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Original article

Synthesis and antiplatelet activity of 2-(diethylamino)-7-ethoxychromone and related compounds

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Summary — 2-(Diethylamino)-7-ethoxychromone **3a** and its 2-(1-piperidinyl)analogue **3b** were synthesized by reaction of 3-ethoxyphenol **1** with 3-(dialkylamino)-3-oxo-propanoic acid ethyl ester **2** in the presence of phosphorus oxychloride. With a view to improve their biological activity the above 7-ethoxychromones **3** were submitted to some structural modifications firstly involving the 4-CO group. The 4H-chromenes **4** and the 4-thiochromones **5** were obtained by action of suitable reagents. The compounds **5** were then easily transformed to 4-(methylthio)chromenylium iodides **6**. Then from the 2-(diethylamino)-7-ethoxychromone **3a** were obtained with suitable reactions the 3,6-diamino derivative **8**, the 3- and 6-formyl derivatives **9a,b** and the Mannich base **10**. By action of acetic anhydride this latter compound yielded the methylenebis derivative **11**. Most of the above compounds were tested *in vitro* for their inhibitory activities against human platelet aggregation induced by collagen, ADP and arachidonic acid. Among the tested compounds the 2-(diethylamino)-7-ethoxychromone **3a** showed the highest activity.

Résumé — Synthèse et activité antiagrégante plaquettaire de la 2-(diéthylamino)-7-éthoxychromone et de ses analogues. *La synthèse de la 2-(diéthylamino)-7-éthoxychromone **3a** et de son analogue 2-(1-pipéridyl) **3b** a été réalisée par réaction du 3-éthoxy-1-phénol **1** avec l'α-(N,N-dialkylcarbamoyl)-acétate d'éthyle **2** en présence d'oxychlorure de phosphore. Les 7-éthoxychromones **3**, soumises à des modifications du carbonyle en 4, permettent d'obtenir les dérivés 4H-chrométnyle **4** et les 4-thiochromones **5**. Ces derniers composés sont convertis en iodures de 4-méthylthiochrométnylium **6**. Par réaction de 2-(diéthylamino)-7-éthoxychromone **3a** avec des réactifs appropriés, on a obtenu le dérivé 3,6-diamino **8**, les dérivés 3-formyl **9a** et 6-formyl **9b** ainsi que la base de Mannich **10**. Cette dernière a été transformée en dérivé* bis-*méthylène **11**. La plupart des composés obtenus ont été testés* in vitro *sur l'agrégation plaquettaire induite par le collagène, l'ADP et l'acide arachidonique. Parmi les composés ayant manifesté une activité antiagrégante plaquettaire vis-à-vis des 3 inducteurs, la 2-(diéthylamino)-7-éthoxychromone **3a** s'est avérée la plus active.*

substituted 2-(dialkylamino)chromones / platelet aggregation inhibition

Introduction

In a previous paper, we reported testing of 23 variously substituted 2-aminochromones for their biological activity towards human platelet aggregation [1]. In that preliminary screening we found that satisfactory activity was shown when the chromones were substituted in the position 2 by the diethylamino group and in the position 7 by a methoxy or hydroxy group. On the other hand, discordant data on 2-(1-piperidinyl)chromones did not allow determination of the effectiveness of 1-piperidinyl substituent in the position 2. For this reason, some 2-(1-piperidinyl) derivatives were still tested in this work.

Furthermore, it is important to note that the presence of electron withdrawing substituents, such as NO_2 or Cl, highly reduced the activity of all 2-(dialkylamino)chromones [1].

Afterwards, of the most active compounds, the 2-(diethylamino)-7-hydroxychromone was selected to investigate the specific mechanism of inhibition [2]. In this regard this compound was found to accumulate cAMP in human platelets treated with prostacyclin, probably inhibiting the cAMP phosphodiesterase. Moreover, thromboxane B_2 formation and granule release reaction are also affected by this drug [2].

Therefore, considering suitably substituted 2-(diethylamino)chromones as a new class of promising antithrombotic drugs, we thought it interesting to extend the knowledge in this field synthesizing 2-(diethylamino)-7-ethoxychromone **3a** and some related compounds to study their behaviour as potential antiplatelet drugs and better clarify the structure–activity relationship (SAR) pattern in this class of compounds.

Chemistry

2-(Dialkylamino)chromones **3a,b** were synthesized by a previously reported method [1] through reaction

of 3-ethoxyphenol **1** with the 3-(dialkylamino)-3-oxopropanoic acids ethyl ester **2a,b** in the presence of phosphorus oxychloride as shown in scheme 1.

Since 2-(dialkylamino)chromones were known to have an interesting antiplatelet activity [1], compounds **3** were submitted to some chemical modifications in an attempt to improve their biological activity.

Compounds **3** were condensed with malononitrile in the presence of acetic anhydride at 110°C to give the 4H-chromene derivatives **4** or were allowed to react with phosphorus pentasulfide in refluxing pyridine to yield the 4-thiochromone derivatives **5a,b**. These latter compounds were then treated with methyl iodide in chloroform at room temperature affording the corresponding 4-(methylthio)chromenylium iodides **6a,b** in very good yield (scheme 1).

Since 2-(diethylamino)-7-ethoxychromone **3a** was found to be the most active as a platelet aggregation inhibitor (see later) the subsequent structural modifications only concerned this compound (scheme 2). Thus **3a** was submitted to nitration with the mixture of sulfuric and nitric acids, affording a mixture of 3,6-dinitro and 3,6,8-trinitrochromones **7a,b** which were separated by column chromatography on silica gel.

It is noteworthy to observe that in this case the strong activation to electrophilic substitution due to ethoxy group produced, although in low yield, a chromone (*ie* **7b**) with 2 nitro groups in the benzo moiety. This behaviour is not common in the nitration of 2-(dialkylamino)chromones substituted in the benzene ring with alkyl or methoxy groups, which usually gave rise to compounds bearing a single nitro group in that portion of the molecule [3, 4].

Only the dinitrochromone **7a** was then hydrogenated into the corresponding diaminochromone **8** using 5% Pd/C as catalyst.

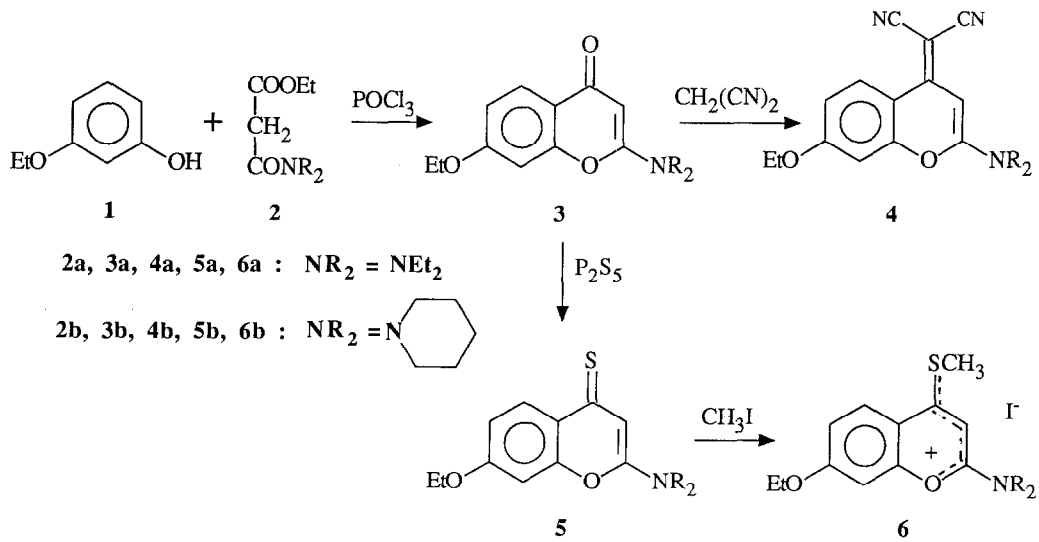
As 7-alkoxy-2-(dialkylamino)chromones do not react under Vilsmeier–Haack conditions, the formyl derivatives were synthesized from **3a** using 1,1-dichloromethyl methyl ether/$TiCl_4$ in refluxing 1,2-dichloroethane [5]. The resulting formyl derivatives **9a,b** were separated by column chromatography on silica gel.

Then by reaction with 42% formaldehyde and morpholine in ethanol containing a small amount of acetic from **3a**, the corresponding Mannich base **10** was obtained. Secondly, this Mannich base was easily transformed into the methylenebis derivative **11** by action of acetic anhydride at 95°C.

The proposed structures of compounds described herein are in agreement with elemental analyses and spectral data (see *Experimental protocols*). In particular, ^{1}HNMR and IR spectral data are in accordance with the literature on chromone field [6] and our previous observations on 2-(dialkylamino)chromones [7–9].

Results and Discussion

In table I are reported data of activity of selected chromones tested as inhibitors of human platelet aggregation induced by 5.0 µg/ml collagen, 5.0 µM ADP and 1.4 mM arachidonic acid. Values for acetylsalicylic acid (ASA) are included for a comparison of the effect of the studied compounds. The compounds **4b** and **11** have not been tested because they were found insoluble in our experimental conditions (see later).



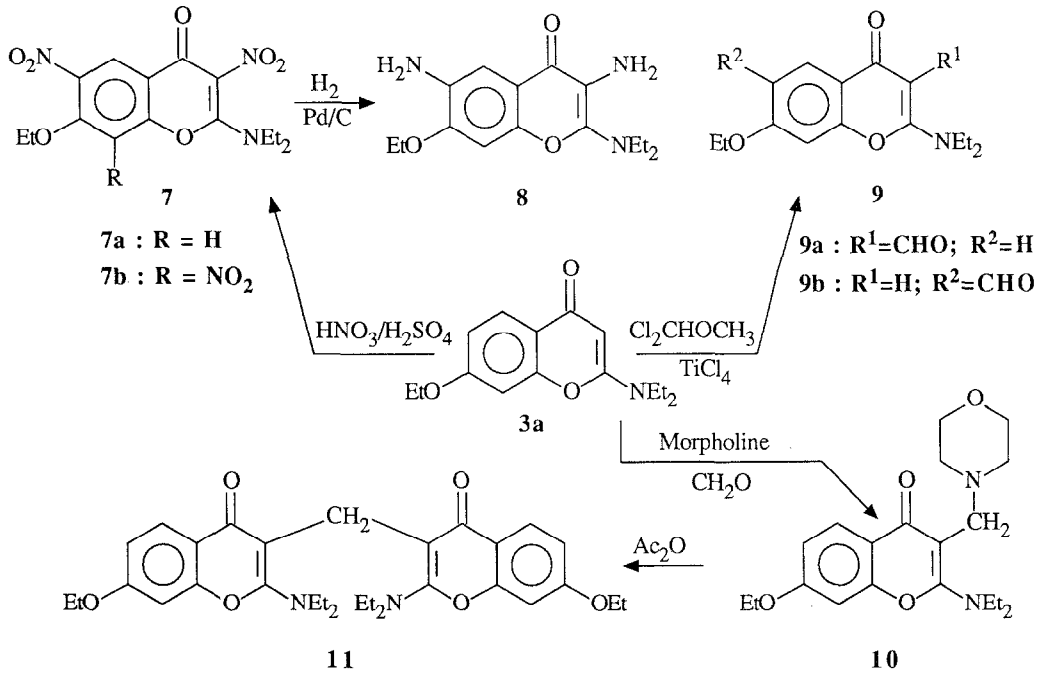
1 2 3 4

2a, 3a, 4a, 5a, 6a : NR_2 = NEt_2

2b, 3b, 4b, 5b, 6b : NR_2 = N

5 6

Scheme 1.



Scheme 2.

IC_{50} values in table I provide evidence that, among the tested compounds, 2-(diethylamino)-7-ethoxychromone **3a** is the most potent inhibitor with all of the 3 inducers used. This occurrence strengthens our previous observations on the effectiveness of matching electron releasing substituents in the position 7 of the chromone ring with the diethylamino group in the position 2 [1].

All other substitutions weaken or cancel out this type of activity. Thus when 2-(diethylamino) group is replaced by 2-(1-piperidinyl) group as in **3b**, the inhibitory effect is less in all cases.

On the other hand, when the carbonyl in 4 is replaced by 4-C=S or 4-SCH_3, the 1-piperidinyl derivatives do not show unequivocal responses in respect to diethylamino derivatives (compare **5a** with **5b** and **6a** with **6b**) but on the whole these compounds must be considered inactive.

Compounds **8–10**, obtained by reactions on **3a** and containing electron-releasing (**8, 10**) and -withdrawing (**9a, 9b**) substituents, retain a certain degree of activity but at a very low level in respect to the parent compound **3a**.

Drawing some SAR conclusions from the above and from previous [1] data concerning 34 tested compounds, we may summarize that for an interesting antiplatelet activity 2-(dialkylamino)chromones should comply with 4 requirements: 1) A diethylamino group in the position 2. 2) An electron-releasing group such as -OH, $-OCH_3$, $-OC_2H_5$ in position 7. 3) No changes on the carbonyl group in position 4. 4) No substituents in the other positions of the molecule.

Considering the influence of the tested compounds on platelet aggregation, we have found that some drugs (in particular **3a** and the previously reported 7-hydroxy and 7-methoxychromones [1]) are active with all the studied inducers, whereas others such as compounds **8** and **10** or compounds **9a** and **9b** show a particular behaviour, which is difficult to explain. In fact compounds **8** and **10** are active in the presence of collagen but ineffective in platelets stimulated by ADP. On the other hand, **9a** and **9b** act on arachidonic acid but not on collagen. It is probable that these compounds act at the level of glycoprotein IIb–IIIa complex, by interfering in different manner on the exposure of fibrinogen receptors. It is possible that the above mentioned compounds could be useful in clarifying the relationship between the fibrinogen binding activity of platelets and changes occurring in the membrane glycoprotein IIb–IIIa complex [10].

Among the compounds with the 4 above-mentioned requirements, **3a** will be examined in order to explore the relationship between aggregation and prostaglandin and cyclic nucleotide metabolism. The determination of endoperoxides and thromboxane A_2 levels after incubation of platelets with thrombin, collagen or arachidonic acid, as well as the measurement of granule secretion (^{14}C serotonin and adenine

Table I. Inhibitory activity of 2-(dialkylamino)-7-ethoxychromones **3** and derivatives (**4–6**, **8–11**) on platelet aggregation induced by collagen, ADP and arachidonic acid.

Comp	*5.0 μg/ml collagen* IC_{50} *(μM) ± SD*	*ADP 5.0 μM* IC_{50} *(μM) ± SD*	*arachidonic acid 1.4 mM* IC_{50} *(μM) ± SD*
ASA	230±110	1000	180±50
3a	290±120	210±130	133±45
3b	550±230	420±190	200±50
4a	>1000	>1000	N.T.
4b	(a)	(a)	(a)
5a	>1000	>1000	390±50
5b	>1000	>1000	>1000
6a	1000	>1000	550±490
6b	650±280	950±80	N.T.
8	540±170	>1000	750±70
9a	775±260	875±95	290±50
9b	760±230	780±150	300±90
10	410±230	>1000	400±65
11	(a)	(a)	(a)

[a]Insoluble in the conditions of the experiments; NT: Not tested

nucleotide) released upon platelet stimulation will be useful for this purpose. We also intend to investigate the effect of **3a** on platelet levels of cAMP, on adenylate cyclase and cAMP phosphodiesterase activity. A series of tests in animal models are also necessary to verify the effectiveness of this chromone derivative in treating thrombotic disease.

Experimental protocols

Chemistry

Melting points were determined using a Fisher–Johns apparatus and are uncorrected. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer. The results of elemental analysis were within ± 0.3% of the theoretical value. ^{1}HNMR spectra were performed on a Hitachi Perkin–Elmer R 600 (60 MHz) spectrometer using TMS as internal standard ($\delta = 0$). IR spectra were recorded on a Perkin–Elmer 398 spectrophotometer.

3-Ethoxyphenol 1

To 30 g (0.27 mol) of resorcinol dissolved in 30 ml of water containing 10.8 g (0.27 mol) of NaOH were added dropwise with stirring 41.6 g (0.27 mol) of diethyl sulfate. The solution was stirred for 4 h at 40°C and then extracted 3 times with chloroform. The pooled organic extracts were washed with water, dried and evaporated under reduced pressure. The resulting liquid was distilled *in vacuo* collecting the fraction at 105–108°C/0.4 mm Hg. 65.5% yield.

2-(Dialkylamino)-7-ethoxychromones 3

In an ice bath-cooled flask, protected from moisture with a calcium chloride drying tube, 7.0 ml (78 mmol) of phosphorus oxychloride were added dropwise with stirring to 55 mmol of 3-(dialkylamino)-3-oxo-propanoic acid ethyl ester **2** [11]. After the addition, the mixture was removed from the ice bath and maintained at room temperature for 0.5 h. To the resulting yellow mixture a solution of 6.9 g (50 mmol) of 3-ethoxyphenol **1** in 40 ml of 1,2-dichloroethane was added slowly with stirring. The reaction mixture was then heated for 5 h at reflux. After cooling, a solution of 68 g of sodium acetate trihydrate in 200 ml of water was added and the mixture was then heated for 1.5 h at 70°C. After cooling the organic phase was discarded and the aqueous one was extracted several times with chloroform. The pooled organic extracts were washed with water, dried and evaporated under reduced pressure to yield a dark red oil. The oil was stirred at room temperature for 2 h together with 200 ml of 2 N NaOH and 50 ml of light petroleum ether. The solid thus obtained was filtered, washed with water and recrystallized from ethyl acetate. Thus obtained were:

*2-(Diethylamino)-7-ethoxychromone **3a**.* 6.9 g (49.8% yield), white solid, mp 107–108°C. Anal ($C_{15}H_{19}NO_3$) C, H, N. IR (KBr) cm^{-1}: 1630 (ν CO), 1610, 1595, 1550. ^{1}HNMR ($CDCl_3$) δ: 1.04–1.63 (9H, m, CH_2-*CH_3*); 3.45 (4H, q, N-*CH_2*-CH_3); 4.10 (2H, q, O-*CH_2*-CH_3); 5.34 (1H, s, H-3); 6.65–7.07 (2H, m, H-6,8); 8.04 (1H, d, H-5).

*7-Ethoxy-2-(1-piperidinyl)chromone **3b**.* 6.1 g (44.7% yield), white solid, mp 135–136°C. Anal ($C_{16}H_{19}NO_3$) C, H, N. IR (KBr) cm^{-1}: 1625 (ν CO), 1590, 1560. ^{1}HNMR ($CDCl_3$) δ: 1.23–1.86 (9H, m, β-CH_2 and CH_2-*CH_3*); 3.26–3.68 (4H, m, α-CH_2); 4.07 (2H, q, *CH_2*-CH_3); 5.40 (1H, s, H-3); 6.62–7.00 (2H, m, H-6,8); 8.04 (1H, d, H-5).

[2-(Dialkylamino)-7-ethoxy-4H-chromen-4-ylidene]malononitriles 4

A mixture of 4 mmol of chromones **3a**,**b**, 0.55 g (8 mmol) of malononitrile and 8 ml of freshly distilled acetic anhydride was heated at 110–115°C for 1 h, cooled and poured onto crushed ice. After stirring for a few minutes the brown solid which separated out was collected, washed with water and recrystallized from ethanol, yielding orange crystals. Thus obtained were:

*[2-(Diethylamino)-7-ethoxy-4H-chromen-4-ylidene]malononitrile **4a**.* 0.88 g (71.1% yield), mp 171–173°C. Anal ($C_{18}H_{19}N_3O_2$) C, H, N. IR (KBr) cm^{-1}: 2190 and 2175 (ν CN), 1629, 1600. ^{1}HNMR ($CDCl_3$) δ: 1.10–1.73 (9H, m, CH_2-*CH_3*); 3.57 (4H, q, N-*CH_2*-CH_3); 4.15 (2H, q, O-*CH_2*-CH_3); 5.69 (1H, s, H-3); 6.61–6.97 (2H, m, H-6,8); 8.62 (1H, d, H-5).

[7-Ethoxy-2-(1-piperidinyl)-4H-chromen-4-ylidene]malononitrile ***4b.*** 0.75 g (58.3% yield), mp 221–223°C. Anal ($C_{19}H_{19}N_3O_2$) C, H, N. IR (KBr) cm^{-1}: 2195 and 2180 (ν CN), 1625, 1595. ^{1}HNMR ($CDCl_3$) δ: 1.24–1.92 (9H, m, β-CH_2 and O-CH_2-CH_3); 3.39-3.82 (4H, m, α-CH_2); 4.11 (2H, q, O-CH_2-CH_3); 5.71 (1H, s, H-3); 6.56–6.93 (2H, m, H-6,8); 8.57 (1H, d, H-5).

2-(Dialkylamino)-7-ethoxy-4-thiochromones ***5***

To 1.0 g of 2-(dialkylamino)-7-ethoxychromone **3a,b** dissolved in 7 ml of pyridine was added 0.8 g of phosphorus pentasulfide and the resulting mixture was refluxed for 1 h. The final solution was poured onto crushed ice and the brown solid which separated out was stirred for 0.5 h and filtered. The solid was then crystallized from ethanol yielding yellow crystals. Thus obtained were:

2-(Diethylamino)-7-ethoxy-4-thiochromone ***5a.*** 0.82 g (77.3% yield), mp 160–161°C. Anal ($C_{15}H_{19}NO_2S$) C, H, N, S. IR (KBr) cm^{-1}: 1621 (ν C=S), 1590, 1550. ^{1}HNMR ($CDCl_3$) δ: 1.10–1.65 (9H, m, CH_2-CH_3); 3.52 (4H, q, N-CH_2-CH_3); 4.09 (2H, q, O-CH_2-CH3); 6.57–7.05 (3H, m, H-3,6,8); 8.58 (1H, d, H-5).

7-Ethoxy-2-(1-piperidinyl)-4-thiochromone ***5b.*** 0.90 g (85.0% yield), mp 183–184°C. Anal ($C_{16}H_{19}NO_2S$) C, H, N, S. IR (KBr) cm^{-1}: 1620 (ν C=S), 1586, 1550. ^{1}HNMR ($CDCl_3$) δ: 1.24–1.91 (9H, β-CH_2 and CH_2-CH_3); 3.38–3.84 (4H, m, α-CH_2); 4.08 (2H, q, CH_2-CH_3); 6.60–7.07 (3H, m, H-3,6,8); 8.56 (1H, d, H-5).

2-(Dialkylamino)-7-ethoxy-4-(methylthio)chromenylium iodides ***6***

To 0.5 g of 2-(dialkylamino)-7-ethoxy-4-thiochromone **5a,b** dissolved in 8 ml of chloroform were added dropwise 10 ml of methyl iodide and the solution was stirred for 1 h. The solid which separated out was collected by filtration, washed with ethyl ether and recrystallized from ethanol, yielding pale yellow crystals. Thus obtained were:

2-(Diethylamino)-7-ethoxy-4-(methylthio)chromenylium iodide ***6a.*** 0.67 g (88.6% yield), mp 173–174°C. Anal ($C_{16}H_{22}INO_2S$) C, H, N, I, S. IR (KBr) cm^{-1}: 1638, 1600. ^{1}HNMR ($CDCl_3$) δ: 1.23–1.70 (9H, m, CH_2-CH_3); 3.00 (3H, s, S-CH_3); 3.78–4.53 (6H, m, CH_2-CH_3); 6.70 (1H, s, H-3); 6.90–7.40 (2H, m, H-6,8); 7.85 (1H, d, H-5).

7-Ethoxy-2-(1-piperidinyl)-4-(methylthio)chromenylium iodide ***6b.*** 0.62 g (83.2% yield), mp 106–108°C. Anal ($C_{17}H_{22}INO_2S$) C, H, N, I, S. IR (KBr) cm^{-1}: 1638, 1600. ^{1}HNMR ($CDCl_3$) δ: 1.47 (3H, t, CH_2-CH_3); 1.70–2.12 (6H, m, β-CH_2); 2.98 (3H, s, S-CH_3); 3.98–4.50 (6H, m, α-CH_2 and CH_2-CH_3); 6.80–7.36 (3H, m, H-3,6,8); 7.82 (1H, d, H-5).

2-(Diethylamino)-7-ethoxy-3,6-dinitrochromone ***7a*** *and 2-(diethylamino)-7-ethoxy-3,6,8-trinitrochromone* ***7b***

In an ice bath-cooled flask, to 1 g of 2-(diethylamino)-7-ethoxychromone **3a** dissolved in 4 ml of conc H_2SO_4, a solution of 0.2 ml of HNO_3 (d = 1.52) in 2 ml of conc H_2SO_4 was added dropwise with stirring within 5 min. The resulting brown solution was stirred for 5 min, then was poured onto crushed ice and the yellow solid which separated out was filtered, washed with water and recrystallized from ethanol. The yellow solid so obtained was chromatographied through a silica gel column using ethyl acetate: light petroleum ether (4:1) as eluent. From the first 120 ml of the eluate it was possible to recover 0.12 g of **7b** (recrystallized from ethanol, mp 146–147°C, 7.9% yield) then in 200 ml 0.65 g of **7a** (recrystallized from ethanol, mp 171–173°C, 48.5% yield).

7a: Anal ($C_{15}H_{17}N_3O_7$) C, H, N. IR (KBr) cm^{-1}: 1630, 1595, 1555. ^{1}HNMR ($CDCl_3$) δ: 1.11–1.75 (9H, m, CH_2-CH_3); 3.55 (4H, q, N-CH_2-CH_3); 4.27 (2H, q, O-CH_2-CH_3); 6.93 (1H, near s, H-8); 8.52 (1H, near s, H-5).

7b: Anal ($C_{15}H_{16}N_4O_9$) C, H, N. IR (KBr) cm^{-1}: 1630, 1610, 1570, 1550. ^{1}HNMR ($CDCl_3$) δ: 1.06–1.67 (9H, m, CH_2-CH_3); 3.49 (4H, q, N-CH_2-CH_3); 4.32 (2H, q, O-CH_2-CH_3); 8.76 (1H, s, H-5).

3,6-Diamino-2-(diethylamino)-7-ethoxychromone ***8***

A solution of 1 g of dinitrochromone **7a** in 30 ml of ethanol and 20 ml of 1,2-dichloroethane was hydrogenated with 0.32 g of 5% palladium on charcoal until gas uptake ceased. The catalyst was filtered off and the solvent was distilled under reduced pressure. The residue was purified on a silica gel column using ethyl acetate: ethanol: light petroleum ether (2:1:2) and the solid obtained after solvent removal was crystallized from benzene/cyclohexane to give 0.61 g (73.6% yield) of **8**, white solid, mp 141–143°C. Anal ($C_{15}H_{21}N_3O_3$) C, H, N. IR (KBr) cm^{-1}: 3435 and 3320 (ν NH_2), 1630, 1605. ^{1}HNMR ($CDCl_3$) δ: 0.95–1.70 (9H, m, CH_2-CH_3); 3.30 (4H, q, N-CH_2-CH_3); 3.75 (4H, near s, NH_2); 4.12 (2H, q, O-CH_2-CH_3); 6.70 (1H, s, H-8); 7.45 (1H, s, H-5).

2-(Diethylamino)-7-ethoxy-3-formylchromone ***9a*** *and 2-(diethylamino)-7-ethoxy-6-formylchromone* ***9b***

To a stirred solution of 1.57 g (6.0 mmol) of chromone **3a** in 20 ml of 1,2-dichloroethane maintained at 0°C were slowly added 7.95 ml (60 mmol) of $TiCl_4$ and 3.21 ml (36 mmol) of 1,1-dichloromethyl methyl ether. The obtained brown solution was heated at reflux for 1.5 h at 60–65°C. After cooling the solution was allowed to stand at room temperature for 6 h. Finally, crushed ice was added to the reaction mixture and the aqueous phase was exhaustively extracted with chloroform. The pooled organic extracts were washed several times with water, diluted $NaHCO_3$ and then water again until neutrality was obtained. After drying over Na_2SO_4 and evaporation of the solvent under reduced pressure the brown solid obtained was chromatographied through a silica gel column using ethyl acetate as eluent. From the first 100 ml of eluate it was possible to recover 0.32 g of **9a** (recrystallized from cyclohexane, mp 108–109°C, 18.4% yield) and from 150 ml 0.65 g of **9b** (recrystallized from ethyl acetate, mp 191–193°C, 37.4% yield).

9a: Anal ($C_{16}H_{19}NO_4$) C, H, N. IR (KBr) cm^{-1}: 1667 (ν CHO), 1620. ^{1}HNMR ($CDCl_3$) δ: 1.05–1.70 (9H, m, CH_2-CH_3); 3.64 (4H, q, N-CH_2-CH_3); 4.12 (2H, q, O-CH_2-CH_3); 6.56–7.05 (2H, m, H-6,8); 8.06 (1H, d, H-5); 10.13 (1H, s, CHO).

9b: Anal ($C_{16}H_{19}NO_4$) C, H, N. IR (KBr) cm^{-1}: 1680 (ν CHO), 1610, 1600. ^{1}HNMR ($CDCl_3$) δ: 1.09–1.75 (9H, m, CH_2-CH_3); 3.48 (4H, q, N-CH_2-CH_3); 4.21 (2H, q, O-CH_2-CH_3); 5.28 (1H, s, H-3); 6.74 (1H, s, H-8); 8.55 (1H, s, H-5); 10.39 (1H, s, CHO).

2-(Diethylamino)-7-ethoxy-3-(morpholinomethyl)chromone ***10***

To 0.82 g (4.6 mmol) of 2-(diethylamino)-7-ethoxychromone **3a** dissolved in 15 ml of ethanol were added 0.80 g (9.2 mmol) of morpholine, 1.28 ml (17.0 mmol) of 42% formaldehyde and 0.26 ml (4.6 mmol) of acetic acid. The resulting mixture was refluxed for 8 h. After cooling, the solvent was evaporated under reduced pressure and the pale yellow oil obtained was chromatographied through a silica gel column using ethyl acetate: light petroleum ether (3:7) as eluent. The solvent removal left a white solid (0.63 g, 55.7% yield) which, after recrystallization from ethyl acetate, yielded **10**, mp 106–107°C. Anal ($C_{20}H_{28}N_2O_4$) C, H, N. IR (KBr) cm^{-1}: 1630, 1610, 1590. ^{1}H NMR ($CDCl_3$) δ: 1.10–1.65 (9H, m, CH_2-CH_3); 2.40–2.75 (4H,

m, N-CH_2-CH_2); 3.40–4.35 (12H, m, O-CH_2-CH_2,N-CH_2-CH_3, =C-CH_2-N and O-CH_2-CH_3); 6.60–7.05 (2H, m, H-6,8); 8.07 (1H, d, H-5).

3,3'-Methylenebis[2-(diethylamino)-7-ethoxychromone] ***11***

To 0.5 g of Mannich base **10** were added 10 ml of freshly distilled acetic anhydride and the mixture was heated at 95–100°C for 1.5 h. The resulting brown solution was poured onto crushed ice and stirred. The solid which separated out was collected by filtration, washed with water and recrystallized from ethanol obtaining 0.30 g (81.1% yield) of methylenebis derivative **11**, white crystals, mp 188–189°C. Anal ($C_{31}H_{38}N_2O_6$) C, H, N. IR (KBr) cm^{-1}: 1625, 1610, 1545. ^1H NMR ($CDCl_3$) δ: 0.67–1.18 (12H, m, CH_2-CH_3); 1.20–1.68 (6H, m, O-CH_2-CH_3); 2.90–3.52 (8H, m, N-CH_2-CH_3); 3.82–4.36 (6H, m, O-CH_2-CH_3 and C-CH_2-C); 6.55–7.07 (4H, m, H-6,8); 8.05 (2H, d, H-5).

Biological methods

Platelet aggregation

Human blood was drawn from normal volunteers and added to a 130 mM trisodium citrate aqueous solution (vol ratio 9:1). Platelet-rich plasma (PRP) was obtained by centrifuging the anti-coagulant-treated blood at 100 *g* for 30 min. The aggregation of PRP was induced by collagen (from bovine tendon at the final concentration of 5 μg/ml), by 5.0 μM ADP (Sigma), or by 1.4 mM arachidonic acid (Sigma) and was monitored according to Born's method [12], using a Menarini platelet aggregometer. The compounds investigated were dissolved in DMSO–H_2O (9:1) and preincubated in PRP at 37°C for 3 min before starting the experiment by adding the platelet aggregating agent. After 5 min, the extent of platelet aggregation was measured.

Calculation of inhibition

The amplitude of aggregation measured in the presence of an inhibitor was always compared with that measured in a control experiment (in the presence of DMSO) carried out under the same conditions, and the percentage of inhibition was calculated. From each series of experiments, an inhibition percentage-concentration curve was derived for the tested compounds, and from this curve the concentration of compound inducing 50% inhibition (IC_{50}) was determined. The IC_{50} values reported in table I are averages (± SD) of those obtained from at least 4 different blood samples.

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