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Coumarin, Chromone, and 4(3*H*)-pyrimidinone Novel Bicyclic and Tricyclic Derivatives as Antiplatelet Agents: Synthesis, Biological Evaluation, and Comparative Molecular Field Analysis

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Abstract—As a further part of our chemical and biological studies in this field, we describe the multistep preparations of the properly substituted 2-(1-piperazinyl)chromone **1b**, 4-(1-piperazinyl)coumarins **5c**–**h**, their linear benzo-fused analogues **4a**,**b** and **8a**,**b**, bicyclic (**15e**–**g**) and tricyclic (**15h**,**i**) fused derivatives of 6-(1-piperazinyl)pyrimidin-4(3*H*)-one, and of the 4*H*-pyrido[1,2-*a*] pyrimidine derivatives **9b**,**c**. The in vitro evaluation of their inhibitory properties towards human platelet aggregation induced in platelet-rich plasma by ADP, collagen, or the Ca^{2+} ionophore A23187 showed the high activity of compounds **5d**–**g** and **15f**,**g**,**i**, among which the coumarins **5g** and **5d** proved to be, in that order, the most effective in vitro antiplatelet agents until now synthesized by us. Thus, in order to consider also the 4-aminocoumarin structural class, we developed a new statistically significant 3-D QSAR model, more general than the one previously obtained, through a further CoMFA study based on the antiplatelet activity data and molecular steric and electrostatic potentials of both the previously studied and herein described compounds.

Introduction

In previous papers we described the synthesis and in vitro inhibitory properties on human platelet aggregation of a number of *N*-substituted 2-aminochromones **1**[1,2] and their angular benzo-fused derivatives **2** and **3**[3] (Chart 1), as well as of the structurally analogous 1,2-fused pyrimidine derivatives **9**, **10**, and **11**[4,5] and their corresponding isomers **12**, **13**, and **14**,[5] respectively (Chart 2). Also the (1-piperazinyl) substituted coumarins **5a**,**b** and benzocoumarins **6a** and **7a** were prepared and their in vitro antiplatelet activity was evaluated[3]

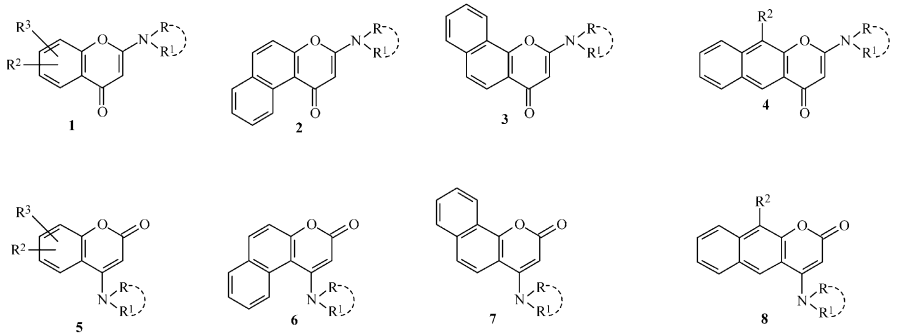

Chart 1.

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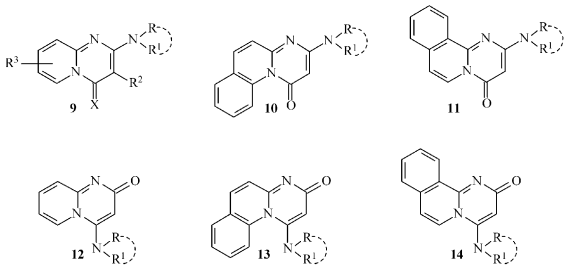


Chart 2.

(Chart 1, Table 1). Actually, in each structural class 1-piperazinyl proved to be the most effective amino substituent among all those used [the (1-piperazinyl) substituted compounds **12**–**14** were not obtainable] and 2-(1-piperazinyl)-4*H*-pyrido(1,2-*a*)pyrimidin-4-one **9a**[5] was the most active of all compounds prepared and tested in the above studies,[1–5] towards all the platelet aggregation inducers used (Table 1). Significantly, both these compounds[6] and 2-(diethylamino)-7-hydroxychromone,[7,8] taken as an example of compounds **1**, exert their antiplatelet activity by specifically inhibiting the activity of cAMP high affinity phosphodiesterase, thus increasing intracellular cAMP concentration.

Starting from these results, we more recently prepared and tested for their in vitro antiplatelet activity a group of novel 1,2-fused pyrimidine derivatives structurally differing from **9a** (taken as a lead) for proper modifications of the pyridine ring (**15a**–**d**) and/or of the 2-substituent (**16** and **17**, respectively) (Chart 3), as well as further compounds **9** with new substitution patterns.[9] This study allowed us to obtain some further significantly active antiplatelet agents and, by adding the new data to those previously afforded by compounds **1**–**3**,[1–3] **5**–**7**,[3] **9**–**14**,[4,5] to submit a wide antiplatelet activity data set to Comparative Molecular Field Analysis (CoMFA). This way a statistically significant 3-D

Table 1. In vitro inhibitory activity of (1-piperazinyl) substituted compounds **1**–**3**, **5**–**7**, and **9**–**11** (Charts 1 and 2) on human platelet aggregation induced in PRP[a] by ADP, collagen and A23187

Compd[b]		R^2	R^3	X	IC_{50} (μM)±SD		
					ADP (5.0 μM)	Collagen (10.0 μg/mL)[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
5a		H	H	—	49±14	36±10	250±38
5b		7-OC_2H_5	H	—	24±14	10±3.6	44±18
6a		—	—	—	53±15	49±17	245±65
7a		—	—	—	81±23	71±27	334±89
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

[a]PRP, platelet-rich plasma.
[b]Compounds **1a**, **2a**, **3a**, **5a**,**b**, **6a**, **7a**: ref 3; compounds **9a**, **10a**, **11a**: ref 5.
[c]5.0 μg/mL for compounds **9a**, **10a**, **11a**.

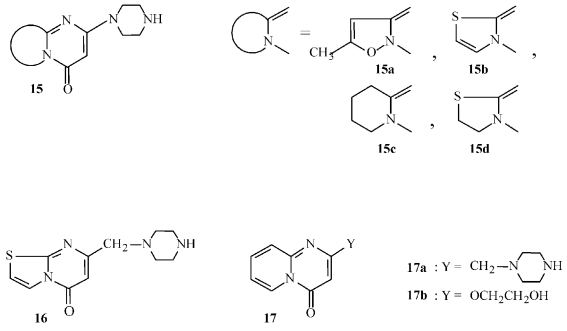


Chart 3.

QSAR model was developed, relating the antiplatelet activity variations to changes of molecular steric and electrostatic potentials.[9]

On the other hand, considering that only two (**5a** and **5b**)[3] 4-(1-piperazinyl)coumarins **5** and four (**15a–d**)[9] (1-piperazinyl)substituted 1,2-fused pyrimidine derivatives **15** were previously synthesized and tested by us, and their antiplatelet activity data submitted to CoMFA,[9] we have now regarded it interesting to prepare and test novel compounds of these promising structural classes. Thus, taking into account that coumarin **5a** was only slightly more active than both its benzo-fused derivatives **6a, 7a** and the isomeric chromone **1a**, but its 7-ethoxy-derivative **5b** was clearly the most effective antiplatelet agent among all the fused pyran derivatives **1–3**, **5–7** previously prepared by us[1–3] (Table 1), and that the 8-methyl-2-morpholino-7-[(1-naphthyl)methoxy]chromone U-84569 was reported to be a potent inhibitor of human platelet aggregation,[10] we have now synthesized and tested in vitro for their human platelet antiaggregating properties the new suitably alkoxy or 7-alkoxy-8-methyl substituted 4-(1-piperazinyl)coumarins **5c–h** (Table 2), aiming at reaching more effective substitution patterns and obtaining further information on structure–antiplatelet activity relationships in this structural class. In this connection, also the linear analogues **4a**,**b** and **8a**,**b** (Chart 1, Table 2) of naphthopyran derivatives **2a**,**3a** and **6a**,**7a** (Chart 1, Table 1), respectively, have been synthesized and tested. Furthermore, chromone **1b** has been prepared in order to compare its activity with that of its very active isomer, coumarin **5g**. In addition, also some properly chosen novel examples of 1,2-fused pyrimidine derivatives (**9b**,**c** and **15e–i**) have been now obtained and their human platelet antiaggregating properties evaluated (Table 2).

Finally, due to the very interesting activity shown by some of these novel compounds, particularly in the previously neglected structural field of coumarins **5**, we considered it convenient to submit to a CoMFA study an extended antiplatelet activity data set including both the results recently analysed[9] and the ones reported here, in order to develop a more general 3-D QSAR model, provided with better predictive ability.

The chemical, biological and 3-D QSAR results of this study are described in the present paper.

Chemistry

The preparation of intermediates **19a–c**, **20a**,**b**,**d**, **22**, **23** and desired 4-(1-piperazinyl)coumarins **5c–h** is illustrated in Scheme 1.

Thus, the dihydroxyacetophenones **18a–c** were treated with proper alkyl iodides (anhydrous K_2CO_3, dry acetone at reflux) to give the corresponding alkoxy derivatives **19a–c**, whose cyclocondensation with diethyl carbonate in the presence of potassium *tert*-butoxide (dry toluene, room temperature) afforded the 4-hydroxycoumarins **20a**,**b**,**d**. By the treatment of these compounds and their analogues **20c**[11] and **20e**[12] with a large excess of piperazine at 160 °C, good yields of the corresponding 4-(1-piperazinyl)coumarins **5c–g** were then obtained.

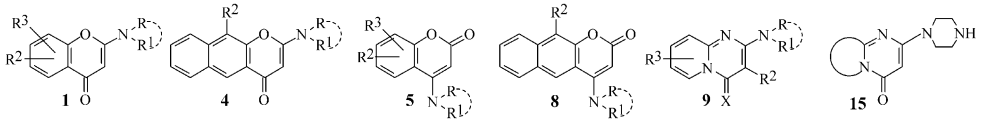
Compound **5h** was not prepared through the above synthetic route due to the difficulty of obtaining a significant amount of the corresponding starting compound **19** (2′-hydroxy-4′,5′-methylenedioxyacetophenone).[13] A very good yield of **5h** was obtained by treating with excess piperazine the 4-chlorocoumarin **23** (ethanol, room temperature), a low amount of which was directly afforded, together with compound **22**, by the reaction of sesamol with the ethyl *N*-phenylmalonamate/$POCl_3$ reagent **21** (chlorobenzene, 110 °C). Compound **22** was in turn nearly completely hydrolysed to the desired **23** (6 N aqueous HCl/tetrahydrofuran 1:1, 100 °C).

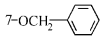
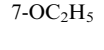
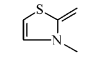
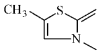
In this reaction (whose mechanism is suggested in Scheme 1)[14] the *N*-monosubstituted reagent **21** shows a particular behaviour with respect to that one we observed in our previous studies where a number of ethyl *N*,*N*-disubstituted malonamate/$POCl_3$ reagents were reacted under similar conditions with phenols or naphthols to give *N*,*N*-disubstituted 2-aminochromones or their benzo-fused derivatives, as main reaction products.[7,15–18]

The substituted 2-(1-piperazinyl)chromone **1b** was prepared as below described (Scheme 2). The 2′-hydroxyacetophenone[19d] was treated under nitrogen with excess carbon disulphide in the presence of potassium *tert*-butoxide (dry toluene, room temperature). After adding water to the reaction mixture and stirring, the corresponding 4-hydroxy-2*H*-1-benzopyran-2-thione was extracted from the previously made acid aqueous phase. The reaction of this raw compound with methyl iodide (anhydrous K_2CO_3, acetone at reflux) afforded the 2-(methylthio)chromone **24** which was finally treated with excess piperazine (ethylene glycol, 160 °C) to give compound **1b** that had previously been reported in a patent.[20]

The 2-(1-piperazinyl)-4*H*-naphtho(2,3-*b*)pyran-4-one **4a** and its 10-methylderivative **4b** were respectively obtained from the reaction of the 2-(methylthio)derivatives **30a** or **30b**[14] with piperazine (ethylene glycol, 160 °C) (Scheme 2). Compound **30a** was prepared starting from 4-hydroxy-2*H*-naphtho(2,3-*b*)pyran-2-one **25**.[21] Actually, the sodium salt (**26**) of latter compound reacted with dimethyl sulphate (acetone at reflux) to give the 4-methoxyderivative **27**, then transformed into 2-thione

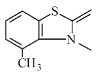
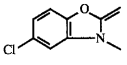
Table 2. In vitro inhibitory activity of compounds **1b**, **4a**,**b**, **5c**–**h**, **8a**,**b**, **9b**,**c**, and **15b**,**e**–**i** on platelet aggregation induced in human PRP by ADP, collagen and A23187



Compd		R^2	R^3	X		IC_{50} (μM) ± SD		
						ADP (5.0 μM)	Collagen (5.0 μg/mL)	A23187 (20.0 μM)
1b			8-CH_3	—	—	137 ± 28	127 ± 35	172 ± 42
4a		H	—	—	—	105 ± 30	159 ± 31	180 ± 18
4b		CH_3	—	—	—	700 ± 132	625 ± 35	900 ± 105
5c		6-OC_2H_5	H	—	—	23.2 ± 6.4	13.4 ± 4.6	12.1 ± 3.5
5d		7-$OCH(CH_3)_2$	H	—	—	5.6 ± 1.4	4.4 ± 2.0	3.8 ± 1.0
5e		7-OCH_3	8-CH_3	—	—	13.0 ± 4.3	6.1 ± 2.1	6.5 ± 2.4
5f		7-OC_2H_5	8-CH_3	—	—	4.0 ± 1.5	5.4 ± 2.0	16.0 ± 4.6
5g			8-CH_3	—	—	1.9 ± 0.2	1.8 ± 0.4	1.1 ± 0.2
5h		6-OCH_2O-7		—	—	86 ± 23	98 ± 37	111 ± 5
8a		H	—	—	—	48 ± 13	60 ± 18	125 ± 16
8b		CH_3	—	—	—	24 ± 4	31 ± 14	32 ± 8
9b		H	H		—	326 ± 16	242 ± 36	850 ± 220
9c		H	H	O	—	> 1000	> 1000	> 1000
15b[a]	—	—	—	—		19.5 ± 4.5	46.4 ± 3.3	51.3 ± 10
15e	—	—	—	—		21 ± 5	50 ± 11	51 ± 16
15f	—	—	—	—		7.4 ± 1.5	11.4 ± 5	22 ± 12
15g	—	—	—	—		5.0 ± 2.2	8.6 ± 3.1	14.0 ± 5.3

(*continued on next page*)

Table 2 (*continued*)

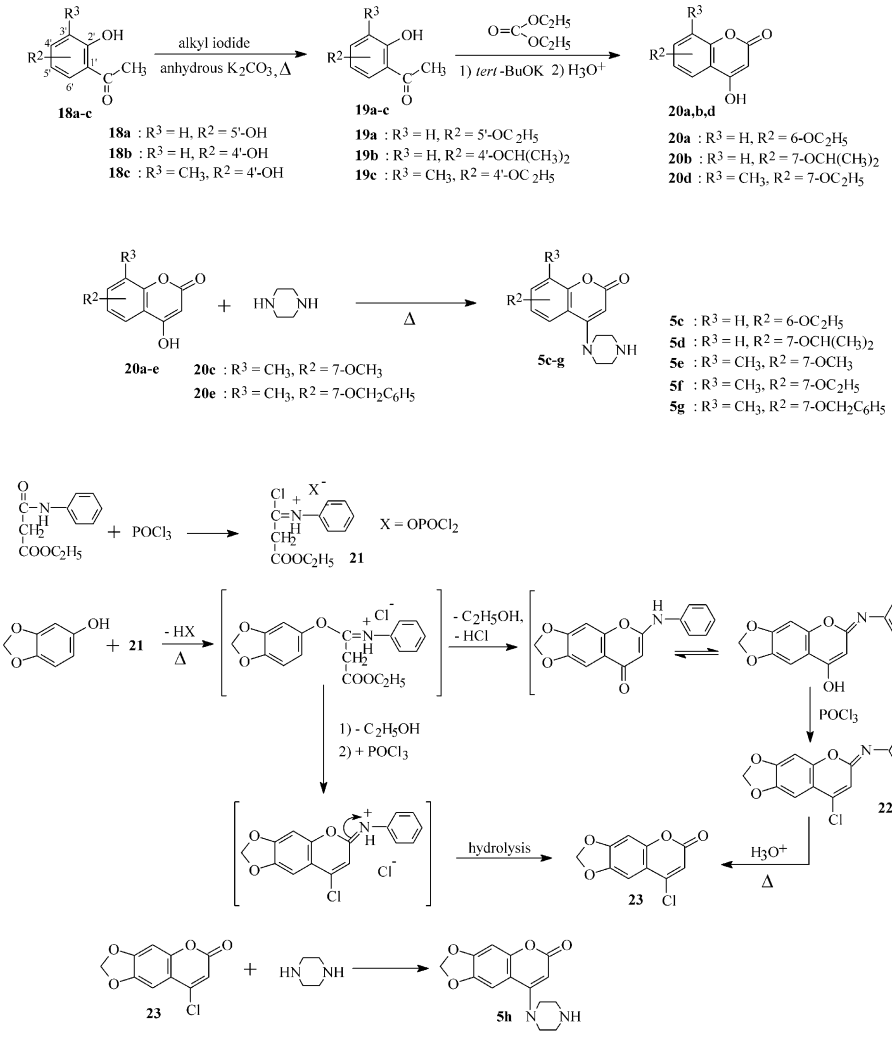
Compd		R^2	R^3	X		IC_{50} (μM) ± SD		
						ADP (5.0 μM)	Collagen (5.0 μg/mL)	A23187 (20.0 μM)
15h	—	—	—	—		213 ± 65	240 ± 116	600 ± 350
15i	—	—	—	—		3.6 ± 1.2	8.8 ± 5.6	13.0 ± 5
ASA						> 1000	159 ± 24	> 1000
Trifluoperazine						180 ± 48	138 ± 41	318 ± 61
Propranolol						291 ± 76	131 ± 31	269 ± 62

[a]The synthesis and IC_{50} values of compound **15b** were reported by us in a previous paper.[9]

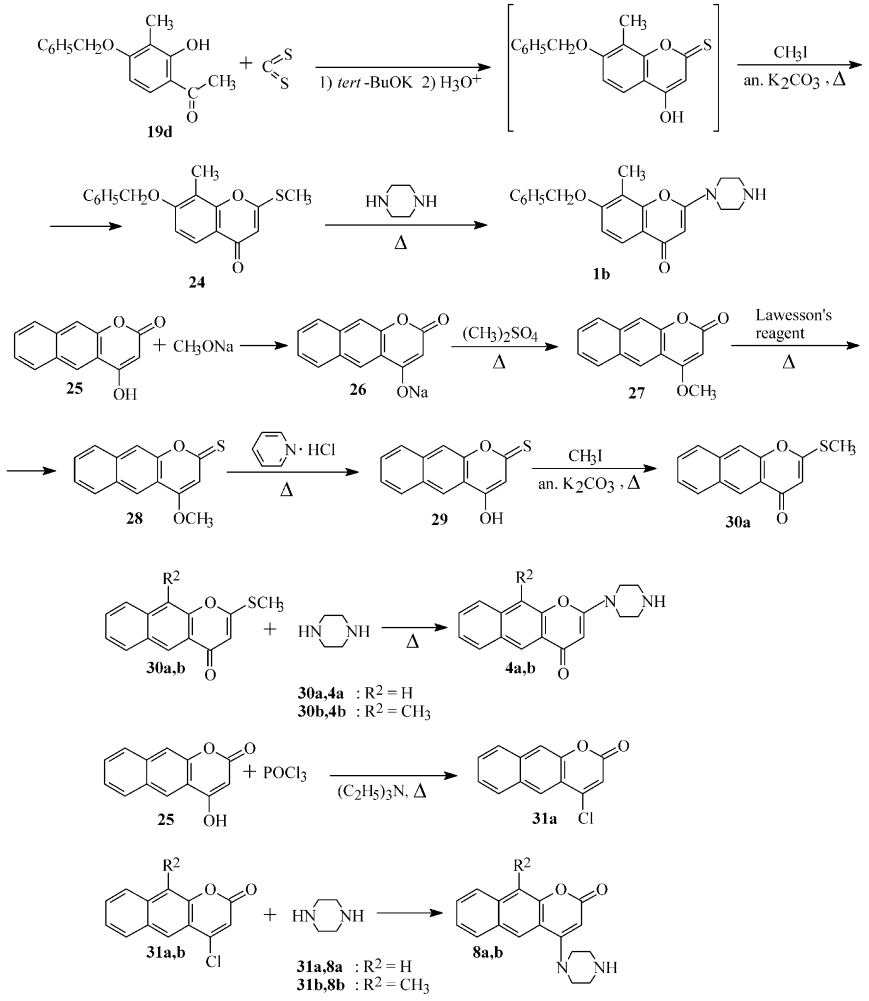
28 by treatment with the Lawesson's reagent in refluxing toluene. The reaction of compound **28** with pyridine hydrochloride (N_2, 230 °C) afforded the 4-hydroxyderivative **29** that was finally treated with excess methyl iodide (anhydrous K_2CO_3, dry acetone at reflux) to give the 2-(methylthio)-4*H*-naphtho(2,3-*b*)pyran-4-one **30a**.

The 4-(1-piperazinyl)-2*H*-naphtho(2,3-*b*)pyran-2-ones **8a**,**b** were prepared by treating the corresponding 4-chloroderivatives **31a** or **31b**,[14] respectively, with an excess of piperazine (ethanol, room temperature). Compound **31a** was obtained by heating (130 °C) the corresponding 4-hydroxyderivative **25**[21] with excess phosphorus oxychloride, in the presence of triethylamine (Scheme 2).

The syntheses of 1,2-fused pyrimidine derivatives **9b**,**c** and **15e**–**i** are depicted in Scheme 3.



Scheme 1.



Scheme 2.

The treatment of the 2-chloro-4*H*-pyrido(1,2-*a*)pyrimidine derivatives **32a**[22] or **32b**[23] with an excess of piperazine or 3-piperidinecarboxylic acid (ethanol at reflux) afforded compounds **9b** or **9c**, respectively.

The reaction of the proper heteroarylamines with ethyl (chloroformyl)acetate (dichloromethane/pyridine, room temperature) gave the ethyl *N*-heteroarylmalonamates **33a**,**d**,**e** which were then heated (130 °C) with a mixture of polyphosphoric acid and phosphorus oxychloride to afford the chloroderivatives **34a**,**d**,**e**, respectively. Compound **34b** was prepared through an analogous route, as previously reported in the literature.[24] On the other hand, the chloroderivative **34c** was obtained by heating in excess phosphorus oxychloride (120 °C) the compound **35** that was in turn afforded by the reaction of 4,5-dimethylthiazol-2-amine with di(2,4,6-trichlorophenyl) malonate (N_2, 180 °C), that is, a method previously reported[25] for the preparation of analogous compounds. Finally, the treatment of chloroderivatives **34a**–**e** with excess piperazine in refluxing ethanol yielded the corresponding (1-piperazinyl)derivatives **15e**–**i**.

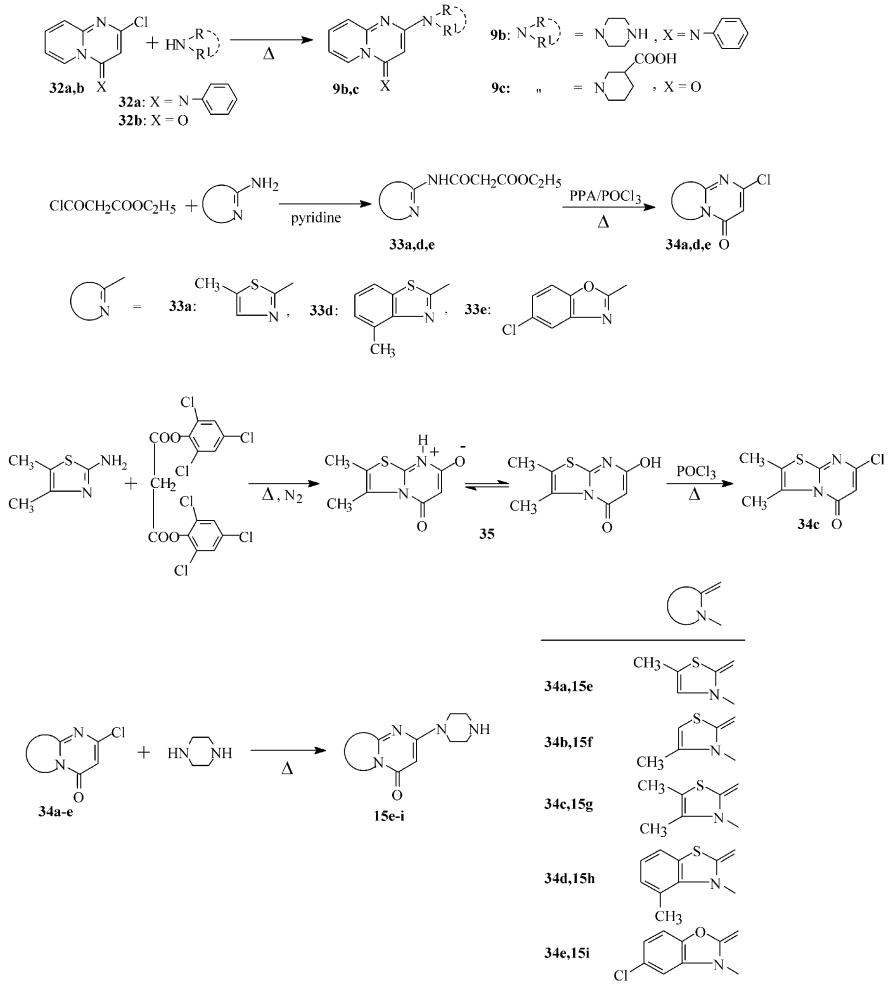
The above intermediates **19a**,[26] **19b**,[27] **23**,[28] **27**,[29] and **35**[30] had been reported before in the literature and were obtained by us in this connection through different or modified procedures.

The results of elemental analyses, and IR and ^{1}H NMR spectral data are consistent with the structures attributed to the compounds described in this paper (see Experimental and Table 4), and the spectral data agree with the ones previously reported by us for analogous compounds.[3,9,14,17]

Results and Discussion

Structure–activity relationships (SARs)

Compounds **1b**, **4a**,**b**, **5c**–**h**, **8a**,**b**, **9b**,**c** and **15e**–**i** were tested in vitro for their inhibitory activity on 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 (i.e., the reference compounds) were tested under the same conditions. According to the antiplatelet activity data obtained (see Table 2) the following observations on the SARs can be made.



Scheme 3.

1-Benzopyran derivatives 1b, 5c–h ($N^{R}_{R1}=N\text{-}NH$). The introduction of a 6- or 7-alkoxy substituent (**5c** or **5b**) increases the activity of **5a** (Tables 1 and 2). Furthermore the antiplatelet activity data afforded by both the series of compounds **5a**, **5b**, **5d** and **5e**, **5f**, **5g** seem to indicate that the effectiveness of 7-alkoxy substituent rises correspondingly with its volume/lipophilicity in the order $-OCH_3 < -OC_2H_5 < -OCH(CH_3)_2$ or $-OCH_2C_6H_5$. In turn, the 8-methyl substituent increases the antiplatelet activity of the 7-alkoxy substituted compounds **5** (compare the activities of compounds **5b** and **5f**). Finally, it must be pointed out that the chromone derivative **1b** is notably less active than its isomer, the coumarin **5g**, which is the most active antiplatelet compound until now synthesised by us, towards all the platelet aggregation inducers used.

Linear naphtho[2,3-*b*]pyran derivatives 4a,b and 8a,b ($N^{R}_{R1}=N\text{-}NH$). In the case of these novel compounds the difference between the antiplatelet activities of coumarin derivative **8a** (higher) and chromone derivative **4a** (lower) is particularly significant compared with the ones previously observed between the activities of their angular isomers **6a** and **2a**, or **7a** and **3a**, respectively (see Table 1). On the other hand, it must be remarked that the introduction of the 10-methyl substituent (**8b**) increases appreciably the antiplatelet activity of **8a**, whereas dramatically lowers that of **4a** (see compound **4b**).

1,2-Fused pyrimidine derivatives 9b,c and 15e–i. Comparison of the inadequate IC_{50} values of compounds **9b** and **9c** with those of lead **9a** (Table 1) further confirms the importance of the 4-carbonyl and 2-(1-piperazinyl) groups, respectively, for a satisfactory in vitro antiplatelet activity of compounds **9**. On the other hand, the IC_{50} values of the previously described[9] 7-(1-piperazinyl)-5*H*-thiazolo(3,2-*a*)pyrimidin-5-one **15b** (see Table 2) and of its novel derivatives **15e-–g**, methyl substituted on the thiazole ring, clearly indicate the influence of the position and number of methyl substituents on the antiplatelet activity of these compounds. On the whole, the tricyclic compound **15i** proved to be the most active of both the previously and here described compounds **15**, and nearly equiactive to compound **9a**.

3-D QSAR study

Comparative molecular field analysis (CoMFA). CoMFA[31] relates the biological activities of a series of molecules with their steric and electrostatic fields calcu-

Table 3. Statistical data of PLS models[a]

Models	Fields	n	ONC	q^2	r^2	s
P_1	Ste	94	4	0.602	0.770	0.361
P_2	Ele	94	4	0.538	0.715	0.402
P_3	Ste + Ele	94	6	0.701	0.883	0.261

[a]For the definition of n, ONC, q^2, r^2, and s see text.

lated at several grid points around the molecule by means of a suitable probe, usually an sp^3 carbon atom with a charge of +1. Partial Least Squares (PLS)[32] is used as the regression method to develop the relationship between steric and electrostatic potentials and biological activity. The graphical representation of CoMFA model, in the form of coefficient isocontour maps, efficiently locates the regions where the variation in steric and electrostatic properties of different molecules in a data set is correlated with the variation of biological activity. CoMFA isocontour maps may furnish useful indications to prioritise future synthesis and to develop sound working hypotheses on the nature of putative ligand-macromolecule interactions.

We previously developed a CoMFA model for a large series of substituted 2-amino-4*H*-pyrido[1,2-*a*]pyrimidin-4-ones, their congeners and isosteric analogues.[9] To derive this model the whole data set was divided in two parts, the *training set* (TS) and the *prediction set* (PS), composed respectively by 73 and 10 molecules. A two fields model (steric + electrostatic) with good fitting and predictive power was obtained: $n = 73$, ONC = 6, $q^2 = 0.682$, $r^2 = 0.874$, $s = 0.242$ where n, ONC, q^2, r^2, and s are the number of data points, the optimal number of components, the squared crossvalidated correlation coefficient, the squared correlation coefficient and the standard deviation of regression equation, respectively.

Incorporation of the training and prediction sets in a unique data set resulted in a model with comparable statistic: $n = 83$, ONC = 6, $q^2 = 0.662$, $r^2 = 0.866$, $s = 0.245$.

In the present work, 11 of the 18 newly synthesized compounds were included in a new TS ($n = 94$), whereas six were chosen for the PS. The PS was built with compounds presenting an acceptable molecular diversity and a good spread of pIC_{50} values (referred to collagen as platelet aggregation inducer).

Molecular modelling and alignment were carried out following methods and procedures described in our previous paper.[9]

The statistical parameters of the models derived for the new TS, on protonated molecules accordingly with previous findings, are listed in Table 3.

PLS models obtained for the new PS showed a slight increase in terms of fitting and predictive power with respect to the previously published ones. It is worth noting that the two field model gives better statistical indices than the ones derived considering only steric or electrostatic fields even considering the same ONC (P_3 ONC = 4, $q^2 = 0.655$, $r^2 = 0.811$, $s = 0.328$).

Model P_3 was therefore selected to test its predictive ability by comparing the observed versus the predicted pIC_{50} values of the six newly synthesized compounds included in the PS. Five out of six compounds of the PS showed a difference between the observed and the predicted pIC_{50} values within the double of standard error of the PLS analysis (**1b** obs = 3.90 pred = 3.82; **9b** obs = 3.62 pred = 3.94; **15e** obs = 4.30 pred = 4.26; **15g** obs = 5.06 pred = 4.56; **15h** obs = 3.62 pred = 4.30), whereas the prediction for compound **15i** (obs = 5.06 pred = 3.94) was very bad. This result could be, at least in part, justified considering that **15i** is the only compound showing a 3,4-fused highly lipophilic chlorobenzene moiety.

Model P_3 was also used to develop the coefficient isocountour maps for the steric and electrostatic field depicted in Figures 1 and 2, respectively.

The steric isocontour map obtained from the PLS analysis performed on the new and larger data set ($n = 94$) highlights the same sterically accessible (green polyhedra) and hindered (red polyhedra) regions already identified in the previous published PLS model based on a lower number of compounds ($n = 73$). In addition to that, the current P_3 model allows the identification of new sterically permitted regions, occupied by the isopropoxy and methyl group of the highly active compounds **5d** and **15f**, respectively (see Fig. 1). On the contrary, the aromatic moiety of the low active compound **9d**[4] ($N(R)(R1) = N{-}C_6H_5$, X = O, R2 = R3 = H) and the alkylamino chain of compound **9e**[9] ($N(R)(R1) = HNCH_2CH_2NH_2$, X = O, R2 = R3 = H) may face unfavourable steric hindrance.

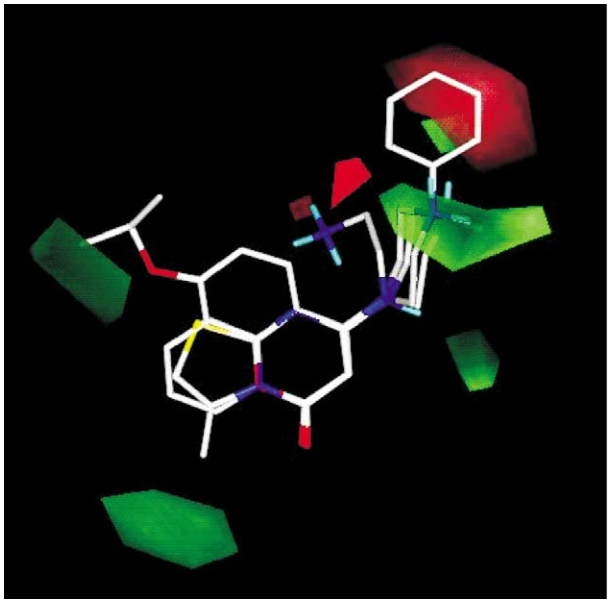


Figure 1. STDEVxCOEFF isocontour steric plots for models P_3 (for the colour code see text). Contour levels as follows: *red* 0.035; *green* 0.025; compounds **5d**, **15f**, **9d**,[4] and **9e**[9] are shown to help interpretation.

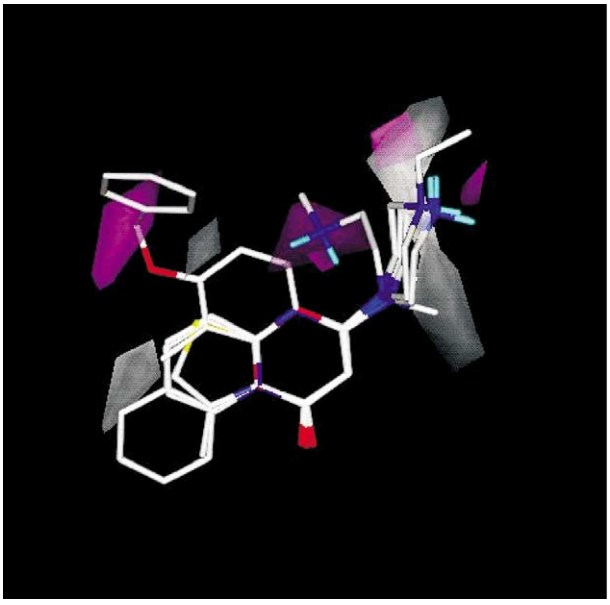


Figure 2. STDEVxCOEFF isocontour electrostatic plots for models P_3 (for the colour code see text). Contour levels as follows: *magenta* 0.020; *white-grey* 0.035; compounds **5g**, **15f**, **9f**,[9] and **2b**[3] are shown to help interpretation.

As far as the electrostatic contour map is concerned, regions where a high electron density might favours/disfavours the biological activity are indicated by magenta and white-grey colours, respectively (see Fig. 2). Interestingly, also in the analysis of the electrostatic isocontour map additional, significant signals in comparison to the previously published model can be detected. Indeed, the electron rich benzyloxy group of compound **5g** is located in a new magenta region whereas the nitrogen and sulphur atoms of the heterocyclic moiety of compound **15f** are facing a similar, but differently located zone.

The same zone is occupied by the positive charged nitrogen of compound **9f**[9] ($N(R)(R) = CH_3NCH_2CH_2NHCH_3$, $X = O$, $R^2 = R^3 = H$), justifying thus its low pIC_{50} value.

Finally, the white-grey regions on the left-hand side of Figure 2 are occupied by aromatic rings of low activity molecules, for example, compound **2b**[3] ($N(R)(R) = N\langle\rangle N-C_2H_5$).

Conclusions

The human platelet aggregation in vitro inhibitory properties shown by the compounds described in this paper (Table 2) suggest the following final remarks on the SARs.

1. The importance of the presence of a β-enaminonic moiety containing 1-piperazinyl as amino group, for a satisfactory antiplatelet activity in this structural field, has been further confirmed.
2. By replacing the fused pyridine ring of **9a** with a proper and properly substituted heteroaromatic ring very active compounds can be obtained. Actually, the 1,2-fused pyrimidine derivatives **15g** and **15i** are almost as active as the lead **9a**.
3. The platelet antiaggregating activity of 4-(1-piperazinyl)coumarin **5a** can be highly increased by the introduction of suitable substituents in proper positions of the fused benzene ring, as we have now clearly observed in the case of compounds **5d** and **5f**, on the whole equiactive to **9a**, and particularly of **5g** which proved to be a very interesting in vitro inhibitor of human platelet aggregation, more active than **9a**.

On the other hand, it can be noted that the 3-D QSAR study of the antiplatelet activity of this large set of compounds (one hundred) gave further insight on the main physicochemical features governing the anti-aggregating inhibitory properties in this chemical field. In particular, the current study allowed the identification of new steric and electrostatic interactions which seem to take place in well definite spatial locations differing from the ones identified in the previously published CoMFA study. Unfortunately, the lack of parameterisation of diverse fragments for the MLP calculations has not allowed to introduce the MLP[33] as third field in our CoMFA study. Therefore, an eventual influence of lipophilic interactions on the antiplatelet activity of the analysed compound cannot be ruled out.

Experimental

Chemistry

Melting points were determined using a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 398 spectrophotometer for compounds **20a**,**b**, **5c**,**e**–**h**, **22**, **23**, **24**, **27**, **28**, **30a**, **4a**,**b**, **31a**, **8a**,**b**, **9b**, **33e**, **34a**,**c**–**e**, **15e**,**g**–**i**, and on a Perkin–Elmer 'Spectrum One' spectrophotometer for **19a**–**c**, **20d**, **5d**, **29**, **9c**, **33a**,**d**, **35**, **15f** (abbreviations relative to IR bands: br = broad, s = strong, w = weak, sh = shoulder). ^{1}H NMR spectra were recorded on a Hitachi Perkin-Elmer R 600 (60 MHz) spectrometer for compounds **19c**, **20a**,**b**,**d**, **5c**–**f**, **22**, **24**, **27**, **28**, **4a**,**b**, **8a**,**b**, **33a**,**e**, **34a**,**c**–**e**, **15e**–**h**, and on a Varian Gemini 200 (200 MHz) spectrometer for **19a**,**b**, **5g**, **23**, **1b**, **29**, **30a**, **31a**, **9b**,**c**, **33d**, **35**, **15i**, 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 were performed, for the indicated elements, by the Laboratorio di Microanalisi del Dipartimento di Scienze Farmaceutiche, University of Genoa, and the results were within ±0.4% of the theoretical values.

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).

Synthesis of substituted acetophenones 19a–c. A mixture of 1-(2,5-dihydroxyphenyl)ethanone **18a** (3.80 g, 25.0 mmol), 1-(2,4-dihydroxyphenyl)ethanone **18b** (3.80 g,

25.0 mmol), or 1-(2,4-dihydroxy-3-methylphenyl)ethanone **18c** (4.15 g, 25.0 mmol), 30.0 mmol of the proper alkyl halide, 5 g of anhyd K_2CO_3 and acetone (150 mL) was heated at reflux for 5 h, with stirring. The mixture was then poured into cold water (500 mL) and exhaustively extracted with ethyl ether. The combined extracts were washed with water, dried over anhyd sodium sulfate, and solvent was removed under reduced pressure. The resulting residue was chromatographed on a silica gel column, eluting with dichloromethane–petroleum ether (1:1) to give, after removal of solvents from the eluate collected, the nearly pure compound **19a** (3.11 g, 69%), **19b** (3.50 g, 72%), or **19c** (3.36 g, 69%), respectively.

1-(5-Ethoxy-2-hydroxyphenyl)ethanone 19a. Obtained from reaction of **18a** with iodoethane (4.68 g); yellow crystals, mp 59–60 °C (petroleum ether) (lit.[26] mp 57 °C). ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H, CH_2CH_3), 2.61 (s, 3H, $COCH_3$), 4.00 (q, 2H, CH_2CH_3), 6.90 (d, $J=9$ Hz, 1H, H-3), 7.05–7.22 (m, 2H, H-4,6), 11.84 (s, 1H, OH; disappeared with D_2O). IR ($CHCl_3$) 3540 w (OH), 3280–2680 br (chelated OH), 1645 (CO), 1620, 1590, 1490 cm^{-1}. Anal. ($C_{10}H_{12}O_3$) C, H.

1-(2-Hydroxy-4-isopropoxyphenyl)ethanone 19b. Obtained from reaction of **18b** with 2-iodopropane (5.10 g); colourless liquid, bp 105–106 °C (0.3 mM Hg). ^{1}H NMR ($CDCl_3$) δ 1.35 [d, 6H, $CH(CH_3)_2$], 2.54 (s, 3H, $COCH_3$), 4.62 [m, 1H, $CH(CH_3)_2$], 6.36–6.45 (m, 2H, H-3,5), 7.62 (d, $J=9$ Hz, 1H, H-6), 12.73 (s, 1H, OH; disappeared with D_2O). IR ($CHCl_3$) 3550 w (OH), 3250–2670 br (chelated OH), 1630 s (CO), 1580, 1500 cm^{-1}. Anal. ($C_{11}H_{14}O_3$) C, H.

1-(4-Ethoxy-2-hydroxy-3-methylphenyl)ethanone 19c. Obtained from reaction of **18c** with iodoethane (4.68 g); white crystals, mp 50–51 °C (petroleum ether). ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, CH_2CH_3), 2.08 (s, 3H, 3-CH_3), 2.53 (s, 3H, $COCH_3$), 4.08 (q, 2H, CH_2CH_3), 6.41 (d of AB q, $J=9$ Hz, 1H, H-5), 7.57 (d of AB q, $J=9$ Hz, 1H, H-6), 12.83 (s, 1H, OH; disappeared with D_2O). IR ($CHCl_3$) 3540 w (OH), 3230–2600 br (chelated OH), 1620 br (CO), 1580 sh, 1500 cm^{-1}. Anal. ($C_{11}H_{14}O_3$) C, H.

Synthesis of substituted 4-hydroxy-2*H*-1-benzopyran-2-ones 20a,b,d. To a stirred suspension of potassium *tert*-butoxide (3.37 g, 30.0 mmol) in 50 mL of anhyd toluene, under nitrogen, a solution of 10.0 mmol of the proper substituted acetophenone **19a**, **19b**, or **19c** and 20.0 mmol (2.36 g) of diethyl carbonate in anhyd toluene (50 mL) was slowly added. The resulting yellow-brown viscous mixture was stirred under nitrogen at room temperature overnight. Cold water (500 mL) and ethyl ether (200 mL) were then added to the mixture, that was further stirred few min, then allowed to stand and reach phase separation. The aqueous phase was collected and the organic one extracted twice with water, then discarded. The whole aqueous phase was acidified with 6 N aq HCl: this way compounds **20a**,**b**,**d** separated out as whitish solids that were collected by filtration, washed with water, dried and crystallized from the proper solvent. Their physical and chemical data are reported in Table 4.

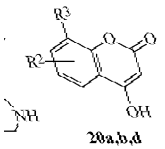
General procedure for synthesis of substituted 4-(1-piperazinyl)-2*H*-1-benzopyran-2-ones 5c–g. A mixture of 3.0 mmol of the proper compound **20** [**20a** (0.62 g), **20b** (0.66 g), **20c**[11] (0.62 g), **20d** (0.66 g), or **20e**[12] (0.85 g)] and an excess (7.0 g) of piperazine was stirred at 160 °C for 1 h, then poured into ice-water. The resulting solution was exhaustively extracted with chloroform. The combined extracts (dried over anhyd sodium sulfate) were evaporated to dryness under reduced pressure to give a thick oil from which, after treatment with a little ethyl ether, nearly pure compound **5** separated out as a whitish solid which was then crystallized from the suitable solvent (compounds **5c,e–g**). Only in the case of **5d** the final oily residue was treated with a solution of 3.0 mmol (0.35 g) of maleic acid in anhyd ethanol to give pure **5d** maleate (**5d**·$H_4C_4O_4$) as a white solid which was then crystallized from anhyd ethanol.

The physical and chemical data of compounds **5c–g** are reported in Table 4.

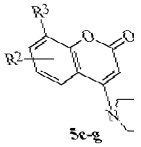
8-Chloro-*N*-phenyl-6*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-6-imine (22) and 8-chloro-6*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-6-one (23). Phosphorus oxychloride (75.0 mmol, 11.50 g) was added dropwise with stirring to an ice-cooled solution of 55.0 mmol (11.40 g) of ethyl *N*-phenylmalonamate[34] in 10 mL of chlorobenzene. The resulting solution was stirred at room temperature for 1 h, then a suspension of 50.0 mmol (6.91 g) of sesamol in 50 mL of chlorobenzene was added and the mixture was heated at 110 °C for 4 h, while stirring. After cooling, a solution of 68 g of trihydrate sodium acetate in 150 mL of water was added and the resulting mixture was stirred at 60 °C for 1 h. The organic layer was then collected and the aqueous one was exhaustively extracted with chloroform. The combined organic phases were dried (anhyd sodium sulfate) and the solvents removed in vacuo to give a dark reddish thick oil which was chromatographed on an aluminium oxide column eluting with toluene–ethyl ether (1:1). The eluate collected, after removal of solvents, afforded an orange thick oil which was treated with 6 N aq HCl (100 mL) and ethyl ether (100 mL), stirring then vigorously at room temperature for few min. This way the nearly pure hydrochloride of the imine **22** separated out as a yellow solid which was collected by filtration, washed with water and a little ethyl ether, then dissolved in a mixture of 10% aq Na_2CO_3 (200 mL) and dichloromethane (200 mL), by stirring. The organic layer was then collected and the aqueous one was extracted twice with dichloromethane. The combined organic phases, after drying and removal of solvent, gave an oily residue from which, by treatment with a little petroleum ether, compound **22** separated out as a yellow solid (4.49 g, 30%); mp 101–102 °C (isopropyl ether). ^{1}H NMR ($CDCl_3$) δ 6.00 (s, 2H, OCH_2O), 6.30–7.60 (m, 7H, H-4,9 + phenyl H's), 6.58 (s, 1H, H-7). IR ($CHCl_3$) 1652 (C=N), 1628, 1596 w, 1584, 1505 w cm^{-1}. Anal. ($C_{16}H_{10}ClNO_3$) C, H, N, Cl.

The mother liquor of the crude **22**·HCl was transferred to a separatory funnel, the ethereal phase was collected and the aqueous one was then extracted thoroughly with ethyl ether. The combined organic phases, after

Table 4. Physical and chemical data of compounds **20a**,**b**,**d** and **5c**–**g**



20a,b,d



5c–g

Compd[a]	Yield (%)	Mp (°C) (solvent)[b]	Molecular formula[c]	IR[d] (cm^{-1})	^1H NMR[e] (δ, ppm)
20a	80	240–242 dec. (A)	$C_{11}H_{10}O_4$	3200–2400 s, br, complex (OH), 1670 s, br (CO), 1611, 1580 s, 1550, 1503 w.	1.38 (t, 3H, CH_2CH_3), 4.10 (q, 2H, CH_2CH_3), 5.62 (s, 1H, H-3), 7.05–7.40 (m, 3H, H-5,7,8), 11.50[f] (broad s, 1H, OH).
20b	59	224–225 (B)	$C_{12}H_{12}O_4$	3150–2530 s, br, complex (OH), 1680 s, br (CO), 1620 s, 1605 sh, 1543, 1515 w.	1.35 [d, 6H, $CH(CH_3)_2$], 4.76 [m, 1H, $CH(CH_3)_2$], 5.50 (s, 1H, H-3), 6.73–7.08 (m, 2H, H-6,8), 7.83 (d, $J=9$ Hz, 1H, H-5), 11.50[f] (broad s, 1H, OH).
20d	69	225–226 (B)	$C_{12}H_{12}O_4$	3150–2450 s, br, complex (OH), 1660 s (CO), 1610 s, br, 1560, 1515.	1.40 (t, 3H, CH_2CH_3), 2.17 (s, 3H, 8-CH_3), 4.16 (q, 2H, CH_2CH_3), 5.48 (s, 1H, H-3), 6.99 (d of AB q, $J=9$ Hz, 1H, H-6), 7.69 (d of AB q, $J=9$ Hz, 1H, H-5), 10.70[f] (broad s, 1H, OH).
5c	69	130–131 (B)	$C_{15}H_{18}N_2O_3$	3360 w (NH), 1692 s, br (CO), 1597 w, 1555 s, br.	1.44 (t, 3H, CH_2CH_3), 1.90[f] (s, 1H, NH), 2.90–3.43 (m, 8H, piperazine CH_2's), 4.18 (q, 2H, CH_2CH_3), 5.72 (s, 1H, H-3), 6.90–7.46 (m, 3H, H-5,7,8).
5d	55	169–171 (C)	$C_{16}H_{20}N_2O_3 \cdot C_4H_4O_4$	3320 w (NH), 1710 s, br (CO), 1613 s, br, 1543, 1506 w.	1.40 [d, 6H, $CH(CH_3)_2$], 2.19[f] (s, 1H, NH), 2.95–3.40 (m, 8H, piperazine CH_2's), 4.65 [m, 1H, $CH(CH_3)_2$], 5.60 (s, 1H, H-3), 6.64–6.97 (m, 2H, H-6,8), 7.55 (d, $J=9$ Hz, 1H, H-5).
5e	57	212–213.5 (B)	$C_{15}H_{18}N_2O_3$	3323 (NH), 1685 s, br (CO), 1600 s, br, 1550, 1498 w.	1.87[f] (s, 1H, NH), 2.27 (s, 3H, 8-CH_3), 2.93–3.43 (m, 8H, piperazine CH_2's), 3.93 (s, 3H, OCH_3), 5.62 (s, 1H, H-3), 6.84 (d of AB q, $J=9$ Hz, 1H, H-6), 7.50 (d of AB q, $J=9$ Hz, 1H, H-5).
5f	72	172–172.5 (B)	$C_{16}H_{20}N_2O_3$	3325 (NH), 1696 s, br (CO), 1615 s, 1606 sh, 1560, 1504 w.	1.47 (t, 3H, CH_2CH_3), 1.83[f] (s, 1H, NH), 2.30 (s, 3H, 8-CH_3), 2.90–3.38 (m, 8H, piperazine CH_2's), 4.15 (q, 2H, CH_2CH_3), 5.61 (s, 1H, H-3), 6.82 (d of AB q, $J=9$ Hz, 1H, H-6), 7.46 (d of AB q, $J=9$ Hz, 1H, H-5).
5g	60	154–155 (B)	$C_{21}H_{22}N_2O_3$	3300 w (NH), 1700 s, br (CO), 1603 s, br, 1558, 1498 w.	1.88[f] (s, 1H, NH), 2.36 (s, 3H, 8-CH_3), 3.02–3.26 (m, 8H, piperazine CH_2's), 5.18 (s, 2H, $CH_2C_6H_5$), 5.61 (s, 1H, H-3), 6.82 (d of AB q, $J=9$ Hz, 1H, H-6), 7.30–7.50 (m, 6H, H-5 + phenyl H's).

[a]The data of compound **5d** refer to the maleate, except for the IR and ^1H NMR data that concern the free base.
[b]Crystallization solvent: A = ethanol, B = ethyl acetate, C = anhyd ethanol.
[c]Anal. C,H,N. (C, H for **20a**,**b**,**d**).
[d]In KBr pellets except for **5d** (film).
[e]In $CDCl_3$ solutions for compounds **5c**–**g**, in DMSO-d_6 solutions for **20a**,**b**,**d**.
[f]Disappeared with D_2O.

drying and removal of solvent, gave an oil which was chromatographed on a silica gel column eluting with dichloromethane. From the eluate collected, after evaporation to dryness in vacuo, a solid residue was obtained which was taken up in a little isopropyl ether and filtered to give pure compound **23** (0.20 g, 1.8%); white crystals, mp 188–189 °C (ethyl acetate) (lit.[28] mp 183–184 °C). ^{1}H NMR ($CDCl_3$) δ 6.11 (s, 2H, OCH_2O), 6.46 (s, 1H, H-7), 6.84 (s, 1H, H-4), 7.22 (s, 1H, H-9). IR ($CHCl_3$) 1743 sh, 1726 s (CO), 1634, 1574, 1505 cm^{-1}. Anal. ($C_{10}H_5ClO_4$) C, H, Cl.

Hydrolysis of compound 22 to 23. A suspension of 10.0 mmol (3.36 g) of **22** hydrochloride in 100 mL of tetrahydrofuran and 100 mL of 6 N aq HCl was heated at reflux for 90 min, while stirring. After cooling, the solution obtained was poured into water (1 L) so that an ivory-white solid separated out. The suspension was stirred at room temperature for 1 h, then filtered to give pure compound **23** that was washed with water and dried (2.04 g, 91%).

8-(1-Piperazinyl)-6*H*-1,3-dioxolo[4,5-g][1]benzopyran-6-one (5h). The mixture of 3.0 mmol (0.67 g) of chlorocoumarin **23**, 30.0 mmol (2.58 g) of piperazine and 100 mL of ethanol was stirred at room temperature for 2 h. The solution obtained was poured into water (300 mL) and the mixture exhaustively extracted with chloroform. The combined extracts, after drying and removal of solvents, afforded a solid residue which was taken up in a little ethyl ether and filtered to yield pure compound **5h** (0.67 g, 81%); white crystals, mp 191.5–192 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 1H, NH; disappeared with D_2O), 3.00–3.25 (m, 8H, piperazine CH_2's), 5.65 (s, 1H, H-7), 6.06 (s, 2H, OCH_2O), 6.81 (s, 1H, H-4), 6.96 (s, 1H, H-9). IR (KBr) 3340 w (NH), 1685 s, br (CO), 1630, 1562, 1510 w cm^{-1}. Anal. ($C_{14}H_{14}N_2O_4$) C, H, N.

7-Benzyloxy-8-methyl-2-(methylthio)-4*H*-1-benzopyran-4-one (24). To a suspension of potassium *tert*-butoxide (5.05 g, 45.0 mmol) in 50 mL of dry toluene, stirred under nitrogen, a solution of 15.0 mmol (3.84 g) of the substituted acetophenone **19d**[19] and 16.5 mmol (1.26 g) of carbon disulphide in dry toluene (50 mL) was slowly added. The resulting orange-yellow viscous mixture was stirred under nitrogen at room temperature overnight. Cold water (500 mL) was then added to this slurry and the resulting mixture was transferred to a separatory funnel, then extracted with ethyl ether. The organic layer was discarded and the orange aqueous solution was collected, then acidified with 6 N aq HCl so that a yellowish solid separated out. The suspension was allowed to stir at room temperature for 1 h (under a fume hood to remove hydrogen sulphide), then subjected to exhaustive extraction with the mixture ethyl ether–tetrahydrofuran (1:1). The solvents were then removed from the dried combined extracts to give a brownish solid residue containing the desired 7-benzyloxy-8-methyl-2*H*-1-benzopyran-2-thione along with some starting compound **19d** and impurities. This raw solid was transferred to a flask and dry acetone (100 mL), anhyd K_2CO_3 (1.40 g), iodomethane (2.0 mL) were added, stirring then the mixture at reflux for 1 h. After cooling, the solvent was removed in vacuo and the residue partitioned between water and dichloromethane. The organic layer was collected and the aqueous one was extracted twice with dichloromethane. The oily residue obtained from the combined extracts after removal of solvents was chromatographed on a silica gel column eluting first with dichloromethane to remove compound **19d** and impurities, then with the mixture dichloromethane–ethyl acetate (1:1) to recover the desired compound **24**. This latter eluate, evaporated to dryness in vacuo, afforded the pure **24** as a pale-brown solid (0.75 g, 16%); whitish crystals, mp 144–145 °C (ethyl acetate–ethyl ether with charcoal). ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, 8-CH_3), 2.52 (s, 3H, SCH_3), 5.17 (s, 2H, $CH_2C_6H_5$), 6.14 (s, 1H, H-3), 6.99 (d of AB q, J = 9 Hz, 1H, H-6), 7.22–7.63 (m, 5H, phenyl H's), 8.01 (d of AB q, J = 9 Hz, 1H, H-5). IR ($CHCl_3$) 1620 s, br (CO), 1587, 1548 cm^{-1}. Anal. ($C_{18}H_{16}O_3S$) C, H, S.

7-Benzyloxy-8-methyl-2-(1-piperazinyl)-4*H*-1-benzopyran-4-one (1b). A mixture of 2.0 mmol (0.62 g) of **24**, 20.0 mmol (1.72 g) of piperazine and 10 mL of ethylene glycol was heated at 160 °C for 1 h, with stirring. The solution finally obtained was poured onto ice-water and the resulting mixture was thoroughly extracted with chloroform. The combined extracts, after drying and removal of solvent, afforded an oily residue from which, after treatment with a little ethyl ether and standing, the nearly pure compound **1b** separated out (0.39 g, 56%); whitish crystals, mp 173–174 °C (ethyl acetate) (lit.[20] mp 165–170 °C). ^{1}H NMR ($CDCl_3$) δ 1.81 (s, 1H, NH; disappeared with D_2O), 2.32 (s, 3H, 8-CH_3), 3.01 (m, 4H, CH_2NHCH_2), 3.51 (m, 4H, CH_2NCH_2), 5.18 (s, 2H, $CH_2C_6H_5$), 5.44 (s, 1H, H-3), 6.97 (d of AB q, J = 9 Hz, 1H, H-6), 7.30–7.50 (m, 5H, phenyl H's), 7.97 (d of AB q, J = 9 Hz, 1H, H-5). IR (KBr) 3270 (NH), 1625 sh, 1610 s (CO), 1585, 1558 s, 1498 w cm^{-1}. Anal. ($C_{21}H_{22}N_2O_3$) C, H, N.

4-Methoxy-2*H*-naphtho[2,3-*b*]pyran-2-one (27). Sodium (0.09 g, 4.0 mmol) was dissolved in 100 mL of dry methanol; compound **25**[21] (0.85 g, 4.0 mmol) was then added and the resulting solution was evaporated to dryness in vacuo. The sodium salt **26** so obtained was taken up in a little anhyd ethyl ether, collected by filtration and dried, then transferred to a flask and suspended in 100 mL of dry acetone. Dimethyl sulfate (1.01 g, 8.0 mmol) was added and the mixture was then refluxed with stirring for 3 h. After cooling, the mixture was poured into water (300 mL) and the whitish solid that separated out was recovered by filtration, washed with water and dried. There was so obtained pure compound **27** (0.65 g, 72%); white crystals, mp 209–210 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 4.04 (s, 3H, OCH_3), 5.73 (s, 1H, H-3), 7.27–8.10 (m, 4H, H-6,7,8,9), 7.70 (s, 1H, H-10), 8.32 (s, 1H, H-5). IR (KBr) 1728 s, br (CO), 1638, 1618, 1596, 1567, 1510 w cm^{-1}. Anal. ($C_{14}H_{10}O_3$) C,H.

4-Methoxy-2*H*-naphtho[2,3-*b*]pyran-2-thione (28). A mixture of compound **27** (0.68 g, 3.0 mmol), Lawesson's reagent (1.82 g, 4.5 mmol) and 40 mL of anhyd toluene was refluxed for 3 h, while stirring. The solvent was then

removed in vacuo and the residue subjected to column chromatography on an aluminium oxide column, eluting with dichloromethane. The eluate collected, evaporated to dryness in vacuo, afforded the pure compound **28** (0.64 g, 88%); yellow crystals, mp 186–187 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 4.06 (s, 3H, OCH_3), 6.80 (s, 1H, H-3), 7.23–8.10 (m, 4H, H-6,7,8,9), 7.80 (s, 1H, H-10), 8.31 (s, 1H, H-5). IR (KBr) 1631, 1611, 1588, 1548, 1105 s (CS) cm^{-1}. Anal. ($C_{14}H_{10}O_2S$) C, H, S.

4-Hydroxy-2*H*-naphtho[2,3-*b*]pyran-2-thione (29). A mixture of compound **28** (0.73 g, 3.0 mmol) and 15 g of pyridine hydrochloride was heated at 230 °C, under nitrogen. The solution obtained was further stirred at 230 °C for 5 min, then allowed to cool. The resulting solid mixture was treated with water (50 mL) at room temperature until the nearly pure compound **29** separated out as amorphous solid which was recovered by filtration, washed with water and dried (0.67 g, 98%); yellow solid, mp 223–225 °C. (tetrahydrofuran/petroleum ether). ^{1}H NMR (DMSO-d_6) δ 6.69 (s, 1H, H-3), 7.62 and 7.68 (2 dd, 1H + 1H, H-7,8), 8.05 and 8.21 (2 near d, 1H + 1H, H-6,9), 8.07 (s, 1H, H-10), 8.56 (s, 1H, H-5); OH signal was not detectable. IR (KBr) 3100–2400 s, br, complex (OH), 1634, 1609, 1593, 1552, 1536, 1088 s (CS) cm^{-1}. Anal. ($C_{13}H_8O_2S$) C, H, S.

2-(Methylthio)-4*H*-naphtho[2,3-*b*]pyran-4-one (30a). A mixture of 3.0 mmol (0.68 g) of thione **29**, 0.70 g of anhyd K_2CO_3, 1.0 mL of iodomethane and 50 mL of dry acetone was stirred at reflux for 1 h. After cooling, the solvent was removed in vacuo and the residue partitioned between water and dichloromethane. The organic layer was collected and the aqueous one was extracted twice with dichloromethane. From the combined extracts, after removal of solvent, an oily residue was obtained which was chromatographed on a silica gel column eluting first with dichloromethane to remove some impurities, then with the mixture dichloromethane–ethyl acetate (1:1). The eluate collected, evaporated to dryness in vacuo, afforded the pure **30a** as a pale-brown solid (0.55 g, 74%); whitish crystals, mp 155–156 °C (isopropyl ether with charcoal). ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H, SCH_3), 6.21 (s, 1H, H-3), 7.52 and 7.63 (2 dd, 1H + 1H, H-7,8), 7.86 (s, 1H, H-10), 7.91 and 8.04 (2 near d, 1H + 1H, H-6,9), 8.76 (s, 1H, H-5). IR ($CHCl_3$) 1650, 1627 s (CO), 1608, 1581 w, 1555, 1503 w cm^{-1}. Anal. ($C_{14}H_{10}O_2S$) C, H, S.

Preparation of 2-(1-piperazinyl)-4*H*-naphtho[2,3-*b*]pyran-4-one (4a) and 10-methyl-2-(1-piperazinyl)-4*H*-naphtho[2,3-*b*]pyran-4-one (4b). A mixture of 1.5 mmol of the (methylthio) derivative **30a** (0.37 g) or **30b**[14] (0.39 g), 15.0 mmol (1.29 g) of piperazine and 10 mL of ethylene glycol was heated at 160 °C for 1 h, with stirring. The solution obtained was poured onto ice-water and the resulting mixture was thoroughly extracted with chloroform. The combined extracts, after drying and removal of solvent, afforded a nearly solid residue which was taken up in a little ethyl ether and filtered to yield compound **4a** (0.25 g, 59%) or **4b** (0.33 g, 75%), respectively.

Compound 4a. Whitish crystals, mp 197–199 °C dec. (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 1H, NH; disappeared with D_2O), 2.99 (m, 4H, CH_2NHCH_2), 3.59 (m, 4H, CH_2NCH_2), 5.52 (s, 1H, H-3), 7.20–8.20 (m, 4H, H-6,7,8,9), 7.71 (s, 1H, H-10), 8.73 (s, 1H, H-5). IR (KBr) 3290 w (NH), 1643, 1596 s, br (CO), 1564 s, 1502 w cm^{-1}. Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

Compound 4b. Whitish crystals, mp 234–235 °C dec. (dichloromethane/ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 1.89 (s, 1H, NH; disappeared with D_2O), 2.67 (s, 3H, 10-CH_3), 3.01 (m, 4H, CH_2NHCH_2), 3.60 (m, 4H, CH_2NCH_2), 5.51 (s, 1H, H-3), 7.24–7.72 (m, 2H, H-7,8), 7.86–8.20 (m, 2H, H-6,9), 8.62 (s, 1H, H-5). IR (KBr) 3285 w (NH), 1633 w, 1592 s, br (CO), 1560 s, br, 1502 w cm^{-1}. Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

4-Chloro-2*H*-naphtho[2,3-*b*]pyran-2-one (31a). A mixture of compound **25**[21] (0.64 g, 3.0 mmol), triethylamine (0.30 g, 3.0 mmol) and phosphorus oxychloride (3.0 mL) was heated at 130 °C for 1 h, while stirring. The dark solution obtained was poured onto ice-water and the resulting mixture was stirred few min at room temperature, then thoroughly extracted with dichloromethane. The combined extracts, after drying and removal of solvent, afforded a thick oil which was chromatographed on a silica gel column, eluting with dichloromethane. The eluate collected, after evaporation to dryness in vacuo, gave compound **31a** (0.30 g, 43%); whitish crystals, mp 198–198.5 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 6.66 (s, 1H, H-3), 7.56 and 7.66 (2 dd, 1H + 1H, H-7,8), 7.74 (s, 1H, H-10), 7.90 and 8.00 (2 near d, 1H + 1H, H-6,9), 8.36 (s, 1H, H-5). IR ($CHCl_3$) 1750 sh, 1722 s (CO), 1631, 1609, 1567 w cm^{-1}. Anal. ($C_{13}H_7ClO_2$) C, H, Cl.

Preparation of 4-(1-piperazinyl)-2*H*-naphtho[2,3-*b*]pyran-2-one (8a) and 10-methyl-4-(1-piperazinyl)-2*H*-naphtho[2,3-*b*]pyran-2-one (8b). A mixture of 1.5 mmol of the chloro derivative **31a** (0.35 g) or **31b**[14] (0.37 g), 15.0 mmol (1.29 g) of piperazine and 60 mL of ethanol was stirred at room temperature for 2 h. The solution obtained was poured into water (200 mL) and the mixture exhaustively extracted with chloroform. The extracts, after drying and removal of solvents, afforded a solid residue which was taken up in a little ethyl ether and filtered to yield compound **8a** (0.29 g, 69%) or **8b** (0.29 g, 66%), respectively.

Compound 8a. Whitish crystals, mp 170–172 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 1H, NH; disappeared with D_2O), 2.92–3.48 (m, 8H, piperazine CH_2's), 5.79 (s, 1H, H-3), 7.29–8.10 (m, 4H, H-6,7,8,9), 7.74 (s, 1H, H-10), 8.18 (s, 1H, H-5). IR (KBr) 3250 (NH), 1706 s,br (CO), 1636, 1610, 1588, 1558 cm^{-1}. Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

Compound 8b. Whitish crystals, mp 192–193 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 1.77 (s, 1H, NH; disappeared with D_2O), 2.77 (s, 3H, 10-CH_3), 3.00–3.48 (m, 8H, piperazine CH_2's), 5.82 (s, 1H, H-3), 7.22–8.24 (m, 4H, H-6,7,8,9), 8.04 (s, 1H, H-5). IR (KBr) 3330 (NH), 1700 s,br (CO), 1622, 1607, 1593 sh, 1563 cm^{-1}. Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

***N*-Phenyl-2-(1-piperazinyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-imine (9b).** A mixture of **32a**[22] (0.38 g, 1.5 mmol), piperazine (1.29 g, 15.0 mmol) and ethanol (100 mL) was refluxed for 16 h, while stirring, then evaporated to dryness under reduced pressure. The residue obtained was partitioned between water and chloroform, and the aqueous phase was extracted several more times with chloroform. The combined extracts (after drying and removal of solvent) afforded a solid residue which was taken up in a little ethyl ether–ethyl acetate (1:1) and filtered to give the pure compound **9b** (0.24 g, 52%); orange-yellow crystals, mp 134–135 °C (ethyl acetate/petroleum ether). ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 1H, NH; disappeared with D_2O), 2.88 (m, 4H, CH_2NHCH_2), 3.46 (m, 4H, CH_2NCH_2), 5.39 (s, 1H, H-3), 6.78 (dd, 1H, H-7), 6.94–7.06 (m, 3H, 3H of NC_6H_5), 7.19 (near d, 1H, H-9), 7.25–7.40 (m, 2H, 2H of NC_6H_5), 7.51 (dd, 1H, H-8), 9.23 (near d, 1H, H-6). IR (KBr) 3260 (NH), 1645 sh, 1623, 1590, 1563, 1540, 1502 cm^{-1}. Anal. ($C_{18}H_{19}N_5$) C, H, N.

1-(4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidin-2-yl)-3-piperidinecarboxylic acid (9c). A mixture of **32b**[23] (0.54 g, 3.0 mmol), 3-piperidinecarboxylic acid (1.94 g, 15.0 mmol) and ethanol (100 mL) was refluxed for 16 h, while stirring. The mixture was then poured into cold water (200 mL) so that a solid separated out which was collected by filtration, washed with water and dried: there was so obtained pure compound **9c** (0.43 g, 52%); white crystalline solid, mp 245–246 °C (methanol/ethyl acetate). ^{1}H NMR (DMSO-d_6) δ 1.30–2.10 (m, 4H, piperidine β-CH_2 + γ-CH_2), 2.35–2.57 (m, 1H, piperidine CH), 2.97–3.28 (m, 2H, piperidine α-CH_2), 4.00–4.22 and 4.36–4.58 (2 m, 1H + 1H, piperidine NCH_2CH), 5.61 (s, 1H, H-3), 7.03 (dd, 1H, H-7), 7.31 (near d, 1H, H-9), 7.76 (dd, 1H, H-8), 8.75 (near d, 1H, H-6), 12.42 (broad s, 1H, COOH; disappeared with D_2O). IR (KBr) 2730–2200 s, br, 1920 br, 1715, 1647 s, 1622 s, 1540 s, 1500 cm^{-1}. Anal. ($C_{14}H_{15}N_3O_3$) C, H, N.

Preparation of ethyl *N*-(5-methyl-2-thiazolyl)malonamate (33a), ethyl *N*-(4-methyl-2-benzothiazolyl)malonamate (33d) and ethyl *N*-(5-chloro-2-benzoxazolyl)malonamate (33e). The solution of 30.0 mmol of 5-methylthiazol-2-amine (3.42 g), 4-methylbenzothiazol-2-amine (4.93 g), or 5-chlorobenzoxazol-2-amine (5.06 g) in 20 mL of dichloromethane and 7.5 mL of dry pyridine was added dropwise at room temperature to a stirred solution of ethyl (chloroformyl)acetate (6.02 g, 40.0 mmol) in 30 mL of dichloromethane (an exothermic reaction with emission of white fumes occurred during the addition). The resulting warm mixture was stirred at room temperature for 30 min then poured into 300 mL of cold water; an excess of sodium carbonate was carefully added, with stirring, and the mixture was further stirred at room temperature for 1 h. The organic layer was then collected and the aqueous phase was extracted several more times with dichloromethane. The combined extracts were washed with water, dried over anhyd sodium sulfate, then evaporated to dryness in vacuo. By treating the nearly solid residue with a little ethyl ether, pure compound **33a** (4.82 g, 70%), **33d** (3.98 g, 48%), or **33e** (4.80 g, 57%), respectively, separated out as a whitish solid which was then crystallized from the suitable solvent.

Compound 33a. White crystals, mp 152–153 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, CH_2CH_3), 2.40 (s, 3H, 5-CH_3), 3.60 (s, 2H, CH_2), 4.26 (q, 2H, CH_2CH_3), 7.13 (s, 1H, H-4), 12.12 (s, 1H, NH; disappeared with D_2O). IR (KBr) 3190 and 3070 (NH), 1747 s (ester CO), 1687 (amide CO), 1575 s cm^{-1}. Anal. ($C_9H_{12}N_2O_3S$) C, H, N, S.

Compound 33d. White crystals, mp 184–185 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, CH_2CH_3), 2.64 (s, 3H, 4-CH_3), 3.62 (s, 2H, CH_2), 4.30 (q, 2H, CH_2CH_3), 7.18–7.28 and 7.60–7.70 (2 m, 2H + 1H, H-5,6,7), 10.75 (s, 1H, NH; disappeared with D_2O). IR (KBr) 3180 and 3073 (NH), 1738 s (ester CO), 1666 (amide CO), 1592, 1564 s cm^{-1}. Anal. ($C_{13}H_{14}N_2O_3S$) C, H, N, S.

Compound 33e. White crystals, mp 133–134 °C (ethyl acetate/petroleum ether). ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, CH_2CH_3), 3.78 (s, 2H, CH_2), 4.29 (q, 2H, CH_2CH_3), 7.26–7.46 (m, 2H, H-6,7), 7.63 (near s, 1H, H-4), 12.00 (broad s, 1H, NH; disappeared with D_2O). IR (KBr) 3215 and 3080 (NH), 1765 s (ester CO), 1640 s (amide CO), 1586, 1575 cm^{-1}. Anal. ($C_{12}H_{11}ClN_2O_4$) C, H, N, Cl.

Preparation of chloro derivatives 34a,d,e. A mixture of 10.0 mmol of the proper compound **33**, phosphorus oxychloride (30.0 mmol, 4.60 g) and polyphosphoric acid (0.70 g) was heated with stirring at 130 °C for 3 h. After cooling, anhyd ethanol (10 mL) was added and the mixture was further heated at reflux and stirred for 30 min, then allowed to cool. The chloro derivative **34** was then recovered from the mixture as described below.

7-Chloro-2-methyl-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (34a). The solution obtained from the reaction of **33a** (2.28 g) was poured into water (200 mL) and the resulting mixture, after addition of $NaHCO_3$ until alkaline was thoroughly extracted with dichloromethane. The oil obtained from the extracts (after drying and removal of solvent) was chromatographed on a silica gel column eluting with dichloromethane. The fraction collected, evaporated to dryness in vacuo, afforded the pure compound **34a** (0.75 g, 37%); white crystals, mp 169.5–170 °C (isopropyl ether). ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, 2-CH_3), 6.33 (s, 1H, H-6), 7.75 (s, 1H, H-3). IR ($CHCl_3$) 1685 s (CO), 1622 w, 1530, 1485 s cm^{-1}. Anal. ($C_7H_5ClN_2OS$) C, H, N, S, Cl.

2-Chloro-6-methyl-4*H*-pyrimido[2,1-*b*]benzothiazol-4-one (34d). By proceeding exactly as above described for the preparation of **34a**, from the reaction of **33d** (2.78 g) an oil was obtained which was chromatographed on a silica gel column eluting with dichloromethane–ethyl acetate (4:1). The fraction collected, evaporated to dryness in vacuo, afforded the pure compound **34d** (0.70 g, 28%); white crystals, mp 168–169 °C (isopropyl ether). ^{1}H NMR ($CDCl_3$) δ 2.66 (s, 3H, 6-CH_3), 6.40 (s, 1H, H-3),

7.28–7.66 (m, 3H, H-7,8,9). IR (KBr) 1693 s, br (CO), 1595 w, 1555 br, 1503 s, br cm^{-1}. Anal. ($C_{11}H_7ClN_2OS$) C, H, N, S, Cl.

2,7-Dichloro-4*H*-pyrimido[2,1-*b*]benzoxazol-4-one (34e). From the reaction carried out with **33e** (2.83 g) a suspension of yellowish crystalline solid was obtained. This solid was collected by filtration then treated with 10% aq Na_2CO_3, and the mixture was exhaustively extracted with dichloromethane. The combined extracts were washed with water and dried. Removal of the solvent gave a solid residue from which, after treatment with a little ethyl ether, pure compound **34e** was recovered (0.66 g, 26%); pale-yellow crystals, mp 202–203 °C (ethyl acetate). ^1H NMR ($CDCl_3$) δ 6.49 (s, 1H, H-3), 7.42–7.60 (m, 2H, H-8,9), 8.46 (near s, 1H, H-6). IR (KBr) 1691 s (CO), 1642, 1607, 1577 w, 1528 s cm^{-1}. Anal. ($C_{10}H_4Cl_2N_2O_2$) C, H, N, Cl.

7-Hydroxy-2,3-dimethyl-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (35). A mixture of 20.0 mmol (2.56 g) of 4,5-dimethylthiazol-2-amine (prepared from its hydrochloride by treatment with 1 N aq NaOH and extraction with chloroform) and 20.0 mmol (9.26 g) of di(2,4,6-trichlorophenyl) malonate[35] was heated at 180 °C, under nitrogen. The solution obtained was further stirred at 180 °C for 5 min, then allowed to cool. The resulting solid mixture was treated with ethyl acetate (50 mL) and the suspension was stirred, then filtered to give the nearly pure compound **35** (1.57 g, 40%); whitish crystals, mp 237–238 °C (ethanol). ^1H NMR (DMSO-d_6) δ 2.24 (s, 3H, 2-CH_3), 2.57 (s, 3H, 3-CH_3), 5.18 (s, 1H, H-6), 11.52 (broad s, 1H, NH^+; disappeared with D_2O). IR (KBr) 2584 s, br (NH^+), 1675 s (CO), 1618 w, 1565, 1500 s, 1470 cm^{-1}. Anal. ($C_8H_8N_2O_2S$) C, H, N, S.

7-Chloro-2,3-dimethyl-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (34c). A mixture of **35** (5.0 mmol, 0.98 g) and phosphorus oxychloride (5 mL) was stirred at 120 °C for 1 h. After cooling, the solution obtained was carefully poured onto ice-water and the mixture was stirred, then made alkaline with $NaHCO_3$ and thoroughly extracted with dichloromethane. The residue obtained from the dried and evaporated extracts was chromatographed on a silica gel column, eluting with dichloromethane-ethyl acetate (1:1), to give pure compound **34c** (0.94 g, 88%); whitish crystals, mp 139.5–140.5 °C (isopropyl ether). ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H, 2-CH_3), 2.72 (s, 3H, 3-CH_3), 6.20 (s, 1H, H-6). IR (KBr) 1690 s, br (CO), 1614 w, 1534, 1490 s, br cm^{-1}. Anal. ($C_8H_7ClN_2OS$) C, H, N, S, Cl.

General procedure for synthesis of compounds 15e–i. A mixture of the proper chloro derivative **34** (3.0 mmol), piperazine (2.58 g, 30.0 mmol) and 60 mL of ethanol was refluxed for 1 h, while stirring, then evaporated to dryness under reduced pressure. The residue obtained was partitioned between water and chloroform, and the aqueous phase was extracted several more times with chloroform. The combined extracts (after drying and removal of solvent) afforded a solid residue which was taken up in a little ethyl ether–ethyl acetate (1:1) and filtered to give the pure compound **15**.

2-Methyl-7-(1-piperazinyl)-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (15e). The reaction carried out with **34a** (0.60 g) yielded 0.46 g (61%) of **15e**; white crystals, mp 158–159 °C (ethyl acetate). ^1H NMR ($CDCl_3$) δ 2.00 (s, 1H, NH; disappeared with D_2O), 2.36 (s, 3H, 2-CH_3), 2.92 (m, 4H, CH_2NHCH_2), 3.59 (m, 4H, CH_2NCH_2), 5.35 (s, 1H, H-6), 7.57 (s, 1H, H-3). IR (KBr) 3305 (NH), 1665 s (CO), 1625 sh, 1563 s, 1515 cm^{-1}. Anal. ($C_{11}H_{14}N_4OS$) C, H, N, S.

3-Methyl-7-(1-piperazinyl)-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (15f). The reaction performed with **34b**[24] (0.60 g) afforded 0.67 g (89%) of **15f**; white crystalline solid, mp 144.5–145.5 °C (ethyl acetate). ^1H NMR ($CDCl_3$) δ 1.87 (s, 1H, NH; disappeared with D_2O), 2.73 (s, 3H, 3-CH_3), 2.90 (m, 4H, CH_2NHCH_2), 3.58 (m, 4H, CH_2NCH_2), 5.26 (s, 1H, H-6), 6.20 (s, 1H, H-2). IR (KBr) 3340 (NH), 1667 s (CO), 1560 s, 1513 cm^{-1}. Anal. ($C_{11}H_{14}N_4OS$) C, H, N, S.

2,3-Dimethyl-7-(1-piperazinyl)-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (15g). The reaction performed with **34c** (0.64 g) gave 0.72 g (91%) of **15g**; white crystals, mp 187–188 °C (ethyl acetate). ^1H NMR ($CDCl_3$) δ 1.86 (s, 1H, NH; disappeared with D_2O), 2.20 (s, 3H, 2-CH_3), 2.66 (s, 3H, 3-CH_3), 2.90 (m, 4H, CH_2NHCH_2), 3.56 (m, 4H, CH_2NCH_2), 5.25 (s, 1H, H-6). IR (KBr) 3315 (NH), 1660 s (CO), 1624, 1558 s, 1520 cm^{-1}. Anal. ($C_{12}H_{16}N_4OS$) C, H, N, S.

6-Methyl-2-(1-piperazinyl)-4*H*-pyrimido[2,1-*b*]benzothiazol-4-one (15h). The reaction carried out with **34d** (0.75 g) yielded 0.61 g (68%) of **15e**; white crystalline solid, mp 167–168 °C (ethyl acetate/ethyl ether). ^1H NMR ($CDCl_3$) δ 2.02 (s, 1H, NH; disappeared with D_2O), 2.55 (s, 3H, 6-CH_3), 2.93 (m, 4H, CH_2NHCH_2), 3.62 (m, 4H, CH_2NCH_2), 5.28 (s, 1H, H-3), 7.10–7.51 (m, 3H, H-7,8,9). IR (KBr) 3210 br (NH), 1690 (CO), 1578 s, 1560 sh, 1525 cm^{-1}. Anal. ($C_{15}H_{16}N_4OS$) C, H, N, S.

7-Chloro-2-(1-piperazinyl)-4*H*-pyrimido[2,1-*b*]benzoxazol-4-one (15i). The reaction carried out with **34e** (0.77 g) afforded 0.33 g (35%) of **15i**·0.5 H_2O; white crystalline solid, mp 227–229 °C dec. (ethyl acetate). ^1H NMR ($CDCl_3$) δ 1.93 (s, 1H + 1H, NH + 1H of crystal. H_2O; disappeared with D_2O), 2.95 (m, 4H, CH_2NHCH_2), 3.62 (m, 4H, CH_2NCH_2), 5.33 (s, 1H, H-3), 7.32–7.45 (m, 2H, H-8,9), 8.34 (near s, 1H, H-6). IR (KBr) 3340 (NH), 1695 s (CO), 1642, 1601, 1545 cm^{-1}. Anal. ($C_{14}H_{13}ClN_4O_2 \cdot 0.5H_2O$) C, H, N, Cl.

Biology

Platelet aggregation. Human blood of 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). Platelet aggregation, performed in an Aggrecoder PA-3210 aggregometer (A. Menarini, Florence, Italy), was measured following the Born's turbidimetric method[36] and quantified by the light transmission reached after 3 min. 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, or 20.0 μM A 23187 (Sigma). Before each experiment the stock solutions of ADP (saline), collagen (saline), and A 23187 (DMSO) were diluted in saline.

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. The IC_{50} values reported in Table 2 are averages (±standard deviation) of those obtained from at least four different batches of platelets (usually 5–8 batches).

3-D QSAR

All the calculations were run on a SGI O2 R10000 workstation. The molecule building, optimisation, alignment and PLS runs were performed as previously described[9] using the Sybyl 6.7 software package.[37]

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