH$_3$	4.9±1.8	7.6±1.4	27.0±6.0
5l	7-OCH$_2$–C$_6$H$_4$(2-CH$_3$)	CH$_3$	9.8±4.2	11.7±2.6	26.8±8.2
5m	7-OCH$_2$–C$_6$H$_4$(3-CH$_3$)	CH$_3$	11.1±2.6	26.5±5.8	43.2±12.5
5n	7-OCH$_2$–C$_6$H$_4$(4-OCH$_3$)	CH$_3$	3.8±1.2	4.3±0.7	15.2±3.8
5o	7-OCH$_2$–C$_6$H$_4$(4-Cl)	CH$_3$	13.1±1.5	3.7±1.4	41.0±17.5
5p	7-OCH(CH$_3$)–C$_6$H$_5$	CH$_3$	2.2±0.8	3.2±0.8	11.6±2.9
5q	7-OCH$_2$CH$_2$–C$_6$H$_5$	CH$_3$	4.4±2.6	7.9±2.7	21.7±6.2
5r	7-OCH$_2$–C$_{10}$H$_7$	CH$_3$	6.6±1.4	6.3±2.8	6.9±1.0
5s	7-OCH$_2$–C$_5$H$_4$N(2)	CH$_3$	2.14±0.63	1.40±0.58	5.12±1.46
5t	7-OCH$_2$–C$_5$H$_4$N(3)	CH$_3$	0.98±0.36	0.51±0.12	1.42±0.43
5u	7-OCH$_2$–C$_5$H$_4$N(4)	CH$_3$	2.9±0.7	2.2±1.0	7.8±1.0
5v	7-OCH$_2$–C$_9$H$_6$N	CH$_3$	5.6±1.5	6.1±1.7	29.9±3.8
21	-	-	273±39	441±71	324±43
ASA			>1000	183±4	>1000
Trifluoperazine			180±48	138±41	318±61
Propranolol			291±76	131±31	269±62

the 7-benzyloxy with the three isomeric 7-(pyridylmethoxy) substituents afforded a group of novel very active antiplatelet agents (compounds **5s-u**), among which the 8-methyl-4-(1-piperazinyl)-7-(3-pyridylmethoxy)coumarin **5t**, clearly due to the particular electronic effect of the 3-pyridyl substituent, proved to be the overall most active in vitro antiplatelet agent until now synthesized by us, and significantly more active than **5a** in the presence of ADP and collagen ($P< 0.0005$).

- With reference to the steric hindrance of 7-alkoxy substituents of compounds **5l-v**, it can be pointed out that

compounds **5l-q** and the 7-(1-naphthylmethoxy) substituted compound **5r** (with bulkier 7-substituents) are all less active than their 7-benzyloxy analogue **5a**, as well as the 7-(2-quinolylmethoxy)derivative **5v** is less active than its 7-(2-pyridylmethoxy) analogue **5s**.

- Finally, the highly different potencies shown by the 4-(1-piperazinyl)-2*H*-pyran-2-one derivatives **21** (Table 2) and **5c** (Table 1) further confirm the importance of the supporting cyclic system for the compound antiplatelet activity in this structural class.

4. Conclusions

The biological results obtained for the compounds **5** described in this paper confirm the high effectiveness of 7-alkoxy-8-methyl-4-(1-piperazinyl)coumarins as in vitro antiplatelet agents, depending on the structure of the 7-substituent.

The now synthesized 8-methyl-4-(1-piperazinyl)-7-(3-pyridylmethoxy)coumarin **5t** proved to be a potent in vitro inhibitor of human platelet aggregation towards all the platelet aggregation inducers used, suggesting proper heteroarylmethoxy groups as novel useful 7-alkoxy substituents.

In this connection, further biological studies have been expressly carried out to investigate the mode of action of **5t** [13]. The results of these experiments have shown that **5t** inhibits human platelet aggregation by increasing intracellular cAMP levels through the specific inhibition of cAMP phosphodiesterase [13], as previously reported by us for 2-(diethylamino)-7-hydroxychromone [9] and 2-(1-piperazinyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one **9a** [10], studied as examples of compounds **1** and **9**, respectively (see Introduction); concerning this, compound **5t** has also proved to reduce intracellular calcium levels and protein kinase C (PKC) activation induced by thrombin [13]. In addition, the coumarin derivative **5t** increases the production of nitric oxide, well known antiaggregating compound, by directly stimulating the platelet nitric oxide synthase enzyme [13].

5. Experimental protocols

5.1. Chemistry

Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer ″Spectrum One″ spectrophotometer (abbreviations relative to IR bands: br = broad, s = strong, w = weak, sh = shoulder). ^{1}H-NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer and chemical shifts (δ) are reported in ppm using $(CH_3)_4Si$ as an internal reference (δ = 0). Spin multiplicities are given as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet). Analyses of all new compounds, indicated by the symbols of the elements, were within ± 0.4% of the theoretical values and were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Genoa.

Thin-layer chromatograms were run on Merck silica gel 60 F_{254} precoated plastic sheets (layer thickness 0.2 mm). Column chromatography was performed using Carlo Erba silica gel (0.05 - 0.20 mm) or Carlo Erba neutral aluminium oxide (Brockmann activity I).

*5.1.1. General procedure for the synthesis of substituted acetophenones **17d,e,h-v***

A mixture of 1-(2,4-dihydroxyphenyl)ethanone **16a** (3.80 g, 25.0 mmol), 1-(2,6-dihydroxyphenyl)ethanone **16b** (3.80 g, 25.0 mmol), or 1-(2,4-dihydroxy-3-methylphenyl)ethanone **16c** (4.15 g, 25.0 mmol), 25.0 mmol of the proper alkyl halide, 1.50 g of finely powdered KOH mixed with 4.0 g of anhydrous K_2CO_3 (in the case of preparation of compounds **17d,h-j** only 5.0 g of anhydrous K_2CO_3 were used) and 2-butanone (150 mL) was heated at reflux for 6 h, with stirring. The mixture was then poured into cold water (500 mL) after which, in many cases , the rough compound **17** separated out as a solid which was filtered, washed with water and dried: the pure compounds **17o** and **17v** were plainly obtained by crystallization of this solid from isopropyl ether. On the contrary, in the case of compounds **17d,h-j,s-u** an emulsion was obtained which was exhaustively extracted with ethyl ether-ethyl acetate (1:1): the combined extracts were then washed with water, dried (anhydrous Na_2SO_4), and solvents were removed under reduced pressure to give an oily or nearly solid residue. Both the solids recovered by filtration (except for **17o** and **17v**) and the residues obtained by extraction were then chromatographed on a silica gel column to recover pure compound **17**, generally eluting with dichloromethane-petroleum ether (1:1); in the only case of the (pyridylmethoxy)derivatives **17s-u** their elution was carried out with ethyl acetate, after discarding some impurities previously eluted with dichloromethane. The pure compounds **17** were finally crystallized from the proper solvent, or distilled in vacuo (**17d**). According to this procedure the following compounds were prepared (^{1}H-NMR and IR data are reported only for compound **17t**, chosen as an example).

*5.1.1.1. 1-[4-(sec-Butoxy)-2-hydroxyphenyl]ethanone (**17d**).* Obtained (3.39 g, 65%) from reaction of **16a** with 2-iodobutane (4.60 g); colourless liquid, b.p. 125-127°C (0.3 mm Hg). Anal. ($C_{12}H_{16}O_3$) C, H.

*5.1.1.2. 1-(6-Benzyloxy-2-hydroxyphenyl)ethanone (**17e**).* Obtained (4.12 g, 68%) from reaction of **16b** with benzyl chloride (3.16 g); white crystals, m.p. 109-110°C (isopropyl ether). Anal. ($C_{15}H_{14}O_3$) C, H.

*5.1.1.3. 1-(2-Hydroxy-3-methyl-4-propoxyphenyl)ethanone (**17h**).* Obtained (4.68 g, 90%) from reaction of **16c** with 1-iodopropane (4.25 g); white crystals, m.p. 36-37°C (petroleum ether). Anal. ($C_{12}H_{16}O_3$) C, H.

*5.1.1.4. 1-(2-Hydroxy-4-isopropoxy-3-methylphenyl)ethanone (**17i**).* Obtained (3.49 g, 67%) from reaction of **16c** with 2-iodopropane (4.25 g); white crystals, m.p. 60-61°C (petroleum ether). Anal. ($C_{12}H_{16}O_3$) C, H.

*5.1.1.5. 1-(4-Butoxy-2-hydroxy-3-methylphenyl)ethanone (**17j**).* Obtained (3.45 g, 62%) from reaction of **16c** with 1-iodobutane (4.60 g); white crystals, m.p. 52-53.5°C (petroleum ether). Anal. ($C_{13}H_{18}O_3$) C, H.

*5.1.1.6. 1-[4-(Cyclohexylmethoxy)-2-hydroxy-3-methylphenyl]ethanone (**17k**).* Obtained (1.18 g, 18%) from reaction of **16c** with (bromomethyl)cyclohexane (4.43 g); white crystals, m.p. 128-129°C (petroleum ether). Anal. ($C_{16}H_{22}O_3$) C, H.

*5.1.1.7. 1-[2-Hydroxy-3-methyl-4-(2-methylbenzyloxy)phenyl]ethanone (**17l**).* Obtained (3.12 g, 46%) from reaction of **16c** with 2-methylbenzyl chloride (3.51 g); white crystals, m.p. 111-112°C (isopropyl ether/petroleum ether). Anal. ($C_{17}H_{18}O_3$) C, H.

*5.1.1.8. 1-[2-Hydroxy-3-methyl-4-(3-methylbenzyloxy)phenyl]ethanone (**17m**).* Obtained (3.04 g, 45%) from reaction of **16c** with 3-methylbenzyl chloride (3.51 g); white crystals, m.p. 118-119°C (isopropyl ether). Anal. ($C_{17}H_{18}O_3$) C, H.

*5.1.1.9. 1-[2-Hydroxy-4-(4-methoxybenzyloxy)-3-methylphenyl]ethanone (**17n**).* Obtained (4.44 g, 62%) from reaction of **16c** with 4-methoxybenzyl chloride (3.91 g); white crystals, m.p. 128-129°C (isopropyl ether). Anal. ($C_{17}H_{18}O_4$) C, H.

*5.1.1.10. 1-[4-(4-Chlorobenzyloxy)-2-hydroxy-3-methylphenyl]ethanone (**17o**).* Obtained (4.65 g, 64%) from reaction of **16c** with 4-chlorobenzyl chloride (4.03 g); white crystals, m.p. 144-145°C (isopropyl ether). Anal. ($C_{16}H_{15}ClO_3$) C, H, Cl.

*5.1.1.11. 1-[2-Hydroxy-3-methyl-4-(1-phenylethoxy)phenyl] ethanone (**17p**).* Obtained (3.72 g, 55%) from reaction of **16c** with (1-bromoethyl)benzene (4.63 g); white crystals, m.p. 94-95°C (petroleum ether). Anal. ($C_{17}H_{18}O_3$) C, H.

*5.1.1.12. 1-[2-Hydroxy-3-methyl-4-(2-phenylethoxy)phenyl] ethanone (**17q**).* Obtained (1.35 g, 20%) from reaction of **16c** with (2-bromoethyl)benzene (4.63 g); white crystals, m.p. 93-94°C (petroleum ether). Anal. ($C_{17}H_{18}O_3$) C, H.

*5.1.1.13. 1-[2-Hydroxy-3-methyl-4-(1-naphthylmethoxy) phenyl]ethanone (**17r**).* Obtained (4.21 g, 55%) from reaction of **16c** with 1-(chloromethyl)naphthalene (4.42 g); white needles, m.p. 135-136°C (isopropyl ether). Anal. ($C_{20}H_{18}O_3$) C, H.

*5.1.1.14. 1-[2-Hydroxy-3-methyl-4-(2-pyridylmethoxy)phenyl]ethanone (**17s**).* Obtained (3.73 g, 58%) from reaction of **16c** with 2-(chloromethyl)pyridine hydrochloride (4.10 g); whitish crystals, m.p. 128-129°C (isopropyl ether). Anal. ($C_{15}H_{15}NO_3$) C, H, N.

*5.1.1.15. 1-[2-Hydroxy-3-methyl-4-(3-pyridylmethoxy)phenyl]ethanone (**17t**).* Obtained (2.64 g, 41%) from reaction of **16c** with 3-(chloromethyl)pyridine hydrochloride (4.10 g); white crystals, m.p. 122-123°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 2.15 (s, 3H, 3-CH_3), 2.57 (s, 3H, $COCH_3$), 5.17 (s, 2H, O$CH_2C_5H_4N$), 6.50 (d of AB q, J = 9 Hz, 1H, H-5), 7.32 (m, 1H, pyridyl H-5'), 7.60 (d of AB q, J = 9 Hz, 1H, H-6), 7.79 (near d, 1H, pyridyl H-4'), 8.60 (near d, 1H, pyridyl H-6'), 8.70 (near s, 1H, pyridyl H-2'), 12.78 (s, 1H, OH; disappeared with D_2O). IR (KBr): 3130-2520 br, w (chelated OH), 1630 s (CO), 1594, 1580, 1500 cm^{-1}. Anal. ($C_{15}H_{15}NO_3$) C, H, N.

*5.1.1.16. 1-[2-Hydroxy-3-methyl-4-(4-pyridylmethoxy)phenyl]ethanone (**17u**).* Obtained (2.78 g, 43%) from reaction of **16c** with 4-(chloromethyl)pyridine hydrochloride (4.10 g); white needles, m.p. 120-121°C (isopropyl ether). Anal. ($C_{15}H_{15}NO_3$) C, H, N.

*5.1.1.17. 1-[2-Hydroxy-3-methyl-4-(2-quinolylmethoxy)phenyl]ethanone (**17v**).* Obtained (3.46 g, 45%) from reaction of **16c** with 2-(chloromethyl)quinoline hydrochloride (5.35 g); white needles, m.p. 134-135°C (isopropyl ether). Anal. ($C_{19}H_{17}NO_3$) C, H, N.

*5.1.2. General procedure for the synthesis of substituted 4-hydroxy-2*H*-1-benzopyran-2-ones **18d,e,h-v***

A mixture of 10.0 mmol of the proper substituted acetophenone **17**, sodium (0.58 g, 25.0 mmol), diethyl carbonate (3.54 g, 30.0 mmol) and Dowtherm A (10 mL) was heated at 160°C with stirring. Within few minutes, after the sodium melting, a sudden reaction occurred resulting in the precipitation of an abundant whitish solid. Xylene (30 mL) was added in order to dilute the mixture and allow the stirring, then the mixture was further heated at 160°C for 1 h. After cooling, methanol (10 mL) was added in order to inactivate residual sodium, then the mixture was poured into cold water (400 mL). By adding then 2N aqueous NaOH (100 mL) and ethyl ether (200 mL) and stirring few minutes at room temperature, in most cases the separation of two limpid phases was obtained. The aqueous phase was collected and the organic one extracted twice with water, then discarded. The whole aqueous phase was acidified with 6N aqueous HCl (or carefully neutralized with 6N aqueous HCl, then acidified down to pH 4 with citric acid, in the case of the amphoteric compounds **18s-v**): this way compounds **18d,e,h-k,n,o,s-v** separated out as whitish solids that were collected by filtration, washed with water, dried and crystallized from the proper solvent. On the other hand, in the case of compounds **18l,m,p-r**, the separation of two clear phases did not occur, therefore the whole resulting mixture was acidified with 6N aqueous HCl. This way compounds **18l,m,p-r**, separated out

as insoluble solids, were recovered by filtration, washed with water, dried, treated with ethyl ether (50 mL) in order to remove the adsorbed Dowtherm A, and finally crystallized from the proper solvent.

*5.1.2.1. 7-(sec-Butoxy)-4-hydroxy-2*H*-1-benzopyran-2-one (**18d**).* Obtained (1.76 g, 75%) from **17d** (2.08 g); white crystals, m.p. 182-183°C (ethyl acetate/isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 0.99 (t, 3H, CH_2CH_3), 1.34 (d, 3H, $CHCH_3$), 1.54-1.92 (m, 2H, $CHCH_2CH_3$), 4.39 [near q, 1H, $OCH(CH_3)CH_2CH_3$], 5.82 (s, 1H, H-3), 6.73-6.89 (m, 2H, H-6,8), 7.77 (d of AB q, *J* = 9 Hz, 1H, H-5), 10.60 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3200-2400 s, br, complex (OH), 1680 s, br (CO), 1612 s, br, 1546, 1518 cm^{-1}. Anal. ($C_{13}H_{14}O_4$) C, H.

*5.1.2.2. 5-Benzyloxy-4-hydroxy-2*H*-1-benzopyran-2-one (**18e**).* Obtained (1.85 g, 69%) from **17e** (2.42 g); white crystals, m.p. 138-139°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 5.28 (s, 2H, $OCH_2C_6H_5$), 5.66 (s, 1H, H-3), 6.90 and 7.07 (2d, *J* = 9 Hz, 1H + 1H, H-6,8), 7.42-7.52 (m, 6H, H-7 + phenyl H's), 9.61 (s, 1H, OH; disappeared with D_2O). IR (KBr): 3296 (OH), 1709 s (CO), 1655, 1608, 1569 w cm^{-1}. Anal. ($C_{16}H_{12}O_4$) C, H.

*5.1.2.3. 4-Hydroxy-8-methyl-7-propoxy-2*H*-1-benzopyran-2-one (**18h**).* Obtained (1.71 g, 73%) from **17h** (2.08 g); white crystals, m.p. 211-212°C (ethanol). ^{1}H-NMR (DMSO-d_6) δ 1.00 (t, 3H, $CH_2CH_2CH_3$), 1.80 (near q, 2H, $CH_2CH_2CH_3$), 2.16 (s, 3H, 8-CH_3), 4.04 (t, 2H, $OCH_2CH_2CH_3$), 5.44 (s, 1H, H-3), 7.00 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.64 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3230-2500 s, br, complex (OH), 1678 s (CO), 1603 s, br, 1550, 1520 cm^{-1}. Anal. ($C_{13}H_{14}O_4$) C, H.

*5.1.2.4. 4-Hydroxy-7-isopropoxy-8-methyl-2*H*-1-benzopyran-2-one (**18i**).* Obtained (1.73 g, 74%) from **17i** (2.08 g); white crystals, m.p. 200-200.5°C (ethyl acetate). ^{1}H-NMR (DMSO-d_6) δ 1.32 [d, 6H, $CH(CH_3)_2$], 2.16 (s, 3H, 8-CH_3), 4.76 [m, 1H, $OCH(CH_3)_2$], 5.46 (s, 1H, H-3), 7.04 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.64 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.26 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3250-2450 br, complex (OH), 1660 (CO), 1595 s, br,1560, 1515 cm^{-1}. Anal ($C_{13}H_{14}O_4$) C, H.

*5.1.2.5. 7-Butoxy-4-hydroxy-8-methyl-2*H*-1-benzopyran-2-one (**18j**).* Obtained (1.99 g, 80%) from **17j** (2.22 g); white crystals, m.p. 232-234°C dec. (ethyl acetate). ^{1}H-NMR (DMSO-d_6) δ 0.96 (t, 3H, $CH_2CH_2CH_2CH_3$), 1.39-1.60 and 1.70-1.86 (2 m, 2H + 2H, $CH_2CH_2CH_2CH_3$), 2.18 (s, 3H, 8-CH_3), 4.11 (t, 2H, $OCH_2CH_2CH_2CH_3$), 5.45 (s, 1H, H-3), 7.03 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.64 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.29 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3100-2300 br, complex (OH), 1660 (CO), 1590 s, br, 1543, 1514 cm^{-1}. Anal. ($C_{14}H_{16}O_4$) C, H.

*5.1.2.6. 7-(Cyclohexylmethoxy)-4-hydroxy-8-methyl-2*H*-1-benzopyran-2-one (**18k**).* Obtained (2.36 g, 82%) from **17k** (2.62 g); white crystals, m.p. 243-244°C (ethanol). ^{1}H-NMR (DMSO-d_6) δ 0.94-1.43 and 1.58-1.95 (2 m, 11H, cyclohexyl H's), 2.17 (s, 3H, 8-CH_3), 3.89 (d, 2H, $OCH_2C_6H_{11}$), 5.44 (s, 1H, H-3), 7.00 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.64 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.28 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3200-2570 br, complex (OH), 1673 br (CO), 1600 s, br, 1562, 1512 cm^{-1}. Anal. ($C_{17}H_{20}O_4$) C, H.

*5.1.2.7. 4-Hydroxy-8-methyl-7-(2-methylbenzyloxy)-2*H*-1-benzopyran-2-one (**18l**).* Obtained (2.07 g, 70%) from **17l** (2.70 g); whitish crystals, m.p. 223-223.5°C (ethyl acetate/isopropyl ether). ^{1}H-NMR (DMSO-d_6) δ 2.21 (s, 3H, 8-CH_3), 2.34 (s, 3H, $C_6H_4CH_3$), 5.25 (s, 2H, $OCH_2C_6H_4CH_3$), 5.48 (s, 1H, H-3), 7.15-7.34 and 7.42-7.52 (2 m, 4H + 1H, H-6 + $C_6H_4CH_3$), 7.70 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3150-2300 br, complex (OH), 1646 br (CO), 1597 s, 1557, 1513 cm^{-1}. Anal. ($C_{18}H_{16}O_4$) C, H.

*5.1.2.8. 4-Hydroxy-8-methyl-7-(3-methylbenzyloxy)-2*H*-1-benzopyran-2-one (**18m**).* Obtained (2.19 g, 74%) from **17m** (2.70 g); white crystals, m.p. 218-219°C (ethyl acetate). ^{1}H-NMR (DMSO-d_6) δ 2.22 (s, 3H, 8-CH_3), 2.34 (s, 3H, $C_6H_4CH_3$), 5.21 (s, 2H, $OCH_2C_6H_4CH_3$), 5.47 (s, 1H, H-3), 7.12 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.17-7.35 (m, 4H, $C_6H_4CH_3$), 7.67 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3130-2300 br, complex (OH), 1662 (CO), 1593 s, 1545, 1514 cm^{-1}. Anal. ($C_{18}H_{16}O_4$) C, H.

*5.1.2.9. 4-Hydroxy-7-(4-methoxybenzyloxy)-8-methyl-2*H*-1-benzopyran-2-one (**18n**).* Obtained (2.44 g, 78%) from **17n** (2.86 g); whitish crystals, m.p. 212-212.5°C (ethanol). ^{1}H-NMR (DMSO-d_6) δ 2.17 (s, 3H, 8-CH_3), 3.76 (s, 3H, OCH_3), 5.16 (s, 2H, $OCH_2C_6H_4OCH_3$), 5.46 (s, 1H, H-3), 6.77 and 7.41 (AB q, *J* = 9 Hz, 2H + 2H, $C_6H_4OCH_3$), 7.12 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.64 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3200-2420 br, complex (OH), 1667 s (CO), 1601 s, br, 1569, 1515 cm^{-1}. Anal. ($C_{18}H_{16}O_5$) C, H.

*5.1.2.10. 7-(4-Chlorobenzyloxy)-4-hydroxy-8-methyl-2*H*-1-benzopyran-2-one (**18o**).* Obtained (2.38 g, 75%) from **17o** (2.90 g); whitish crystals, m.p. 273-275°C (ethanol). ^{1}H-NMR (DMSO-d_6) δ 2.11 (s, 3H, 8-CH_3), 5.25 (s, 2H, $OCH_2C_6H_4Cl$), 5.47 (s, 1H, H-3), 7.09 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.48 and 7.50 (near AB q, *J* = 9 Hz, 4H, C_6H_4Cl), 7.64 (d of AB q, *J* = 9 Hz, 1H, H-5), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3230-2550 br, complex (OH), 1681 s (CO), 1602 s, br, 1568, 1515 w cm^{-1}. Anal. ($C_{17}H_{13}ClO_4$) C, H, Cl.

*5.1.2.11. 4-Hydroxy-8-methyl-7-(1-phenylethoxy)-2*H*-1-benzopyran-2-one (**18p**).* Obtained (1.96 g, 66%) from **17p** (2.70 g); white crystals, m.p. 190-191.5°C (ethyl

acetate/isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 1.67 (d, 3H, $C_6H_5CHCH_3$), 2.33 (s, 3H, 8-CH_3), 5.37 [q, 1H, $OCH(CH_3)C_6H_5$], 5.80 (s, 1H, H-3), 6.50 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.23-7.44 (m, 6H, H-5 + phenyl H's), 11.45 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3220-2500 s, br, complex (OH), 1704 s and 1680 sh (CO), 1606 s, 1552, 1512 w cm^{-1}. Anal. ($C_{18}H_{16}O_4$) C, H.

*5.1.2.12. 4-Hydroxy-8-methyl-7-(2-phenylethoxy)-2*H*-1-benzopyran-2-one (**18q**).* Obtained (2.23 g, 75%) from **17q** (2.70 g); white crystals, m.p. 191-192°C (ethyl acetate/isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 2.22 (s, 3H, 8-CH_3), 3.14 (t, 2H, $OCH_2CH_2C_6H_5$), 4.22 (t, 2H, $OCH_2CH_2C_6H_5$), 5.81 (s, 1H, H-3), 6.70 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.20-7.42 (m, 5H, phenyl H's), 7.64 (d of AB q, *J* = 9 Hz, 1H, H-5), 11.20 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3230-2530 br, complex (OH), 1712 and 1690 sh (CO), 1605 s, br, 1562, 1511 w cm^{-1}. Anal. ($C_{18}H_{16}O_4$) C, H.

*5.1.2.13. 4-Hydroxy-8-methyl-7-(1-naphthylmethoxy)-2*H*-1-benzopyran-2-one (**18r**).* Obtained (2.66 g, 80%) from **17r** (3.06 g); white crystals, m.p. 231.5-233°C dec. (ethanol). ^{1}H-NMR (DMSO-d_6) δ 2.15 (s, 3H, 8-CH_3), 5.47 (s, 1H, H-3), 5.70 (s, 2H, $OCH_2C_{10}H_7$), 7.34 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.48-8.25 (m, 8H, naphthyl H's + H-5), 12.32 (near s, 1H, OH; disappeared with D_2O). IR (KBr): 3220-2300 s, br, complex (OH), 1684 s (CO), 1607 s, br, 1563 s, 1511 cm^{-1}. Anal. ($C_{21}H_{16}O_4$) C, H.

*5.1.2.14. 4-Hydroxy-8-methyl-7-(2-pyridylmethoxy)-2*H*-1-benzopyran-2-one (**18s**).* Obtained (1.76 g, 62%) from **17s** (2.57 g); ivory-white crystals, m.p. 229-230°C (ethanol). ^{1}H-NMR (DMSO-d_6) δ 2.69 (s, 3H, 8-CH_3), 5.33 (s, 2H, $OCH_2C_5H_4N$), 5.48 (s, 1H, H-3), 7.13 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.40 (m, 1H, pyridyl H-5'), 7.57 (near d, 1H, pyridyl H-3'), 7.65 (d of AB q, *J* = 9 Hz, 1H, H-5), 7.87 (m, 1H, pyridyl H-4'), 8.61 (near d, 1H, pyridyl H-6'), 12.31 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3170-2400 br, complex (OH), 1710 (CO), 1659, 1601 s, br, 1561 cm^{-1}. Anal. ($C_{16}H_{13}NO_4$) C, H, N.

*5.1.2.15. 4-Hydroxy-8-methyl-7-(3-pyridylmethoxy)-2*H*-1-benzopyran-2-one (**18t**).* Obtained (1.93 g, 68%) from **17t** (2.57 g); white crystals, m.p. 235-237°C dec. (pyridine/petroleum ether). ^{1}H-NMR (DMSO-d_6) δ 2.21 (s, 3H, 8-CH_3), 5.31 (s, 2H, $OCH_2C_5H_4N$), 5.47 (s, 1H, H-3), 7.16 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.48 (m, 1H, pyridyl H-5'), 7.68 (d of AB q, *J* = 9 Hz, 1H, H-5), 7.94 (m, 1H, pyridyl H-4'), 8.59 (near d, 1H, pyridyl H-6'), 8.74 (near s, 1H, pyridyl H-2'), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3300-2340 br, complex (OH), 1709 br (CO), 1607 s, br, 1566, 1508 w cm^{-1}. Anal. ($C_{16}H_{13}NO_4$) C, H, N.

*5.1.2.16. 4-Hydroxy-8-methyl-7-(4-pyridylmethoxy)-2*H*-1-benzopyran-2-one (**18u**).* Obtained (2.01 g, 71%) from **17u** (2.57 g); whitish crystals, m.p. 282-283°C dec. (methanol). ^{1}H-NMR (DMSO-d_6) δ 2.27 (s, 3H, 8-CH_3), 5.33 (s, 2H, $OCH_2C_5H_4N$), 5.44 (s, 1H, H-3), 7.06 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.47 (near d, 2H, pyridyl H-3',5'), 7.65 (d of AB q, *J* = 9 Hz, 1H, H-5), 8.60 (near d, 2H, pyridyl H-2',6'), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3200-2400 br, complex (OH), 1702 br (CO), 1608 s, br, 1562, 1512 w cm^{-1}. Anal. ($C_{16}H_{13}NO_4$) C, H, N.

*5.1.2.17. 4-Hydroxy-8-methyl-7-(2-quinolylmethoxy)-2*H*-1-benzopyran-2-one (**18v**).* Obtained as monohydrate (**18v** • H_2O) (2.42 g, 69%) from **17v** (3.07 g); whitish crystals, m.p. 254-256°C dec. (ethanol). ^{1}H-NMR (DMSO-d_6) δ 2.30 (s, 3H, 8-CH_3), 5.47 (s, 1H, H-3), 5.52 (s, 2H, $OCH_2C_9H_6N$), 7.12 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.57-7.87 (m, 4H, 3H of C_9H_6N + H-5), 7.96-8.09 (m, 2H, 2H of C_9H_6N), 8.44 (d, *J* = 8.5 Hz, 1H, 1H of C_9H_6N), 12.30 (broad s, 1H, OH; disappeared with D_2O). IR (KBr): 3396 (water of crystallization), 3220-2400 br, complex (OH), 1678 (CO), 1611 s, br, 1567, 1508 cm^{-1}. Anal. ($C_{20}H_{15}NO_4 \bullet H_2O$) C, H, N.

*5.1.3. General procedure for the synthesis of substituted 4-chloro-2*H*-1-benzopyran-2-ones **19d-h,l,m,p-t***

A mixture of the proper 4-hydroxycoumarin derivative **18** (5.0 mmol), triethylamine (0.51 g, 5.0 mmol) and phosphorus oxychloride (5.0 mL) was stirred at 130°C for the time below reported for each compound. The final reaction mixture was poured onto ice-water and stirred, then exhaustively extracted with dichloromethane (after careful addition of sodium bicarbonate until neutral, in the case of the pyridyl-derivatives **19s,t**). The combined extracts, after drying (anhydrous Na_2SO_4) and removal of solvents, afforded a dark oily or nearly solid residue which was chromatographed on a silica gel column eluting with the mixture dichloromethane-petroleum ether (2:1)[dichloromethane-ethyl acetate (1:1) in the case of compounds **19s,t**]. The eluate collected, after removal of solvents, afforded the desired 4-chlorocoumarin derivative as a solid which was taken up in a little petroleum ether, separated by filtration, then crystallized from the proper solvent.

*5.1.3.1. 7-(sec-Butoxy)-4-chloro-2*H*-1-benzopyran-2-one (**19d**).* Obtained (0.49 g, 39%) from the reaction carried out (30 min) with **18d** (1.17 g); whitish crystals, m.p. 70-71°C (petroleum ether). ^{1}H-NMR ($CDCl_3$) δ 0.99 (t, 3H, CH_2CH_3), 1.34 (d, 3H, $CHCH_3$), 1.50-1.91 (m, 2H, $CHCH_2CH_3$), 4.40 [near q, 1H, $OCH(CH_3)CH_2CH_3$], 6.41 (s, 1H, H-3), 6.70-7.05 (m, 2H, H-6,8), 7.75 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 1743 s (CO), 1603 s, 1550 w, 1500 cm^{-1}. Anal. ($C_{13}H_{13}ClO_3$) C, H, Cl.

*5.1.3.2. 5-Benzyloxy-4-chloro-2*H*-1-benzopyran-2-one (**19e**).* Obtained (0.82 g, 57%) from the reaction carried out (30 min) with **18e** (1.34 g); ivory-white crystals, m.p. 128-129°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 5.21 (s, 2H, $OCH_2C_6H_5$), 6.48 (s, 1H, H-3), 6.89 and 7.00 (2d, *J* = 9 Hz, 1H + 1H, H-6,8), 7.32-7.55 (m, 6H, H-7 + phenyl H's). IR

(KBr): 1737 and 1717 s (CO), 1602, 1558, 1499 w cm^{-1}. Anal. ($C_{16}H_{11}ClO_3$) C, H, Cl.

*5.1.3.3. 6-Benzyloxy-4-chloro-2*H*-1-benzopyran-2-one (**19f**).* Obtained (1.05 g, 73%) from the reaction carried out (3 h) with **18f** [11] (1.34 g); whitish crystals, m.p. 128-129°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 5.14 (s, 2H, O$CH_2$$C_6H_5$), 6.62 (s, 1H, H-3), 7.22-7.53 (m, 8H, H-5,7,8 + phenyl H's). IR (KBr): 1750 and 1723 s (CO), 1599, 1572, 1489 cm^{-1}. Anal. ($C_{16}H_{11}ClO_3$) C, H, Cl.

*5.1.3.4. 7-Benzyloxy-4-chloro-2*H*-1-benzopyran-2-one (**19g**).* Obtained (0.76 g, 53%) from the reaction carried out (3 h) with **18g** [11] (1.34 g); whitish crystals, m.p. 127-128°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 5.16 (s, 2H, O$CH_2$$C_6H_5$), 6.44 (s, 1H, H-3), 6.91 (d, $J_{8,6}$ = 2.5 Hz, 1H, H-8), 7.00 (dd, $J_{6,5}$ = 9 Hz, $J_{6,8}$ = 2.5 Hz, 1H, H-6), 7.34-7.48 (m, 5H, phenyl H's), 7.76 (d, $J_{5,6}$ = 9 Hz, 1H, H-5). IR (KBr): 1734 s and 1715 (CO), 1613 s, 1560 w, 1503 cm^{-1}. Anal. ($C_{16}H_{11}ClO_3$) C, H, Cl.

*5.1.3.5. 4-Chloro-8-methyl-7-propoxy-2*H*-1-benzopyran-2-one (**19h**).* Obtained (0.51 g, 40%) from the reaction carried out (40 min) with **18h** (1.17 g); white crystals, m.p. 107-108°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 1.08 (t, 3H, $CH_2CH_2CH_3$), 1.88 (m, 2H, $CH_2CH_2CH_3$), 2.30 (s, 3H, 8-CH_3), 4.04 (t, 2H, O$CH_2CH_2CH_3$), 6.40 (s, 1H, H-3), 6.87 (d of AB q, J = 9 Hz, 1H, H-6), 7.67 (d of AB q, J = 9 Hz, 1H, H-5). IR (KBr): 1726 s, br (CO), 1606 s, 1560, 1496 cm^{-1}. Anal. ($C_{13}H_{13}ClO_3$) C, H, Cl.

*5.1.3.6. 4-Chloro-8-methyl-7-(2-methylbenzyloxy)-2*H*-1-benzopyran-2-one (**19l**).* Obtained (0.79 g, 50%) from the reaction carried out (30 min) with **18l** (1.48 g); whitish crystals, m.p. 154-155°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 2.35 (s, 3H, 8-CH_3), 2.39 (s, 3H, $C_6H_4CH_3$), 5.18 (s, 2H, O$CH_2$$C_6H_4CH_3$), 6.44 (s, 1H, H-3), 7.00 (d of AB q, J = 9 Hz, 1H, H-6), 7.14-7.46 (m, 4H, $C_6H_4CH_3$), 7.69 (d of AB q, J = 9 Hz, 1H, H-5). IR (KBr): 1724 s (CO), 1609 sh, 1602 s, 1560, 1494 cm^{-1}. Anal. ($C_{18}H_{15}ClO_3$) C, H, Cl.

*5.1.3.7. 4-Chloro-8-methyl-7-(3-methylbenzyloxy)-2*H*-1-benzopyran-2-one (**19m**).* Obtained (0.79 g, 50%) from the reaction carried out (30 min) with **18m** (1.48 g); whitish crystals, m.p. 141-142°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 2.37 and 2.39 (2 s, 3H + 3H, 8-CH_3 + $C_6H_4CH_3$), 5.17 (s, 2H, O$CH_2$$C_6H_4CH_3$), 6.43 (s, 1H, H-3), 6.94 (d of AB q, J = 9 Hz, 1H, H-6), 7.13-7.37 (m, 4H, $C_6H_4CH_3$), 7.67 (d of AB q, J = 9 Hz, 1H, H-5). IR (KBr): 1724 s (CO), 1603 s, 1560, 1497 cm^{-1}. Anal. ($C_{18}H_{15}ClO_3$) C, H, Cl.

*5.1.3.8. 4-Chloro-8-methyl-7-(1-phenylethoxy)-2*H*-1-benzopyran-2-one (**19p**).* Obtained (0.47 g, 30%) from the reaction carried out (30 min) with **18p** (1.48 g); white crystals, m.p. 137.5-139°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 1.71 (d, 3H, $C_6H_5CHCH_3$), 2.41 (s, 3H, 8-CH_3), 5.47 [q, 1H, OCH(CH_3)C_6H_5], 6.40 (s, 1H, H-3), 6.73 (d of AB q, J = 9 Hz, 1H, H-6), 7.24-7.41 (m, 5H, phenyl H's), 7.54 (d of AB q, J = 9 Hz, 1H, H-5). IR (KBr): 1728 s (CO), 1609 s, 1601 sh, 1562, 1490 cm^{-1}. Anal. ($C_{18}H_{15}ClO_3$) C, H, Cl.

*5.1.3.9. 4-Chloro-8-methyl-7-(2-phenylethoxy)-2*H*-1-benzopyran-2-one (**19q**).* Obtained (0.63 g, 40%) from the reaction carried out (30 min) with **18q** (1.48 g); whitish crystals, m.p. 132-133°C (isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 2.28 (s, 3H, 8-CH_3), 3.16 (t, 2H, O$CH_2$$CH_2$$C_6H_5$), 4.29 (t, 2H, O$CH_2$$CH_2C_6H_5$), 6.42 (s, 1H, H-3), 6.87 (d of AB q, J = 9 Hz, 1H, H-6), 7.22-7.42 (m, 5H, phenyl H's), 7.66 (d of AB q, J = 9 Hz, 1H, H-5). IR (KBr): 1724 s, br (CO), 1606 s, 1563, 1494 cm^{-1}. Anal. ($C_{18}H_{15}ClO_3$) C, H, Cl.

*5.1.3.10. 4-Chloro-8-methyl-7-(1-naphthylmethoxy)-2*H*-1-benzopyran-2-one (**19r**).* Obtained (0.63 g, 36%) from the reaction carried out (3 h) with **18r** (1.66 g); whitish crystals, m.p. 197-198°C (ethyl acetate/isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 2.33 (s, 3H, 8-CH_3), 5.66 (s, 2H, O$CH_2$$C_{10}H_7$), 6.46 (s, 1H, H-3), 7.12 (d of AB q, J = 9 Hz, 1H, H-6), 7.46-7.68 and 7.85-8.13 (2 m, 4H + 3H, naphthyl H's), 7.72 (d of AB q, J = 9 Hz, 1H, H-5). IR (KBr): 1716 s (CO), 1603 s, 1558, 1496 cm^{-1}. Anal. ($C_{21}H_{15}ClO_3$) C, H, Cl.

*5.1.3.11. 4-Chloro-8-methyl-7-(2-pyridylmethoxy)-2*H*-1-benzopyran-2-one (**19s**).* Obtained (0.66 g, 44%) from the reaction carried out (2 h) with **18s** (1.42 g); whitish crystals, m.p. 228.5-229°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 2.42 (s, 3H, 8-CH_3), 5.34 (s, 2H, O$CH_2$$C_5H_4N$), 6.44 (s, 1H, H-3), 6.95 (d of AB q, J = 9 Hz, 1H, H-6), 7.26 (m, 1H, pyridyl H-5'), 7.54 (near d, 1H, pyridyl H-3'), 7.67 (d of AB q, J = 9 Hz, 1H, H-5), 7.78 (m, 1H, pyridyl H-4'), 8.62 (near d, 1H, pyridyl H-6'). IR (KBr): 1725 s (CO), 1608, 1600 sh, 1574, 1498 cm^{-1}. Anal. ($C_{16}H_{12}ClNO_3$) C, H, N, Cl.

*5.1.3.12. 4-Chloro-8-methyl-7-(3-pyridylmethoxy)-2*H*-1-benzopyran-2-one (**19t**).* Obtained (0.85 g, 56%) from the reaction carried out (20 min) with **18t** (1.42 g); whitish crystals, m.p. 222°C (dichloromethane/ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 2.35 (s, 3H, 8-CH_3), 5.22 (s, 2H, O$CH_2$$C_5H_4N$), 6.45 (s, 1H, H-3), 6.95 (d of AB q, J = 9 Hz, 1H, H-6), 7.39 (m, 1H, pyridyl H-5'), 7.71 (d of AB q, J = 9 Hz, 1H, H-5), 7.82 (near d, 1H, pyridyl H-4'), 8.64 (near d, 1H, pyridyl H-6'), 8.72 (near s, 1H, pyridyl H-2'). IR (KBr): 1727 s (CO), 1602 s, 1562, 1497 cm^{-1}. Anal. ($C_{16}H_{12}ClNO_3$) C, H, N, Cl.

*5.1.4. General procedures for the synthesis of substituted 4-(1-piperazinyl)-2*H*-1-benzopyran-2-ones **5***

*Method A). From the proper 4-hydroxycoumarin derivatives **18** (compounds **5i-k,n,o,u,v**)*

A mixture of 3.0 mmol of the proper compound **18** and excess piperazine (7.0 g) was stirred at 160°C for 1 h, then final hot solution was poured into ice-water. The resulting solution was exhaustively extracted with chloroform. The

combined extracts (dried over anhydrous Na_2SO_4) were evaporated to dryness under reduced pressure to give a thick oil from which (compounds **5k,n,o,u,v**), after treatment with a little ethyl ether and standing, nearly pure compound **5** separated out as a whitish solid which was then crystallized from the suitable solvent. In the case of compounds **5i,j** the final oily residue was treated with a solution of maleic acid (0.35 g, 3.0 mmol) in anhydrous ethanol to give the corresponding pure maleates (**5i** • $H_4C_4O_4$ or **5j** • $H_4C_4O_4$) as a white solid which was then crystallized from anhydrous ethanol. The free amines **5i,j** were obtained, as thick oils, from the analytical samples of these maleates, by treatment with aqueous sodium bicarbonate, exhaustive extraction with chloroform and removal of solvent, then were crystallized from the proper solvents.

*Method B). From 4-chlorocoumarin derivatives **19** (compounds **5d-h,l,m,p-t**)*

A mixture of 3.0 mmol of the proper chloroderivative **19**, piperazine (2.58 g, 30.0 mmol) and ethanol (100 mL) was stirred at room temperature for 2 h. The solution obtained was poured into water (250 mL) and the mixture exhaustively extracted with chloroform. The combined extracts, after drying and removal of solvents, afforded an oily or nearly solid residue which, in most cases, was treated with a little ethyl ether to give pure compound **5** as a whitish solid which was then crystallized from the suitable solvent (compounds **5e,g,h,l,m,p-t**). Compounds **5d,f** were obtained as maleates by the treatment described in Method A for **5i,j; 5d** was stored as maleate, while **5f** was transformed in the corresponding free amine as described above, then crystallized.

According to procedures above described, the following compounds were prepared.

*5.1.4.1. 7-(sec-Butoxy)-4-(1-piperazinyl)-2*H*-1-benzopyran-2-one (**5d**).* Obtained as maleate (**5d** • $H_4C_4O_4$) (1.19 g, 95%) from **19d** (0.76 g); whitish crystals, m.p. 169-170.5°C (anhydrous ethanol/ethyl ether). The ^{1}H-NMR and IR spectra were recorded on the free amine obtained (thick oil) from the corresponding maleate as above described. ^{1}H-NMR ($CDCl_3$) δ 0.98 (t, 3H, CH_2CH_3), 1.33 (d, 3H, $CHCH_3$), 1.72 (m, 2H, $CHCH_2CH_3$), 1.79 (s, 1H, NH; disappeared with D_2O), 2.99-3.34 (m, 8H, piperazine CH_2's), 4.37 [m, 1H, $OCH(CH_3)CH_2CH_3$], 5.57 (s, 1H, H-3), 6.69-6.84 (m, 2H, H-6,8), 7.50 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (film): 3330 (NH), 1709 s, br (CO), 1611 s, br, 1549, 1507 cm^{-1}. Anal. ($C_{17}H_{22}N_2O_3$ • $H_4C_4O_4$) C, H, N.

*5.1.4.2. 5-Benzyloxy-4-(1-piperazinyl)-2*H*-1-benzopyran-2-one (**5e**).* Obtained (0.83 g, 82%) from **19e** (0.86 g); ivory-white crystals, m.p. 159-159.5°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.78 (s, 1H, NH; disappeared with D_2O), 2.68-2.83 and 2.97-3.20 (2 m, 4H + 4H, piperazine CH_2's), 5.14 (s, 2H, $OCH_2C_6H_5$), 5.60 (s, 1H, H-3), 6.84 and 6.99 (2 d, *J* = 9 Hz, 1H + 1H, H-6,8), 7.32-7.50 (m, 6H, H-7 + phenyl H's). IR (KBr): 3345 w (NH), 1702 s (CO), 1599 s, 1543 cm^{-1}. Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

*5.1.4.3. 6-Benzyloxy-4-(1-piperazinyl)-2*H*-1-benzopyran-2-one (**5f**).* Obtained (0.56 g, 55%) from **19f** (0.86 g); whitish crystals, m.p. 104-105°C (ethyl acetate/petroleum ether). ^{1}H-NMR ($CDCl_3$) δ 1.82 (s, 1H, NH; disappeared with D_2O), 2.93-3.18 (m, 8H, piperazine CH_2's), 5.14 (s, 2H, $OCH_2C_6H_5$), 5.71 (s, 1H, H-3), 6.99-7.47 (m, 8H, H-5,7,8 + phenyl H's). IR (KBr): 3343 (NH), 1695 s (CO), 1604 w, 1558, 1487 w cm^{-1}. Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

*5.1.4.4. 7-Benzyloxy-4-(1-piperazinyl)-2*H*-1-benzopyran-2-one (**5g**).* Obtained (0.79 g, 78%) from **19g** (0.86 g); white crystals, m.p. 149-150°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.73 (s, 1H, NH; disappeared with D_2O), 2.98-3.30 (m, 8H, piperazine CH_2's), 5.12 (s, 2H, $OCH_2C_6H_5$), 5.59 (s, 1H, H-3), 6.80-6.94 (m, 2H, H-6,8), 7.30-7.56 (m, 6H, H-5 + phenyl H's). IR (KBr): 3220 (NH), 1703 s (CO), 1610 s, br, 1548 cm^{-1}. Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

*5.1.4.5. 8-Methyl-4-(1-piperazinyl)-7-propoxy-2*H*-1-benzopyran-2-one (**5h**).* Obtained (0.59 g, 65%) from **19h** (0.76 g); white crystals, m.p. 150-150.5°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.07 (t, 3H, $CH_2CH_2CH_3$), 1.80 (s, 1H, NH; disappeared with D_2O), 1.87 (m, 2H, $CH_2CH_2CH_3$), 2.30 (s, 3H, 8-CH_3), 3.02-3.30 (m, 8H, piperazine CH_2's), 4.00 (t, 2H, $OCH_2CH_2CH_3$), 5.60 (s, 1H, H-3), 6.79 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.43 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 3322 (NH), 1695 s (CO), 1604 s, 1557, 1501 w cm^{-1}. Anal. ($C_{17}H_{22}N_2O_3$) C, H, N.

*5.1.4.6. 7-Isopropoxy-8-methyl-4-(1-piperazinyl)-2*H*-1-benzopyran-2-one (**5i**).* Obtained (0.58 g, 64%) from **18i** (0.70 g); ivory-white crystals, m.p. 139-140°C (ethyl acetate/petroleum ether). ^{1}H-NMR ($CDCl_3$) δ 1.36 [d, 6H, $CH(CH_3)_2$], 2.20 (s, 1H, NH; disappeared with D_2O), 2.27 (s, 3H, 8-CH_3), 2.96-3.37 (m, 8H, piperazine CH_2's), 4.62 [m, 1H, $OCH(CH_3)_2$], 5.60 (s, 1H, H-3), 6.79 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.39 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 3341 w (NH), 1710 s (CO), 1606 s, 1557, 1497 w cm^{-1}. Anal. ($C_{17}H_{22}N_2O_3$) C, H, N.

*5.1.4.7. 7-Butoxy-8-methyl-4-(1-piperazinyl)-2*H*-1-benzopyran-2-one (**5j**).* Obtained (0.43 g, 45%) from **18j** (0.74 g); ivory-white crystals, m.p. 142-143°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.01 (t, 3H, $CH_2CH_2CH_2CH_3$), 1.54 (m, 2H, $CH_2CH_2CH_2CH_3$), 1.76 (s, 1H, NH; disappeared with D_2O), 1.84 (m, 2H, $CH_2CH_2CH_2CH_3$), 2.32 (s, 3H, 8-CH_3), 3.05-3.30 (m, 8H, piperazine CH_2's), 4.07 (t, 2H, $OCH_2CH_2CH_2CH_3$), 5.62 (s, 1H, H-3), 6.80 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.43 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 3321 (NH), 1693 s (CO), 1605 s, 1557, 1503 w cm^{-1}. Anal. ($C_{18}H_{24}N_2O_3$) C, H, N.

*5.1.4.8. 7-(Cyclohexylmethoxy)-8-methyl-4-(1-piperazinyl)-2*H*-1-benzopyran-2-one (**5k**).* Obtained (0.62 g, 58%) from **18k** (0.87 g); whitish crystals, m.p. 177-178°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.00-1.46 and 1.66-1.96 (2 m,

11H, cyclohexyl H's), 1.77 (s, 1H, NH; disappeared with D_2O), 2.32 (s, 3H, 8-CH_3), 3.06-3.28 (m, 8H, piperazine CH_2's), 3.85 (d, 2H, O$CH_2C_6H_{11}$), 5.61 (s, 1H, H-3), 6.78 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.42 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 3320 w (NH), 1709 s and 1690 s (CO), 1607 s, 1551, 1503 w cm^{-1}. Anal. ($C_{21}H_{28}N_2O_3$) C, H, N.

*5.1.4.9. 8-Methyl-7-(2-methylbenzyloxy)-4-(1-piperazinyl)-2H-1-benzopyran-2-one (**5l**).* Obtained (0.99 g, 91%) from **19l** (0.94 g); whitish crystals, m.p. 162-163°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.69 (s, 1H, NH; disappeared with D_2O), 2.34 (s, 3H, 8-CH_3), 2.39 (s, 3H, $C_6H_4CH_3$), 3.04-3.28 (m, 8H, piperazine CH_2's), 5.15 (s, 2H, O$CH_2C_6H_4CH_3$), 5.62 (s, 1H, H-3), 6.89 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.18-7.32 (m, 3H, 3H of $C_6H_4CH_3$), 7.40-7.50 (m, 2H, 1H of $C_6H_4CH_3$ + H-5). IR (KBr): 3308 w (NH), 1689 s (CO), 1604 s, 1555, 1504 w cm^{-1}. Anal. ($C_{22}H_{24}N_2O_3$) C, H, N.

*5.1.4.10. 8-Methyl-7-(3-methylbenzyloxy)-4-(1-piperazinyl)-2H-1-benzopyran-2-one (**5m**).* Obtained (1.01 g, 92%) from **19m** (0.94 g); ivory-white crystals, m.p. 143-144°C (ethyl acetate/petroleum ether). ^{1}H-NMR ($CDCl_3$) δ 1.74 (s, 1H, NH; disappeared with D_2O), 2.37 and 2.38 (2 s, 3H + 3H, 8-CH_3 + $C_6H_4CH_3$), 3.00-3.29 (m, 8H, piperazine CH_2's), 5.14 (s, 2H, O$CH_2C_6H_4CH_3$), 5.61 (s, 1H, H-3), 6.84 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.11-7.37 (m, 4H, $C_6H_4CH_3$), 7.41 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 3322 (NH), 1698 s (CO), 1605 s, 1558, 1501 w cm^{-1}. Anal. ($C_{22}H_{24}N_2O_3$) C, H, N.

*5.1.4.11. 7-(4-Methoxybenzyloxy)-8-methyl-4-(1-piperazinyl)-2H-1-benzopyran-2-one (**5n**).* Obtained (0.32 g, 28%) from **18n** (0.94 g); ivory-white crystals, m.p. 174.5-175°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.70 (s, 1H, NH; disappeared with D_2O), 2.36 (s, 3H, 8-CH_3), 3.05-3.16 and 3.18-3.27 (2 m, 4H + 4H, piperazine CH_2's), 3.84 (s, 3H, OCH_3), 5.12 (s, 2H, O$CH_2C_6H_4OCH_3$), 5.63 (s, 1H, H-3), 6.86 (d of AB q, *J* = 9 Hz, 1H, H-6), 6.95 and 7.38 (AB q, *J* = 9 Hz, 2H + 2H, $C_6H_4OCH_3$), 7.43 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 3313 w (NH), 1700 s (CO), 1601 s, 1554, 1514 cm^{-1}. Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

*5.1.4.12. 7-(4-Chlorobenzyloxy)-8-methyl-4-(1-piperazinyl)-2H-1-benzopyran-2-one (**5o**).* Obtained (0.53 g, 46%) from **18o** (0.95 g); ivory-white crystals, m.p. 188-189°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.79 (s, 1H, NH; disappeared with D_2O), 2.36 (s, 3H, 8-CH_3), 3.03-3.13 and 3.17-3.27 (2 m, 4H + 4H, piperazine CH_2's), 5.15 (s, 2H, O$CH_2C_6H_4Cl$), 5.62 (s, 1H, H-3), 6.81 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.38 (s, 4H, C_6H_4Cl), 7.43 (d of AB q, *J* = 9 Hz, 1H, H-5). IR (KBr): 3305 w (NH), 1703 s (CO), 1605 s, 1556, 1493 w cm^{-1}. Anal. ($C_{21}H_{21}ClN_2O_3$) C, H, N, Cl.

*5.1.4.13. 8-Methyl-7-(1-phenylethoxy)-4-(1-piperazinyl)-2H-1-benzopyran-2-one (**5p**).* Obtained (0.76 g, 69%) from **19p** (0.94 g); white crystals, m.p. 156-157°C (ethyl acetate/petroleum ether). ^{1}H-NMR ($CDCl_3$) δ 1.69 (d + s, 3H + 1H, $C_6H_5CHCH_3$ + NH; d, 3H, after treatment with D_2O), 2.40 (s, 3H, 8-CH_3), 3.00-3.22 (m, 8H, piperazine CH_2's), 5.40 [q, 1H, O$CH(CH_3)C_6H_5$], 5.58 (s, 1H, H-3), 6.64 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.22-7.40 (m, 6H, phenyl H's + H-5). IR (KBr): 3334 w (NH), 1701 s (CO), 1603 s, 1553, 1495 cm^{-1}. Anal. ($C_{22}H_{24}N_2O_3$) C, H, N.

*5.1.4.14. 8-Methyl-7-(2-phenylethoxy)-4-(1-piperazinyl)-2H-1-benzopyran-2-one (**5q**).* Obtained (0.98 g, 90%) from **19q** (0.94 g); white crystals, m.p. 144-145°C (ethyl acetate/isopropyl ether). ^{1}H-NMR ($CDCl_3$) δ 1.68 (s, 1H, NH; disappeared with D_2O), 2.28 (s, 3H, 8-CH_3), 3.04-3.26 (m, 8H + 2H, piperazine CH_2's + $OCH_2CH_2C_6H_5$), 4.26 (t, 2H, O$CH_2CH_2C_6H_5$), 5.61 (s, 1H, H-3), 6.77 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.20-7.45 (m, 6H, phenyl H's + H-5). IR (KBr): 3267 w (NH), 1705 s (CO), 1604 s, 1558, 1499 w cm^{-1}. Anal. ($C_{22}H_{24}N_2O_3$) C, H, N.

*5.1.4.15. 8-Methyl-7-(1-naphthylmethoxy)-4-(1-piperazinyl)-2H-1-benzopyran-2-one (**5r**).* Obtained (0.77 g, 64%) from **19r** (1.05 g); whitish crystals, m.p. 196-197°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.70 (s, 1H, NH; disappeared with D_2O), 2.34 (s, 3H, 8-CH_3), 3.03-3.28 (m, 8H, piperazine CH_2's), 5.64 (overlapped singlets, 2H + 1H, O$CH_2C_{10}H_7$ + H-3), 7.00 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.40-7.65 (m, 5H, 4H of $C_{10}H_7$ + H-5), 7.85-8.12 (m, 3H, 3H of $C_{10}H_7$). IR (KBr): 3311 w (NH), 1700 s (CO), 1606 s, 1556, 1506 w cm^{-1}. Anal. ($C_{25}H_{24}N_2O_3$) C, H, N.

*5.1.4.16. 8-Methyl-4-(1-piperazinyl)-7-(2-pyridylmethoxy)-2H-1-benzopyran-2-one (**5s**).* Obtained (0.99 g, 94%) from **19s** (0.91 g); white crystals, m.p. 217-218°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.74 (s, 1H, NH; disappeared with D_2O), 2.41 (s, 3H, 8-CH_3), 2.97-3.32 (m, 8H, piperazine CH_2's), 5.30 (s, 2H, O$CH_2C_5H_4N$), 5.61 (s, 1H, H-3), 6.84 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.26 (m, 1H, pyridyl H-5'), 7.42 (d of AB q, *J* = 9 Hz, 1H, H-5), 7.55 (near d, 1H, pyridyl H-3'), 7.76 (m, 1H, pyridyl H-4'), 8.62 (near d, 1H, pyridyl H-6'). IR (KBr): 3289 w (NH), 1713 s (CO), 1611 s, 1593 sh, 1560, 1500 w cm^{-1}. Anal. ($C_{20}H_{21}N_3O_3$) C, H, N.

*5.1.4.17. 8-Methyl-4-(1-piperazinyl)-7-(3-pyridylmethoxy)-2H-1-benzopyran-2-one (**5t**).* Obtained (0.83 g, 79%) from **19t** (0.91 g); white crystals, m.p. 197-198°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.74 (s, 1H, NH; disappeared with D_2O), 2.34 (s, 3H, 8-CH_3), 3.00-3.30 (m, 8H, piperazine CH_2's), 5.19 (s, 2H, O$CH_2C_5H_4N$), 5.62 (s, 1H, H-3), 6.84 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.37 (m, 1H, pyridyl H-5'), 7.43 (d of AB q, *J* = 9 Hz, 1H, H-5), 7.80 (near d, 1H, pyridyl H-4'), 8.62 (near d, 1H, pyridyl H-6'), 8.70 (near s, 1H, pyridyl H-2'). IR (KBr): 3268 (NH), 1705 s (CO), 1605 s, 1557, 1505 w cm^{-1}. Anal. ($C_{20}H_{21}N_3O_3$) C, H, N.

*5.1.4.18. 8-Methyl-4-(1-piperazinyl)-7-(4-pyridylmethoxy)-2H-1-benzopyran-2-one (**5u**).* Obtained (0.51 g, 48%) from **18u** (0.85 g); whitish crystals, m.p. 208-208.5°C (ethyl ac-

etate). ^{1}H-NMR ($CDCl_3$) δ 1.82 (s, 1H, NH; disappeared with D_2O), 2.42 (s, 3H, 8-CH_3), 3.03-3.27 (m, 8H, piperazine CH_2's), 5.22 (s, 2H, O$CH_2C_5H_4N$), 5.64 (s, 1H, H-3), 6.77 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.38 (near d, 2H, pyridyl H-3',5'), 7.43 (d of AB q, *J* = 9 Hz, 1H, H-5), 8.65 (near d, 2H, pyridyl H-2',6'). IR (KBr): 3335 (NH), 1692 s (CO), 1600 s, 1556 cm^{-1}. Anal. ($C_{20}H_{21}N_3O_3$) C, H, N.

*5.1.4.19. 8-Methyl-4-(1-piperazinyl)-7-(2-quinolylmethoxy)-2H-1-benzopyran-2-one (**5v**).* Obtained (0.53 g, 44%) from **18v** (1.05 g); whitish crystals, m.p. 237-238°C (dichloromethane/ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.76 (s, 1H, NH; disappeared with D_2O), 2.47 (s, 3H, 8-CH_3), 3.02-3.26 (m, 8H, piperazine CH_2's), 5.51 (s, 2H, O$CH_2C_9H_6N$), 5.63 (s, 1H, H-3), 6.88 (d of AB q, *J* = 9 Hz, 1H, H-6), 7.40 (d of AB q, *J* = 9 Hz, 1H, H-5), 7.51-7.92 (m, 4H, 4H of C_9H_6N), 8.11 (near d, 1H, 1H of C_9H_6N), 8.24 (d, *J* = 8.5 Hz, 1H, 1H of C_9H_6N). IR (KBr): 3241 (NH), 1716 s (CO), 1608 s, 1559, 1500 cm^{-1}. Anal. ($C_{24}H_{23}N_3O_3$) C, H, N.

*5.1.5. 6-Methyl-4-(1-piperazinyl)-2H-pyran-2-one (**21**).* A mixture of 4-chloro-6-methyl-2*H*-pyran-2-one **20** [12] (0.43 g, 3.0 mmol), piperazine (2.58 g, 30.0 mmol) and ethanol (100 mL) was stirred at room temperature for 1 h. The resulting solution was poured into water (250 mL) and the mixture exhaustively extracted with chloroform. The extracts, after drying and removal of solvents, afforded a thick oil from which, after treatment with ethyl ether and standing, pure **21** separated out as whitish solid (0.39 g, 67%); ivory-white crystals, m.p. 144-145°C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ 1.77 (s, 1H, NH; disappeared with D_2O), 2.18 (s, 3H, CH_3), 2.88-3.04 and 3.28-3.43 (2 m, 4H + 4H, piperazine CH_2's), 5.12 (near s, 1H, H-5), 5.85 (near s, 1H, H-3). IR (KBr): 3288 (NH), 1678 s (CO), 1634, 1539 s cm^{-1}. Anal. ($C_{10}H_{14}N_2O_2$) C, H, N.

5.2. Biology

5.2.1. Platelet aggregation

Human blood from healthy volunteers was added to a 130 mM trisodium citrate aqueous solution (volume ratio 9:1), then centrifuged at 100 *g* for 30 min to give platelet-rich plasma (PRP) which was diluted to 2.0 x 10^8 plts mL^{-1} with PPP (platelet-poor plasma). The diluted PRP (500 µL) was preincubated at 37°C for 2 min with solvent (dimethylsulfoxide, 5 µL) or drug solution before the addition of the platelet aggregation agent. PRP aggregation was induced by 5.0 µM ADP (Sigma), collagen from bovine tendon (Mascia Brunelli) at the final concentration of 5.0 µg mL^{-1}, or 20.0 µM A23187 (Sigma). Before each experiment the stock solutions of ADP (saline), collagen (saline), and A23187 (DMSO) were diluted in saline. Platelet aggregation, performed in an Aggrecoder PA-3210 aggregometer (A. Menarini, Florence, Italy), was measured following the Born's turbidimetric method [14] and quantified by the light transmission reached after 3 min.

5.2.2. Calculation of inhibition

In order to calculate the percentage of inhibition, the extent of aggregation measured in the presence of the compounds tested was always compared with that measured for a control sample containing the solvent, in an experiment carried out under the same conditions. From each series of experiments, in which the inhibitors were tested in at least five concentrations, a percentage inhibition-concentration curve was derived. From this curve the IC_{50} value was calculated as the concentration of inhibitor causing a 50% inhibition of the aggregation.

5.2.3. Statistical analysis

The IC_{50} values reported in Table 2 are averages (± standard deviation) of those obtained from at least four independent determinations carried out on different batches of platelets (usually 5-8 batches). Statistical analysis was performed using the Student's *t*-test and considering significant the activity difference between two compounds when corresponding to $P < 0.05$.

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