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1,2-Fused pyrimidines VII. 3-(Dialkylamino)-1*H*-pyrimido[1,2-*a*]quinolin-1-ones and 2-(dialkylamino)-4*H*-pyrimido[2,1-*a*]isoquinolin-4-ones as antiplatelet compounds

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Summary — A number of 3-(dialkylamino)-1*H*-pyrimido[1,2-*a*]quinolin-1-ones **3** and 2-(dialkylamino)-4*H*-pyrimido[2,1-*a*]isoquinolin-4-ones **4** were prepared by treating the corresponding chloro derivatives with an excess of dialkylamines. The highest *in vitro* antiplatelet activity was obtained when the dialkylamino substituent was 1-piperazinyl (compounds **3g** and **4e**). The novel 2-(1-piperazinyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one **2a** was also prepared by an analogous procedure, which resulted in the most active compound towards all the platelet aggregation inducers used (ADP, collagen, A 23187). Moreover, some examples of 1-(dialkylamino)-3*H*-pyrimido[1,2-*a*]quinolin-3-ones **5** and 4-(dialkylamino)-2*H*-pyrimido[2,1-*a*]isoquinolin-2-ones **6** were also obtained (together with negligible or lower amounts of the corresponding isomers **3** and **4**, respectively) from the cyclocondensation of the appropriate ethyl *N,N*-dialkylmalonamate/phosphorus oxychloride reagents **13** with 2-aminoquinoline or 1-aminoisoquinoline. These latter compounds showed a rather low antiplatelet activity.

3-(dialkylamino)-1*H*-pyrimido[1,2-*a*]quinolin-1-one / 2-(dialkylamino)-4*H*-pyrimido[2,1-*a*]isoquinolin-4-one / *in vitro* antiplatelet activity

Introduction

Some pyrido[1,2-*a*]pyrimidine derivatives have been reported to show platelet aggregation inhibitory properties [1] and the *in vitro* antiplatelet activity of some 7-substituted 2-(dialkylamino)chromones **1** has also been described [2, 3]. In the light of these reports, we have recently evaluated the *in vitro* inhibitory activity on human platelet aggregation of some N-substituted 2-amino-4*H*-pyrido[1,2-*a*]pyrimidin-4-ones **2** [4], which are isosteric analogs of 2-aminochromones **1** (fig 1). The most active compounds **2** were the 2-(diethylamino) derivative (**2**: $NR_2 = N(C_2H_5)_2$), which is more active than acetylsalicylic acid (ASA) when platelet aggregation was induced by the Ca^{2+} ionophore A 23187 (calcimycin), and the 2-(4-methyl-1-piperazinyl) derivative (**2**: NR_2 = 4-methyl-1-piperazinyl), when the inducers were adenosine diphosphate (ADP) (more active than ASA) or collagen (as active as ASA) [4]. The antiplatelet activity of the latter derivative towards ADP and collagen was comparable to that previously exhibited by the most active chromones **1** [2, 3]. On the whole, compounds **2** showed a higher inhibitory activity towards platelet aggregation induced by collagen [4].

Prompted by these results, we continued our studies in this field in order to obtain novel compounds related to the pyrido[1,2-*a*]pyrimidines **2** with higher platelet aggregation inhibitory properties.

Thus, we have now prepared compounds **3** and **4**, *ie* two isomeric benzo-fused derivatives of compounds **2**, and tested them for antiplatelet activity. In this respect, we also believed that it would be interesting, from both chemical and biological points of view, to synthesize and test some examples of compounds **5** and **6**, which are isomers of **3** and **4**, respectively. Furthermore, since the 1-piperazinyl group was the most suitable dialkylamino substituent to yield antiplatelet activity in compounds **3** and **4**, we also prepared and tested the 2-(1-piperazinyl)-substituted compound **2a**, which has not been described previously.

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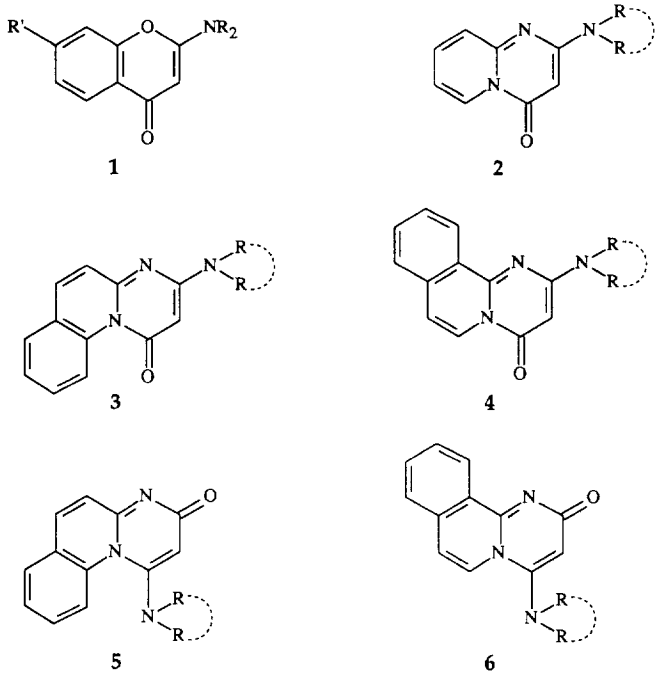


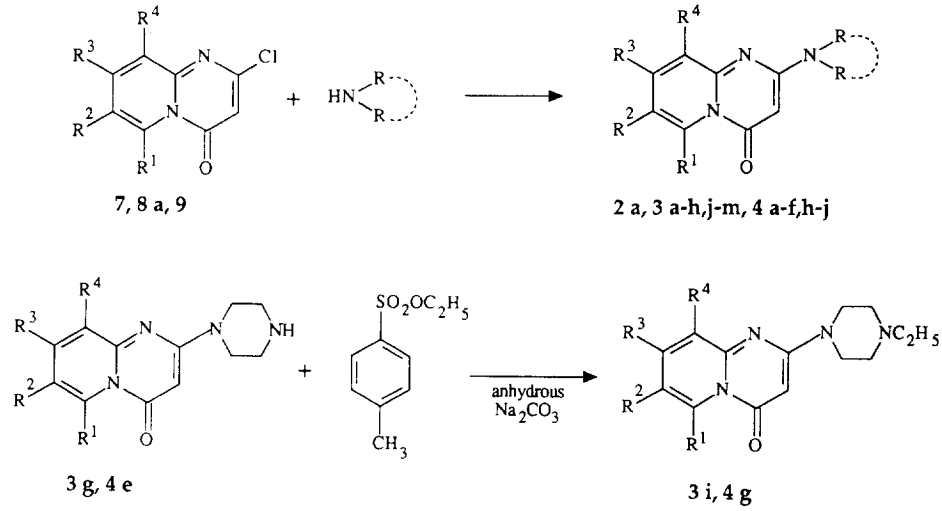
Fig 1. Structures of compounds **1–6**.

Chemistry

The reaction of chloro derivatives **7** [5], **8a** [6] or **9** [7] with excess dialkylamines (ethanol at reflux, 1–5 h; or ethylene glycol, 160°C, 2 h, for compounds **3c**, **d**) afforded high yields of 2-(1-piperazinyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one **2a**, 3-(dialkylamino)-1*H*-pyrimido[1,2-*a*]quinolin-1-ones **3a–h**, **j–m**, or 2-(dialkylamino)-4*H*-pyrimido[2,1-*a*]isoquinolin-4-ones **4a–f**, **h–j**, respectively (scheme 1, table I). The (4-ethyl-1-piperazinyl)derivatives **3i**, **4g** were in turn obtained by treating compounds **3g**, **4e** with ethyl *p*-toluenesulphonate, in the presence of anhydrous sodium carbonate (ethanol at reflux, 3 h) [8] (scheme 1, table I).

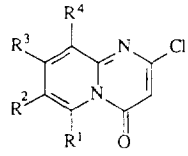
1-(Dialkylamino)-3*H*-pyrimido[1,2-*a*]quinolin-3-ones **5a,b** and 4-(dialkylamino)-2*H*-pyrimido[2,1-*a*]-isoquinolin-2-ones **6a–c** were prepared *via* the synthetic routes shown in scheme 2. The one-pot reaction of *N,N*-dialkylmalonamic acids **10a,b** [9] with phosphorus pentachloride (room temperature, 3 h) followed by condensation of the acyl chloride with 2-aminoquinoline (room temperature, 30 min), in anhydrous tetrahydrofuran, gave the intermediate compounds **11a,b** in good yields. The cyclization of malonamides **11a,b** by treatment with phosphorus oxychloride (refluxing 1,2-dichloroethane, 3–4 h), afforded satisfactory yields of the desired compounds **5a,b**. Compounds **5a,b** were also obtained from the cyclocondensation of 2-aminoquinoline with the iminium compounds **13a** [10] or **13b** [11], respectively (refluxing 1,2-dichloroethane, 8 h). Only traces of the corresponding isomers **3** were present in the final reaction mixtures (TLC).

On the other hand, the reaction of 1-aminoisoquinoline with the appropriate reagents **13a–c** [10–12] under the same conditions gave compounds **6a–c**, together with smaller amounts of the isomers **4b–d**, respectively.

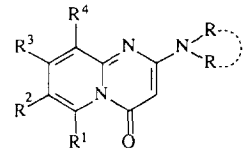


Scheme 1. Synthesis of compounds **2a**, **3a–m** and **4a–j**.

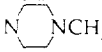
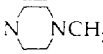
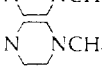
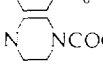
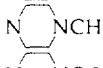
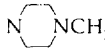
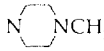
Table I. Structures of compounds **7**, **8a**, **9**, **2a**, **3a–m**, **4a–j**.

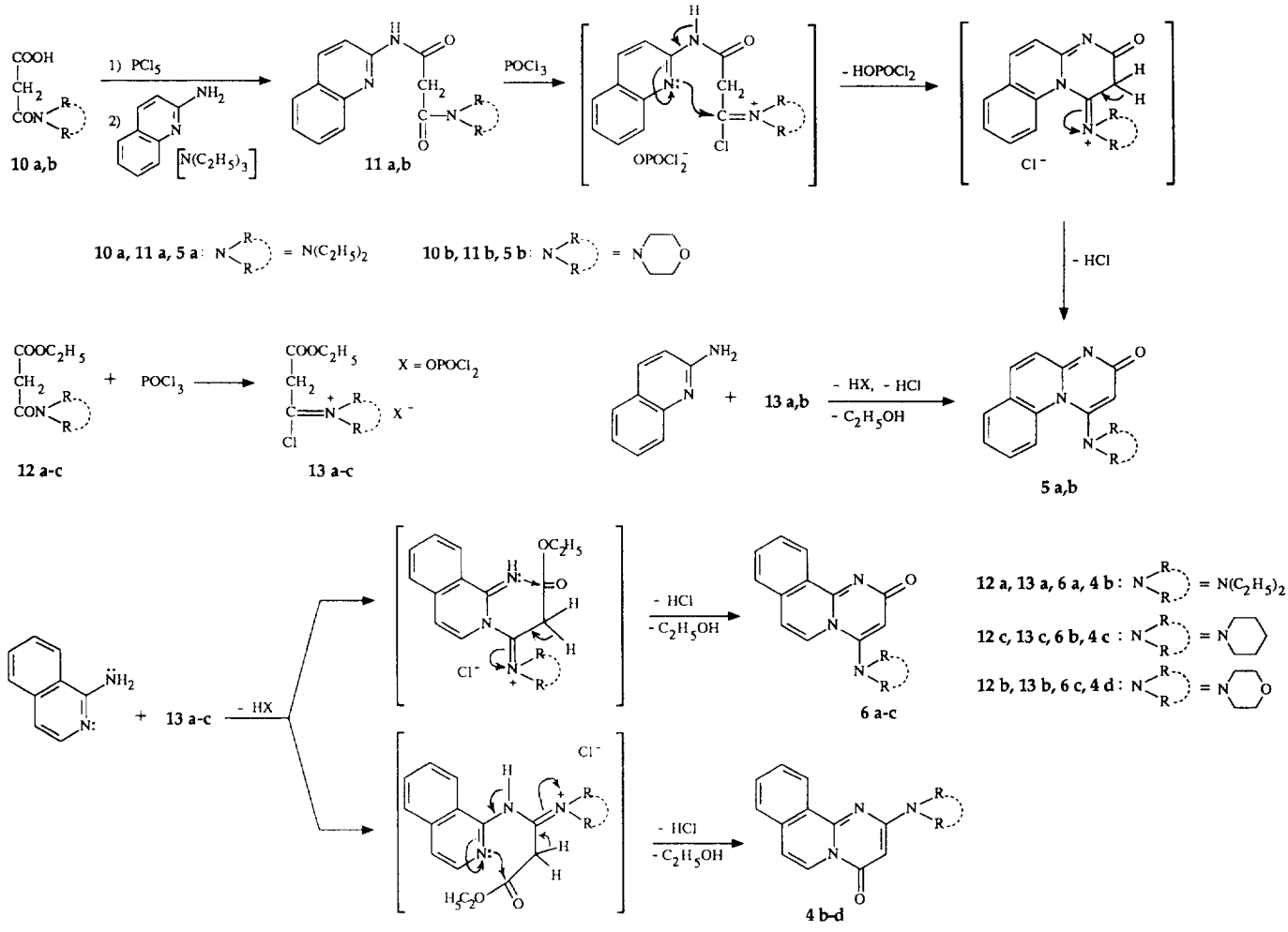


7, 8 a, 9



2 a, 3 a-m, 4 a-j

Compound		R^1	R^2	R^3	R^4
7	-	H	H	H	H
8 a	-	-(CH=CH)$_2$-		H	H
9	-	H	H	-(CH=CH)$_2$-	
2 a		H	H	H	H
3 a	$N(CH_3)_2$	-(CH=CH)$_2$-		H	H
3 b	$N(C_2H_5)_2$	-(CH=CH)$_2$-		H	H
3 c	$N(C_4H_9)_2$	-(CH=CH)$_2$-		H	H
3 d	$N(CH_2CH_2OH)_2$	-(CH=CH)$_2$-		H	H
3 e		-(CH=CH)$_2$-		H	H
3 f		-(CH=CH)$_2$-		H	H
3 g		-(CH=CH)$_2$-		H	H
3 h		-(CH=CH)$_2$-		H	H
3 i		-(CH=CH)$_2$-		H	H
3 j		-(CH=CH)$_2$-		H	H
3 k		-(CH=CH)$_2$-		H	H
3 l		-(CH=CH)$_2$-		H	H
3 m		-(CH=CH)$_2$-		H	H
4 a	$N(CH_3)_2$	H	H	-(CH=CH)$_2$-	
4 b	$N(C_2H_5)_2$	H	H	-(CH=CH)$_2$-	
4 c		H	H	-(CH=CH)$_2$-	
4 d		H	H	-(CH=CH)$_2$-	
4 e		H	H	-(CH=CH)$_2$-	
4 f		H	H	-(CH=CH)$_2$-	
4 g		H	H	-(CH=CH)$_2$-	
4 h		H	H	-(CH=CH)$_2$-	
4 i		H	H	-(CH=CH)$_2$-	
4 j		H	H	-(CH=CH)$_2$-	



Scheme 2. Synthetic routes to compounds **5a,b** and **6a–c**.

Although compounds **5a,b** and **6a–c** were obtained in this way in rather low yields, the above cyclocondensations involving reagents **13**, appear to be, as far as we can see, the most direct synthetic route to the formation of these novel classes of compounds.

The reaction pattern we suggest for the syntheses of compounds **5a,b** and **6a–c** by the reactions of the appropriate reagents **13** with 2-aminoquinoline or 1-aminoisoquinoline, respectively, is illustrated for the formation of compounds **6a–c** (scheme 2). The preparation of the 1-piperazinyl-substituted compounds **5** and **6** could not be achieved by the synthetic methods depicted in scheme 2.

The results of elemental analyses, and IR and ^{1}H-NMR spectral data were consistent with the structures attributed to the compounds described in this paper (see *Experimental protocols* and table II). The characteristic differences previously observed [13, 14] between both IR and ^{1}H-NMR spectra of dialkylamino-substituted isomers **2** and **14** were again significant between spectra of their isomeric benzo-fused derivatives **3**, **4**, and **5**, **6**. In this respect, the positions of the vCO IR band and the ^{1}H-NMR signal of the pyrimidine hydrogen were especially meaningful. The ^{1}H-NMR H-10 signals of compounds **3** are shifted downfield (multiplet center (mc), δ 9.80–9.92) due to the deshielding anisotropy effect of coplanar 1-CO [15], as we previously reported for the corresponding signals of their isosteric analogs 3-(dialkylamino)-1*H*-naphtho[2,1-*b*]pyran-1-ones [16, 17].

The comparison of the ^{1}H-NMR spectrum of compound **3n** (H-5: s, δ 7.01) prepared by a univocal

synthetic pathway (scheme 3), with those of compounds **3a–m** and **5a,b**, made it possible to unequivocally assign the doublet at δ 6.98–7.14 to the proton H-5 of the latter compounds.

Finally, the νNH band was not observed when the IR spectra of (1-piperazinyl) derivatives **2a**, **3g**, **4e** were recorded in $CHCl_3$ solutions, whereas this band was present when KBr pellets were used (see *Experimental protocols* and table II).

None of the compounds described in this paper has been reported previously in the literature, except for compound **4c**, which has been recently obtained by a different procedure [18].

Biological results and discussion

Compounds **2a**, **3a–l**, **4b–j**, **5a,b**, and **6a,c** were tested *in vitro* for their inhibitory activity on the aggregation of human platelets induced in platelet-rich plasma (PRP) by adenosine diphosphate (ADP), collagen, or the Ca^{2+} ionophore A 23187 (calcimycin) (see *Experimental protocols*). Compounds **2c** and **14a,b** (fig 2), which were prepared previously by us [13], were also tested under the same conditions.

The IC_{50} values obtained for the above compounds and ASA, trifluoperazine, and propranolol (reference compounds) are reported in table III. The values recently determined by us [4] for compound **2b** [13] are also reported for comparison.

Among the compounds tested, the 1-piperazinyl-substituted **2a**, **3g** and **4e** were generally the most active, and were more active than all reference compounds whatever the platelet aggregation inducer used. Compounds **2c**, **3a,b,e,h**, **4h**, **5a**, and **6c** were more active than all the reference compounds when platelet aggregation was induced by A 23187.

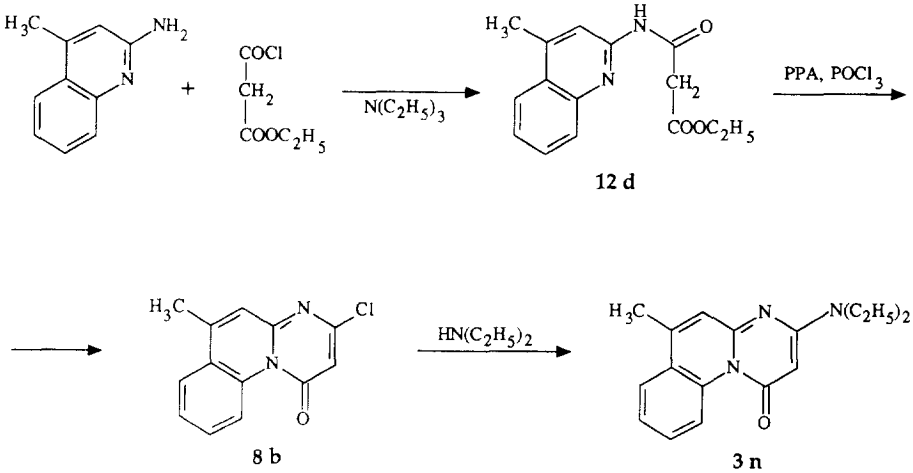
The platelet aggregation inhibitory properties of compounds **2a**, **3g**, and **4e**, particularly those shown by **2a**, are very satisfactory and seem to indicate that the 1-piperazinyl-substituent plays a significant role in the activity both of the pyrido[1,2-*a*]pyrimidines **2** and their angular benzo-fused derivatives **3** and **4**.

The antiplatelet activities of the diethylamino-substituted compounds **2b** (towards A 23187 [4]) and **3b** (towards collagen and A 23187), the morpholino-substituted compounds **2c** (towards A 23187) and **3f** (towards collagen), and the 1-piperidinyl-substituted compound **3e** (towards A 23187) were also interesting, particularly in the case of **3b**, which afforded the second best result when platelet aggregation was induced by A 23187 (IC_{50} = 24 ± 15 μM). As regards the tricyclic isomers **3** and **4**, the IC_{50} values obtained (table III) clearly indicate that, on the whole, structure **3** is more suitable for antiplatelet activity.

Finally, for the isomeric compounds **2b,c** and **14a,b**, **3b,f** and **5a,b**, **4b,d** and **6a,c**, it can be observed that, while compounds **2** and **3** appear to be more active than the corresponding isomers **14** and **5**, respectively, the data for isomers **4** and **6** are of poor quality and are contradictory.

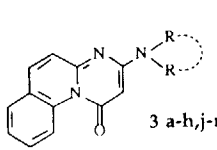
Conclusions

From the data reported in table III the following conclusions can be drawn about the *in vitro* antiplatelet activity of the compounds tested. Between the two

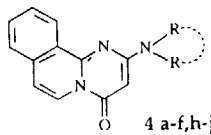


Scheme 3. Synthesis of compound **3n**.

Table II. Data of 3-(dialkylamino)-1*H*-pyrimido[1,2-*a*]quinolin-1-ones **3a–h, j–m** and 2-(dialkylamino)-4*H*-pyrimido[2,1-*a*]isoquinolin-4-ones **4a–f, h–j**.



3 a-h,j-m



4 a-f,h-j

Compd.	*Yield (%)*	*mp °C (solv.)*[a]	*Molecular formula*[b]	*IR*[c] *(cm^{-1})*	^1H-*NMR*[d] *(δ, ppm)*
3 a	81	115-116 (A)	$C_{14}H_{13}N_3O$	1656 (CO),1627,1579, 1561,1533.	3.10(s,6H,CH_3), 5.51(s,1H,H-2), 7.07(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.32-7.85(m,4H, H-6,7,8,9), 9.92(mc,1H,H-10).
3b	86	109-109.5 (A)	$C_{16}H_{17}N_3O$	1656 (CO),1627,1578, 1562,1534.	1.19(t,6H,CH_3), 3.50(q,4H,CH_2), 5.49(s,1H,H-2), 7.00(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.24-7.78(m,4H,H-6,7,8,9), 9.85(mc,1H,H-10).
3 c	80	59-60.5 (B)	$C_{20}H_{25}N_3O$	1656 (CO),1627,1573, 1562,1534.	0.76-2.19(m,14H,$CH_2CH_2CH_2CH_3$), 3.50(t,4H,$CH_2CH_2CH_2CH_3$), 5.52(s,1H,H-2), 7.07(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.34-7.89(m,4H,H-6,7,8,9), 9.91(mc,1H,H-10).
3 d	63	161-162 (C)	$C_{16}H_{17}N_3O_3$	3310s,br and 3150s,br (OH),1649 (CO),1622, 1573,1560,1535.	3.69(mc,8H,CH_2CH_2), 4.88[e] (near s,2H,OH), 5.53(s,1H,H-2), 7.14(d,$J_{5,6}$ = 9.6 Hz, 1H,H-5), 7.39-8.19(m,4H,H-6,7,8,9), 9.80(mc,1H,H-10).
3 e	97	153-153.5 (D)	$C_{17}H_{17}N_3O$	1656 (CO),1627,1572, 1560,1536.	1.65(mc,6H,β-CH_2 + γ-CH_2), 3.64(mc,4H,α-CH_2), 5.62(s,1H,H-2), 7.08(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.29-7.88(m,4H,H-6,7,8,9), 9.89(mc,1H,H-10).
3 f	91	239-240 (E)	$C_{16}H_{15}N_3O_2$	1660 (CO),1629,1573, 1561,1534.	3.51-4.04(m,8H,morpholine CH_2's), 5.62(s,1H,H-2), 7.11(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.40-7.91(m,4H,H-6,7,8,9), 9.90(mc,1H,H-10).
3 g	80	187-188 (D)	$C_{16}H_{16}N_4O$	3324 (NH),1657 (CO), 1629,1573,1561,1531.	1.79[e] (s,1H,NH), 2.93[mc,4H,HN(CH_2)$_2$], 3.64[mc,4H,N(CH_2)$_2$], 5.61(s,1H,H-2), 7.07(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.35-7.86(m,4H,H-6,7,8,9), 9.88(mc,1H,H-10).
3 h	88	151.5-152 (F)	$C_{17}H_{18}N_4O$	1657 (CO),1627,1573, 1561,1535.	2.35(s,3H,CH_3), 2.49[mc,4H,CH_3N(CH_2)$_2$], 3.71[mc,4H,N(CH_2)$_2$], 5.64(s,1H,H-2), 7.10(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.32-7.90(m,4H,H-6,7,8,9), 9.91(mc,1H,H-10).
3j	91	142-143 (D)	$C_{18}H_{20}N_4O_2$	3380br (OH),1656 (CO), 1627,1572,1560,1533.	2.61[mc,6H,N(CH_2)$_3$], 2.88[e] (s,1H,OH), 3.72[mc,6H,N(CH_2)$_2$ + OCH_2], 5.64 (s,1H,H-2), 7.11(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.29-7.89(m,4H,H-6,7,8,9), 9.88(mc, 1H,H-10).
3 k	82	120-121 (F)	$C_{23}H_{22}N_4O$	1658 (CO),1630,1574, 1562,1536.	2.51[mc,4H,$C_6H_5CH_2$N(CH_2)$_2$], 3.56(s,2H,$CH_2C_6H_5$), 3.67[mc,4H,N(CH_2)$_2$], 5.60(s,1H,H-2), 7.07(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.24-7.90(m,9H,H-6,7,8,9 + phenyl H's), 9.88(mc,1H,H-10).
3 l	94	185-186 (D)	$C_{22}H_{20}N_4O$	1660 (CO),1629,1600, 1574,1562,1535.	3.26[mc,4H,C_6H_5N(CH_2)$_2$], 3.84[mc,4H,N(CH_2)$_2$] , 5.68(s,1H,H-2), 6.71-7.93(m, 9H,H-6,7,8,9 + phenyl H's), 6.98(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 9.90(mc,1H,H-10).

Table II . *Continued.*

Compd.	*Yield (%)*	*mp °C (solv.)*[a]	*Molecular formula*[b]	*IR*[c] *(cm^{-1})*	*^{1}H-NMR*[d] *(δ, ppm)*
3m	92	228 (G)	$C_{19}H_{20}N_4O_3$	1690 (urethane CO), 1660 (1-CO),1628, 1574,1561,1534.	1.29(t,3H,CH_2CH_3), 3.66(s,8H,piperazine CH_2's), 4.23(q,2H,CH_2CH_3), 5.66 (s,1H, H-2), 7.13(d,$J_{5,6}$ = 9.6 Hz,1H,H-5), 7.42-7.93 (m,4H,H-6,7,8,9), 9.92(mc,1H,H-10).
4a	86	191-193 (H)	$C_{14}H_{13}N_3O$	1658 (CO),1578,1554, 1523.	3.20(s,6H,CH_3), 5.50(s,1H,H-3), 7.05(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.30-7.83(m,3H,H-8,9, 10), 8.66(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.86(mc,1H,H-11).
4b	82[f]	110-111 (A)	$C_{16}H_{17}N_3O$	1658 (CO),1573,1548, 1519.	1.28(t,6H,CH_3), 3.64(q,4H,CH_2), 5.59(s,1H,H-3), 7.00(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.30- -7.83(m,3H,H-8,9,10), 8.70(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.86(mc,1H,H-11).
4c	81	179-180[g] (H)	$C_{17}H_{17}N_3O$	1658 (CO),1570,1546, 1515.	1.69(mc,6H,β-CH_2 + γ-CH_2), 3.75(mc,4H,α-CH_2), 5.68(s,1H,H-3),7.02(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.42-7.84(m,3H,H-8,9,10), 8.67(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.86(mc, 1H,H-11).
4d	94	210-211.5 (D)	$C_{16}H_{15}N_3O_2$	1660 (CO),1572,1547, 1513.	3.81(near s,8H,morpholine CH_2's), 5.68(s,1H,H-3), 7.07(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.40-7.88(m,3H,H-8,9,10), 8.72(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.87(mc,1H,H-11).
4e	84	192-193 (D)	$C_{16}H_{16}N_4O$	3300 (NH),1658 (CO), 1570,1544,1514.	1.91[e] (s,1H,NH), 3.00[mc,4H,HN(CH_2)$_2$], 3.77[mc,4H,N(CH_2)$_2$], 5.68(s,1H,H-3), 7.05(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.45-7.88(m,3H,H-8,9,10), 8.70(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.87(mc,1H,H-11).
4f	80	151.5-153 (I)	$C_{17}H_{18}N_4O$	1660 (CO),1572,1547, 1513.	2.37(s,3H,CH_3), 2.54[mc,4H,CH_3N(CH_2)$_2$], 3.80[mc,4H,N(CH_2)$_2$], 5.68(s,1H,H-3), 7.05(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.36-7.87(m,3H,H-8,9,10), 8.68(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.87(mc,1H,H-11).
4h	91	146-147 (D)	$C_{18}H_{20}N_4O_2$	3370br (OH),1658 (CO), 1571,1547,1513.	2.66[mc,6H,N(CH_2)$_3$], 2.95[e] (s,1H,OH), 3.79[mc,6H,N(CH_2)$_2$ + OCH_2], 5.70(s,1H, H-3), 7.07(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.45-7.88(m,3H,H-8,9,10), 8.70(d,$J_{6,7}$ = 8 Hz,1H, H-6), 8.89(mc,1H,H-11).
4i	81	194-194.5 (D)	$C_{23}H_{22}N_4O$	1660 (CO),1572,1548, 1514.	2.57[mc,4H,$C_6H_5CH_2$N(CH_2)$_2$], 3.58(s,2H,$CH_2C_6H_5$), 3.79[mc,4H,N(CH_2)$_2$], 5.67(s,1H,H-3), 7.02(d,$J_{7,6}$ = 8 Hz,1H,H-7), 7.39(near s,5H,phenyl H's), 7.48- -7.81(m,3H,H-8,9,10), 8.68(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.86(mc,1H,H-11).
4j	79	177-178 (D)	$C_{22}H_{20}N_4O$	1660 (CO),1600,1571, 1546,1510.	3.31[mc,4H,C_6H_5N(CH_2)$_2$], 3.94[mc,4H,N(CH_2)$_2$], 5.72(s,1H,H-3), 6.69-7.86 (m,9H,H-7,8,9,10 + phenyl H's), 8.70(d,$J_{6,7}$ = 8 Hz,1H,H-6), 8.88(mc,1H,H-11).

[a]Crystallization solvent: A = ethyl ether/petroleum ether 40–70°C, B = petroleum ether 40–70°C, C = acetone, D = ethyl acetate, E = chloroform/ethyl acetate, F = isopropyl ether, G = ethanol, H = ethyl acetate/isopropyl ether, I = ethyl acetate/petroleum ether 40–70°C. [b]Anal C, H, N. [c]In $CHCl_3$ solutions, except for **3d**,**g** and **4e** for which a KBr pellet was used. Abbreviations: s = strong, br = broad. [d]Solvents: $CDCl_3$ for all compounds except **3d**, for which $(CD_3)_2SO$ was used. [e]Disappeared with D_2O. [f]Purified by column chromatography (silica gel/chloroform). [g]Lit [18]: mp 169–171°C.

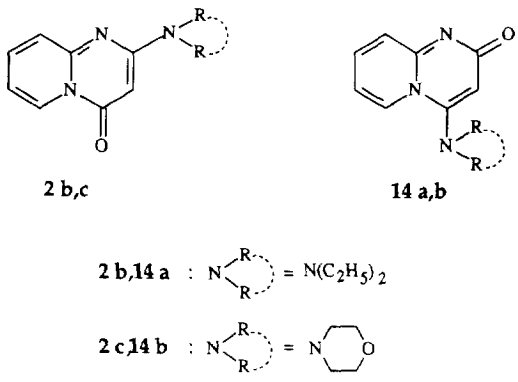


Fig 2. Structures of compounds **2b.c** and **14a.b**.

tricyclic isomeric structures **3** and **4**, structure **3** seems to be the most convenient for antiplatelet activity. On the other hand, the *in vitro* inhibitory properties on human platelet aggregation shown by bicyclic compounds **2** (see table III and reference [4]) appear about equivalent to those exhibited by their corresponding benzo-fused derivatives **3**.

Among the dialkylamino substituents used, 1-piperazinyl gives rise to the highest antiplatelet activity towards all platelet aggregation inducers. In fact, the 1-piperazinyl derivatives **2a**, **3g** and **4e** are, in order of decreasing activity, the most active of all the compounds tested, and clearly more active than all the reference compounds used. In particular compound **2a** (IC_{50} (μM): 6 ± 1.8 (ADP), 3.6 ± 1.2 (collagen), 19 ± 9 (A 23187)) can be regarded as a very interesting *in vitro* antiplatelet agent. The presence of a substituent in position 4 of the 1-piperazinyl group always lowered the activity in all structures examined but to variable extents.

Experimental protocols

Chemical synthesis

Melting points were determined using a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 398 spectrophotometer. ^{1}H-NMR spectra were recorded on a Hitachi Perkin-Elmer R 600 (60 MHz) spectrometer using $(CH_3)_4Si$ as an internal reference (δ = 0). Analyses of all new compounds, indicated by the symbols of the elements, were within ±0.4% of the theoretical values and were performed by the Laboratorio di Microanalisi, Istituto di Scienze Farmaceutiche, Università di Genova.

Thin-layer chromatography was run on Merck silica-gel 60 F_{254} precoated plastic sheets (0.2 mm thick). Column chromatography was performed using Carlo Erba silica-gel (0.05–0.20 mm).

2-(1-Piperazinyl)-4H-pyrido[1,2-a]pyrimidin-4-one ***2a***

A mixture of 5.0 mmol (0.90 g) of 2-chloro-4*H*-pyrido[1,2-*a*]-pyrimidin-4-one **7** [5], 50.0 mmol (4.31 g) of piperazine and 50 ml of ethanol was heated at reflux for 2 h, with stirring. The solvent was then removed *in vacuo* and water (50 ml) was added to the solid residue; the resulting alkaline solution was exhaustively extracted with chloroform. The combined extracts were washed with water, dried (anhydrous sodium sulphate), then evaporated to dryness under reduced pressure to give a thick oil from which, after addition of a little ethyl ether and standing, pure compound **2a** (1.01 g, 88%) separated out as a whitish crystalline solid; mp: 125–126°C after recrystallization from ethyl acetate/petroleum ether 40–70°C. Anal $C_{12}H_{14}N_4O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1664 (CO), 1640 shoulder, 1560, 1536, 1504; νNH: 3305 (in KBr). ^{1}H-NMR ($CDCl_3$), δ: 1.90 (s, 1H, NH; disappeared after treatment with D_2O), 2.95 (mc, 4H, $HN(CH_2)_2$), 3.69 (mc, 4H, $N(CH_2)_2$), 5.66 (s, 1H, H-3), 6.89 (mc, 1H, H-7), 7.30 (mc, 1H, H-9), 7.63 (mc, 1H, H-8), 8.95 (mc, 1H, H-6).

3-(Dialkylamino)-1H-pyrimido[1,2-a]quinolin-1-ones ***3a–h, j–m*** *and 2-(dialkylamino)-4H-pyrimido[2,1-a]isoquinolin-4-ones* ***4a–f, h–j****. General procedure*

A mixture of 2.0 mmol (0.46 g) of 3-chloro-1*H*-pyrimido[1,2-*a*]-quinolin-1-one **8a** [6] (preparation of compounds **3a–h, j–m**) or 2-chloro-4*H*-pyrimido[2,1-*a*]isoquinolin-4-one **9** [7] (preparation of compounds **4a–f, h–j**), 20.0 mmol of the appropriate amine and 80 ml ethanol was heated at reflux for the following time, depending on the compound prepared: 1 h (**4c**), 2 h (**3a,e,f, 4a,d–f,h,i**), 3 h (**3b,g,j–l, 4j**), 4 h (**3h,m**) and 5 h (**4b**). In the case of the preparations of compounds **3a** and **4a**, 20.0 mmol dimethylamine hydrochloride and 20.0 mmol triethylamine were used instead of the free amine. The final solution was evaporated to dryness under reduced pressure, the residue partitioned between 5% aqueous sodium bicarbonate and chloroform, and the aqueous phase was extracted several more times with chloroform. The combined extracts were dried (anhydrous sodium sulphate) and the solvent was removed to give an oily or nearly solid residue, which was treated with a little ethyl ether so that a solid separated out. The nearly pure compounds **3** (pale-yellow solids) or **4** (white or whitish solids) were collected by filtration, and then crystallized from a suitable solvent (see table II).

In the case of compounds **3c,d** (reaction of **8a** with dibutylamine or diethanolamine, respectively), 10 ml ethylene glycol was used as a solvent and the reaction mixture was heated at 160°C for 2 h. After cooling, the final solution was poured onto crushed ice and water (100 ml) and the mixture obtained was exhaustively extracted with chloroform. The combined extracts were dried over anhydrous sodium sulphate and the solvent was removed. This gave crude compounds **3c** (oil) or **3d** (nearly solid). The recovery of pure compound **3d** was achieved by the above-described treatment with ethyl ether, whereas **3c** was purified through the preparation of the picrate (yellow crystals from ethanol, mp 143–143.5°C). Treatment of an analytically pure sample of this picrate with aqueous 1 N sodium hydroxide, followed by extraction with ethyl ether and removal of solvent, gave pure compound **3c** as a thick oil, which, after addition of a little petroleum ether 40–70°C and standing, easily crystallized.

Data for compounds **3a–h, j–m** and **4a–f,h–j** are reported in table II.

3-(4-Ethyl-1-piperazinyl)-1H-pyrimido[1,2-a]quinolin-1-one ***3i*** *and 2-(4-ethyl-1-piperazinyl)-4H-pyrimido[2,1-a]isoquinolin-4-one* ***4g***

A mixture of 3.0 mmol (0.84 g) of **3g** or **4e**, 4.0 mmol (0.80 g) of ethyl *p*-toluene sulphonate, 0.20 g anhydrous sodium carbonate and 40 ml anhydrous ethanol was heated at reflux for 3 h,

Table III. *In vitro* inhibitory activity of compounds **2a–c**, **3a–l**, **4b–j**, **14a,b**, **5a,b** and **6a,c** on human platelet aggregation induced in PRP by ADP, collagen, and A 23187.

Compound [a]	IC_{50} (μM) ± SD		
	ADP (5.0 μM)	*Collagen (5.0 μg/ml)*	*A 23187 (20.0 μM)*
2 a	6±1.8	3.6±1.2	19±9
2b	>1000±0 [b]	330±136 [b]	100±87 [b]
2c	370±50	240±70	100±52
3 a	>1000±0	371±100	144±51
3b	>1000±0	78±8	24±15
3c	>1000±0	510±120	490±130
3 d	710±120	500±180	>1000±0
3 e	>1000±0	240±100	75±0
3 f	970±70	76±18	850±140
3 g	13±4	15±8	28±8
3 h	360±150	210±90	91±44
3 i	420±50	60±33	370±170
3j	910±46	86±30	840±150
4b	>1000±0	890±130	>1000±0
4 d	500±70	120±43	>1000±0
4 e	38±10	21±9	54±17
4 f	820±150	290±87	>1000±0
4 g	900±140	700±130	>1000±0
4 h	310±39	160±48	160±50
5 a	410±66	390±100	250±80
5b	710±200	550±130	450±90
6 a	>1000±0	360±150	>1000±0
6 c	730±200	380±170	200±47
ASA	>1000±0	150±50	>1000±0
Trifluoperazine	110±13	37±20	290±50
Propranolol	490±220	110±4	290±70

[a]The IC_{50} values of compounds **3k,l**, **4c,i,j**, **14a,b** were > 1000 μM towards all the three platelet aggregation inducers. [b]Reference [4].

with stirring. The solvent was then removed *in vacuo* and the residue was partitioned between chloroform and 1 N aqueous sodium hydroxide. The organic layer was collected and the aqueous phase extracted several more times with chloroform. The combined extracts were washed with water and dried over anhydrous sodium sulphate. Removal of the solvent afforded an oily residue, which was chromatographed on a silica-gel column, eluting first with ethyl acetate until some impurities were removed, then with chloroform/petroleum ether 40–70°C/triethylamine (6:2:1). The thick oil obtained was treated with a little ethyl ether to give pure compound **3i** or **4g** as a crystalline solid.

Compound ***3i***. 0.48 g (52%), pale-yellow needles, mp 122–122.5°C, after crystallization from isopropyl ether. Anal $C_{18}H_{20}N_4O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1657 (CO), 1626, 1573, 1560, 1534. ^{1}H-NMR ($CDCl_3$), δ: 1.11 (t, 3H, CH_3), 2.23–2.81 (m, 6H, $N(CH_2)_3$), 3.69 (mc, 4H, $N(CH_2)_2$), 5.63 (s, 1H, H-2), 7.07 (d, $J_{5,6}$ = 9.6 Hz, 1H, H-5), 7.29–7.87 (m, 4H, H-6,7,8,9), 9.88 (mc, 1H, H-10).

Compound ***4g***. 0.72 g (78%), white needles, mp 134–134.5°C after crystallization from isopropyl ether. Anal $C_{18}H_{20}N_4O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1659 (CO), 1572, 1548, 1514. ^{1}H-NMR ($CDCl_3$), δ: 1.13 (t, 3H, CH_3), 2.26–2.85 (m, 6H,

$N(CH_2)_3$), 3.79 (mc, 4H, $N(CH_2)_2$), 5.66 (s, 1H, H-3), 7.00 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 7.33–7.84 (m, 3H, H-8,9,10), 8.67 (d, $J_{6,7}$ = 8 Hz, 1H, H-6), 8.83 (mc, 1H, H-11).

N,N-Dialkyl-N'-(2-quinolinyl)malonamides ***11a,b***
In an ice-cooled flask, protected from moisture with a calcium chloride drying tube, 18.8 mmol (3.91 g) phosphorus pentachloride was slowly added to a stirred solution of 18.0 mmol of the appropriate *N,N*-dialkylmalonamic acid **10a** or **10b** in 80 ml of anhydrous tetrahydrofuran. The resulting solution was stirred at room temperature for 3 h, and then 8.0 mmol (1.15 g) 2-aminoquinoline [19, 20] dissolved in 40 ml anhydrous tetrahydrofuran was added and the mixture was cooled in an ice-bath. Triethylamine (10 ml) was then added dropwise and an exothermic reaction occurred with emission of white fumes and formation of a precipitate. This suspension was further stirred at room temperature for 30 min, and then poured into ice-water. The mixture was made alkaline with sodium carbonate and exhaustively extracted with chloroform. The combined extracts, dried over anhydrous sodium sulphate and evaporated to dryness, afforded an oily residue from which compound **11a** or **11b** was recovered as described below.

N,N-Diethyl-N'-(2-quinolinyl)malonamide ***11a***
The residue obtained from the reaction carried out with 2.87 g of *N,N*-diethylmalonamic acid **10a** [9] was chromatographed on a silica-gel column eluting with a chloroform/ethyl acetate mixture (1:1). The thick oil obtained was treated with a little isopropyl ether, to afford 1.19 g (52%) pure compound **11a** as white crystals; mp: 98–99°C after recrystallization from the same solvent. Anal $C_{16}H_{19}N_3O_2$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 3210 broad (NH), 1687 (CO), 1626 (CO), 1598, 1577, 1526, 1500. ^{1}H-NMR ($CDCl_3$), δ: 1.18 (mc, 6H, CH_2CH_3), 3.12–3.79 (m, 4H, CH_2CH_3), 3.55 (s, 2H, CH_2), 7.20–8.59 (m, 6H, quinoline Hs), 10.70 (broad s, 1H, NH; disappeared after treatment with D_2O).

3-Morpholino-3-oxo-N-(2-quinolinyl)propanamide ***11b***
The thick oil obtained from the reaction of 3-morpholino-3-oxopropanoic acid **10b** (3.12 g) [9] was treated with a little ethyl acetate. After standing, 1.25 g of pure **11b** crystallized as a white solid which was collected by filtration; mp: 167–168°C after recrystallization from the same solvent. Anal $C_{16}H_{17}N_3O_3$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 3220 broad (NH), 1685 (CO), 1633 (CO), 1597, 1578, 1525, 1498. ^{1}H-NMR ($CDCl_3$), δ: 3.40–3.90 (m, 10H, morpholine CH_2s + CH_2CO), 7.27–8.54 (m, 6H, quinoline Hs), 10.34 (broad s, 1H, NH; disappeared after treatment with D_2O). From the filtrate, by the same chromatographic procedure used for the recovery of compound **11a**, an additional crop was obtained (0.16 g) of **11b** (total yield: 59%).

Cyclization of malonamides ***11a,b*** *to 1-(dialkylamino)-3H-pyrimido[1,2-a]quinolin-3-ones* ***5a,b***
Phosphorus oxychloride (8.18 mmol, 1.25 g) was added dropwise with stirring to an ice-cooled solution of 3.0 mmol of **11a** (0.86 g) or **11b** (0.90 g) in 5 ml 1,2-dichloroethane. The mixture was allowed to stir at room temperature for 30 min, and then heated at reflux for 3 h (compound **11a**) or 4 h (compound **11b**), while stirring. After cooling, 15 ml 1,2-dichloroethane and a solution of 7.5 g trihydrate sodium acetate in 20 ml water were added, and the resulting mixture was stirred at room temperature for 15 min. The organic layer was then collected and the aqueous phase was exhaustively extracted with chloroform. The combined extracts were dried (anhydrous sodium sulphate) and solvents removed *in vacuo* to give an oily residue, which was chromatographed on a silica-gel column eluting first with a chloroform/ethyl acetate mixture (1:1) to remove impurities and then with a chloroform/methanol mixture (95:5). This eluate, after removal of solvents, afforded a thick oil which was treated with some ethyl acetate/ethyl ether (1:1); the nearly pure compound **5a** or **5b** which thus separated out as a crystalline solid was then recrystallized from ethyl acetate.

1-(Diethylamino)-3H-pyrimido[1,2-a]quinolin-3-one ***5a***. 0.52 g (65%), whitish crystals, mp 164.5–165°C. Anal $C_{16}H_{17}N_3O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1620 (CO), 1563, 1520. ^{1}H-NMR ($CDCl_3$), δ: 1.10 (t, 6H, CH_3), 2.75–3.36 (m, 4H, CH_2), 5.93 (s, 1H, H-2), 7.11 (d, $J_{5,6}$ = 9.6 Hz, 1H, H-5), 7.35–7.88 (m, 4H, H-6,7,8,9), 8.60 (mc, 1H, H-10).

1-Morpholino-3H-pyrimido[1,2-a]quinolin-3-one ***5b***. 0.57 g (68%), whitish crystals, mp 231–232°C. Anal $C_{16}H_{15}N_3O_2$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1623 (CO), 1562, 1521. ^{1}H-NMR ($CDCl_3$), δ: 2.56–3.35 (m, 4H, NCH_2), 3.45–4.13 (m, 4H, OCH_2), 5.90 (s, 1H, H-2), 7.09 (d, $J_{5,6}$ = 9.6 Hz, 1H, H-5), 7.30–7.90 (m, 4H, H-6,7,8,9), 8.78 (mc, 1H, H-10).

An alternative route to 1-(dialkylamino)-3H-pyrimido[1,2-a]-quinolin-3-ones ***5a,b***
Phosphorus oxychloride (37.5 mmol, 5.75 g) was added dropwise with stirring to 27.5 mmol of ethyl *N,N*-diethylmalonamate **12a** [10] (5.15 g) or ethyl 3-morpholino-3-oxopropanoate **12b** [11] (5.53 g dissolved in 10 ml 1,2-dichloroethane) which was contained in a flask cooled in an ice-bath and protected from moisture with a calcium chloride tube. The resulting solution was stirred at room temperature for 30 min, and then a suspension of 25.0 mmol (3.60 g) of 2-aminoquinoline in 30 ml 1,2-dichloroethane was added and the mixture was heated at reflux for 8 h, with stirring. A solution of 34 g trihydrate sodium acetate in 70 ml water was then added to the hot reaction mixture; the mixture obtained was briefly stirred, cooled, and allowed to stir at room temperature for further 15 min. The organic layer was then collected and the aqueous phase was exhaustively extracted with chloroform. The combined organic phases were dried (anhydrous sodium sulphate) and the solvents removed *in vacuo* to give an oily residue which was chromatographed on a silica-gel column, eluting first with the mixture chloroform/ethyl acetate (1:1) to remove several impurities and traces of compound **3b** or **3f** (TLC), and then with the chloroform/triethylamine mixture (3:1). The eluate collected was evaporated to dryness under reduced pressure to give an oil from which, after treatment with a little ethyl acetate, crude compound **5** separated out as yellowish solid; by crystallizing this solid from ethyl acetate with charcoal, the whitish crystalline compound **5a** (1.46 g, 22%) or **5b** (1.70 g, 24%) was obtained.

4-(Dialkylamino)-2H-pyrimido[2,1-a]isoquinolin-2-ones ***6a–c*** *and 2-(dialkylamino)-4H-pyrimido[2,1-a]isoquinolin-4-ones* ***4b–d***. *General procedure*
The reaction of the appropriate ethyl *N,N*-dialkylmalonamate **12** (27.5 mmol) with 1-aminoisoquinoline [21, 19] (25.0 mmol, 3.60 g) in the presence of phosphorus oxychloride (37.5 mmol, 5.75 g) was carried out exactly as described above in the analogous procedure for the preparation of compounds **5a,b**. Only the quantity of 1,2-dichloroethane was larger (80 ml) due to the lower solubility of 1-aminoisoquinoline in this solvent. After an identical treatment of the final reaction mixture with an aqueous solution of trihydrate sodium acetate and the subsequent exhaustive extraction of the aqueous phase with chloroform, the combined organic phases finally afforded a dark, oily residue from which compounds **4** and **6** were recovered according to the following procedures.

4-(Diethylamino)-2H-pyrimido[2,1-a]isoquinolin-2-one ***6a*** *and 2-(diethylamino)-4H-pyrimido[2,1-a]isoquinolin-4-one* ***4b***. The oil obtained from the reaction carried out with 5.15 g of ethyl *N,N*-diethylmalonamate **12a** [10] was dissolved in a little chloroform and chromatographed on a silica-gel column, eluting with the mixture chloroform/ethyl acetate (1:1). The first fractions of this eluate, containing **4b** along with several impurities, were evaporated to dryness *in vacuo* to give a residue which was subjected to a second column chromatography (silica gel and benzene/petroleum ether 40–70°C/triethylamine (3:3:1) as eluent). The eluate collected after removal of solvents afforded an oil, which was dissolved in ethanol and treated with 70% aqueous $HClO_4$; after addition of a little ethyl ether and standing at 4°C a pale-yellow solid (nearly pure **4b**·$HClO_4$) separated out. From this solid, after treatment with 10% aqueous sodium carbonate and extraction with chloroform, pure compound **4b** (0.11 g, 1.6%) was finally recovered.

The first chromatography of the crude reaction product was then pursued by eluting with chloroform/triethylamine (3:1). The eluate collected was evaporated to dryness *in vacuo* to give an oil from which, after treatment with a little ethyl acetate, compound **6a** (1.35 g, 20%) separated out as a yellowish solid; mp: 132°C, whitish crystals, after crystallization from ethyl acetate/petroleum ether 40–70°C with charcoal. Anal $C_{16}H_{17}N_3O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1625 (CO), 1608 shoulder, 1557 weak, 1498. ^{1}H-NMR ($CDCl_3$), δ: 1.15 (t, 6H, CH_3), 3.16 (q, 4H, CH_2), 6.20 (s, 1H, H-3), 7.08 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 7.48–7.95 (m, 3H, H-8,9,10), 8.14 (d, $J_{6,7}$ = 8 Hz, 1H, H-6), 9.15 (mc, 1H, H-11).

4-(1-Piperidinyl)-2H-pyrimido[2,1-a]isoquinolin-2-one ***6b*** *and 2-(1-piperidinyl)-4H-pyrimido[2,1-a]isoquinolin-4-one* ***4c***. The residue derived from the reaction of 5.48 g ethyl 3-oxo-3-(1-piperidinyl)propanoate **12c** [12] was worked-up exactly as described above for the corresponding oily residue in the previous case. From the chromatographic fraction eluted with chloroform/ethyl acetate (1:1), after the same purifying procedures used above for **4b**, compound **4c** (0.17 g; 2.4%) was obtained.

The fraction eluted with chloroform/triethylamine (3:1), after a procedure analogous to that employed above for the recovery of **6a**, afforded a first amount (0.46 g) of nearly pure **6b** as yellowish solid; whitish crystals, mp 199.5–200.5°C, after crystallization from ethyl acetate with charcoal. Anal $C_{17}H_{17}N_3O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1630 (CO), 1613 shoulder, 1562 shoulder, 1500. ^{1}H-NMR ($CDCl_3$), δ: 1.50–2.16 (m, 6H, β-CH_2 + γ-CH_2), 2.40–3.63 (m, 4H, α-CH_2), 6.09 (s, 1H, H-3), 7.05 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 7.47–7.93 (m, 3H, H-8,9,10), 7.96 (d, $J_{6,7}$ = 8 Hz, 1H, H-6), 9.07 (mc, 1H, H-11).

The oil obtained by removing ethyl acetate from the mother liquor of compound **6b** was dissolved in a little anhydrous ethanol and treated with a saturated solution of hydrogen chloride in anhydrous ethyl ether. After standing at 4°C, the rough hydrochloride of **6b** separated out as a reddish solid which was collected and dissolved in water. The aqueous solution was stirred with charcoal, filtered, made alkaline by the addition of sodium carbonate, and then exhaustively extracted with chloroform. The combined extracts were evaporated under reduced pressure to give an additional crop (0.39 g) of pure **6b** (total yield: 12%).

4-Morpholino-2H-pyrimido[2,1-a]isoquinolin-2-one ***6c*** *and 2-morpholino-4H-pyrimido[2,1-a]isoquinolin-4-one* ***4d***. The oil obtained from the reaction carried out with 5.53 g of ethyl 3-morpholino-3-oxopropanoate **12b** [11] was subjected to a chromatographic procedure identical to that used for the corresponding oils in the preceding cases. The fraction eluted with chloroform/ethyl acetate (1:1), after removal of solvents, gave a nearly solid residue from which, after addition of some ethyl acetate, a first amount (0.69 g) of pure **4d** separated out. By evaporating the mother liquor, an oil was obtained which was dissolved in a little ethanol and treated with 70% aqueous $HClO_4$. After addition of a little ethyl ether and standing at 4°C, the perchlorate of compound **4d** separated out as a pale-yellow solid from which, after treatment with 10% aqueous sodium carbonate and extraction with chloroform, a further amount (0.16 g) of pure **4d** was recovered (total yield: 12%).

By proceeding as described above in the case of the preparation of **6a**, nearly pure compound **6c** (1.26 g, 18%) was then obtained from the fraction eluted with chloroform/triethylamine (3:1); whitish crystals, mp 249–250°C after crystallization from dichloromethane/ethyl acetate with charcoal. Anal $C_{16}H_{15}N_3O_2$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1631 (CO), 1618 shoulder, 1564 weak, 1502. ^{1}H-NMR ($CDCl_3$), δ: 2.88–3.31 (m, 4H, NCH_2), 3.73–4.25 (m, 4H, OCH_2), 6.09 (s, 1H, H-3), 7.07 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 7.37–7.88 (m, 3H, H-8,9,10), 8.04 (d, $J_{6,7}$ = 8 Hz, 1H, H-6), 8.99 (mc, 1H, H-11).

Ethyl N-(4-methyl-2-quinolinyl)malonamate ***12d***
The solution of 3.01 g (20.0 mmol) of ethyl 3-chloro-3-oxopropanoate in 15 ml dry benzene was added dropwise to an ice-cooled solution of 3.16 g (20.0 mmol) of 2-amino-4-methylquinoline [19] (mp 130–132°C, lit [22]: mp 130–131°C) and 2.02 g (20.0 mmol) of triethylamine in 25 ml dry benzene. The resulting mixture was then heated at reflux for 1 h, with stirring. After cooling, the mixture was poured into ice-water and, after addition of sodium bicarbonate, the benzene layer was collected and the aqueous phase was thoroughly extracted with chloroform. The combined organic phases were washed with water, dried over anhydrous sodium sulphate and evaporated to dryness *in vacuo*. The oily residue was dissolved in a little chloroform and was chromatographed on a silica-gel column eluting with the mixture chloroform/ethyl acetate (1:1). By removing the solvents from this eluate, pure compound **12d** (3.27 g, 60%) was obtained; white needles, mp 168–168.5°C after crystallization from ethanol. Anal $C_{15}H_{16}N_2O_3$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 3400 and 3300 (free and assoc NH), 1724 (ester CO), 1692 (amide CO), 1600, 1580, 1526, 1500. ^{1}H-NMR ($CDCl_3$), δ: 1.25 (t, 3H, CH_2CH_3), 2.67 (s, 3H, CH_3), 3.52 (s, 2H, CH_2CO), 4.24 (q, 2H, CH_2CH_3), 7.20–8.36 (m, 5H, quinoline Hs), 9.83 (broad s, 1H, NH; disappeared after treatment with D_2O).

3-Chloro-6-methyl-1H-pyrimido[1,2-a]quinolin-1-one ***8b***
The mixture of 10.0 mmol (2.72 g) of **12d**, 30.0 mmol (4.60 g) of phosphorus oxychloride and 0.70 g of polyphosphoric acid was heated with stirring at 130°C for 3 h. Anhydrous ethanol (10 ml) was then added to the resulting hot, brown slurry and the mixture was refluxed for 30 min, with stirring. After cooling, the resulting reddish suspension was poured into water (about 800 ml) so that the crude compound **8b** separated out as a yellowish amorphous solid. This solid was recovered by filtration, washed with water, dried (IR lamp), dissolved in a little chloroform and chromatographed on a silica-gel column eluting with the mixture chloroform/ethyl acetate (1:1). The eluate collected was evaporated to dryness *in vacuo* to afford 1.57 g (64%) of pure **8b**; yellow needles, mp 182.5–183°C, after crystallization from ethanol. Anal $C_{13}H_9ClN_2O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1677 (CO), 1638, 1571, 1542, 1513. ^{1}H-NMR ($CDCl_3$), δ: 2.64 (s, 3H, 6-CH_3), 6.52 (s, 1H, H-2), 7.22 (s, 1H, H-5), 7.48–8.10 (m, 3H, H-7,8,9), 9.88 (mc, 1H, H-10).

3-(Diethylamino)-6-methyl-1H-pyrimido[1,2-a]quinolin-1-one ***3n***

A mixture of 2.0 mmol (0.49 g) of compound **8b**, 20.0 mmol (1.46 g) of diethylamine and 10 ml ethylene glycol was heated at 160°C for 2 h, with stirring. After cooling, the solution was poured into 200 ml water and the mixture was exhaustively extracted with ethyl ether. The combined extracts were dried (anhydrous sodium sulphate), and then evaporated to dryness to give an oily residue which was chromatographed on a silica-gel column eluting with chloroform/ethyl acetate (1:1). By removing the solvents from the fraction collected, a thick oil was obtained which was treated with some petroleum ether 40–70°C and allowed to stand at 4°C until nearly pure compound **3n** (0.41 g, 73%) separated out as a yellowish solid: pale-yellow crystals, mp 124.5–125°C, after crystallization from the same solvent with charcoal. Anal $C_{17}H_{19}N_3O$ (C, H, N). IR ($CHCl_3$), cm^{-1}: 1658 (CO), 1631, 1573, 1563, 1528. ^{1}H-NMR ($CDCl_3$), δ: 1.22 (t, 6H, CH_2CH_3), 2.52 (s, 3H, 6-CH_3), 3.55 (q, 4H, CH_2CH_3), 5.50 (s, 1H, H-2), 7.01 (s, 1H, H-5), 7.28–7.95 (m, 3H, H-7,8,9), 9.93 (mc, 1H, H-10).

Biological evaluation

Platelet aggregation

Human blood obtained from healthy volunteers was collected in a 130 mM trisodium citrate aqueous solution (volume ratio 9:1). Platelet-rich plasma (PRP) was prepared by centrifuging the anticoagulant-treated blood at 100 *g* for 30 min.

Platelet aggregation, performed in a Aggrecoder PA-3210 aggregometer (A Menarini, Florence, Italy), was measured following the Born's turbidimetric method [23] and quantified by the light transmission reached after 3 min.

PRP (500 μl) was preincubated at 37°C for 2 min with solvent (dimethylsulphoxide, 5 μl), or drug solution before the addition of the platelet aggregation agent. PRP aggregation was induced by 5.0 μM ADP (Sigma), by collagen from bovine tendon (Mascia Brunelli) at the final concentration of 5.0 μg/ml, or by 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 50% inhibition of the aggregation. The IC_{50} values reported in table III are averages (± SD) of those obtained from at least four different batches of platelets (usually 5–8 batches).

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