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Synthesis and Pharmacological Evaluation of 2,5-Cycloamino-5*H*-[1]benzopyrano[4,3-*d*]pyrimidines Endowed with In Vitro Antiplatelet Activity

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Abstract—A series of new 2,5-cycloamino-5*H*-[1]benzopyrano[4,3-*d*]pyrimidines **3a–i** have been synthesized and tested in vivo for the anti-inflammatory/analgesic/antipyretic effects and in vitro to evaluate the antiplatelet activity on guinea-pig platelet-rich plasma aggregated by collagen, adenosine-5′-diphosphate (ADP) and arachidonic acid (AA). Title compounds were ineffective in vivo; however, the pyrrolidino derivatives **3a** and **3c** exhibited an antiplatelet activity against all the aggregants differing from that of acetylsalicylic acid (ASA) while the 5-morpholino derivatives **3g–i** showed the most potent ASA-like antiplatelet activity.

We recently reported the synthesis and the pharmacological properties of original benzopyrano[4,3-*d*]pyrimidine derivatives **1a–f** and **2a–f** (Fig. 1). Compounds **1a–f**,[1] which are characterized by the presence of free and substituted amino groups in position 2 and 5, respectively, of the tricycle system, showed interesting antipyretic, analgesic and antiphlogistic activities. They were also endowed with antiplatelet and gastroprotective properties, and were lacking in ulcerogenicity. In particular, the pharmacological data indicated that, generally, the 5-cycloamino derivatives were more active than the 5-alkylamino congeners. The presence of the 2-methylthio instead of the 2-amino group in compounds **2a–f**[2] markedly impaired the in vivo biological activities and, furthermore, seemed to increase the in vitro antiplatelet effect. In fact, the 2-methylthio derivatives **2a**, **2c** and **2d** were more effective than the corresponding 2-amino analogues as inhibitors of the ADP- and AA-induced aggregation. Moreover, only the 5-pyrrolidino derivative **2d** prevented ethanol-induced gastric ulceration sharing the gastroprotective effect displayed by almost all the 2-amino derivatives. Therefore, we concluded that the nature of the substituent in position 2 can discriminate between the in vivo or in vitro activities, while the substituent in position 5 could be crucial to determine the potential of the biological activities in vitro. Finally, the pyrrolidino moiety in position 5 is associated with remarkable pharmacological properties apart from the presence of an amino or methylthio 2-substitution.

Now, we planned new modifications of the same scaffold in order to examine the structure–activity relationships of these compounds and we therefore synthesized a series of 2,5-cycloaminobenzopyrano[4,3-*d*]pyrimidines **3a–i** (Fig. 1). In these new molecules, we have maintained in position 5 the pyrrolidino, piperidino or morpholino ring, present in the most interesting previous compounds **1** and **2**, whereas in position 2 we have inserted a more hindered basic moiety instead of the simple amino or methylthio groups. In this paper, we report the synthesis and the evaluation of the anti-inflammatory/analgesic/antipyretic activities and the anti-aggregatory effect of these novel molecules. The compounds were tested in vivo on rat paw edema, mice writhing test and rat lipopolysaccaridic (LPS) fever and in vitro on guinea-pig platelet-rich plasma aggregated by collagen, adenosine-5′-diphophate (ADP) and arachidonic acid (AA).

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Synthesis

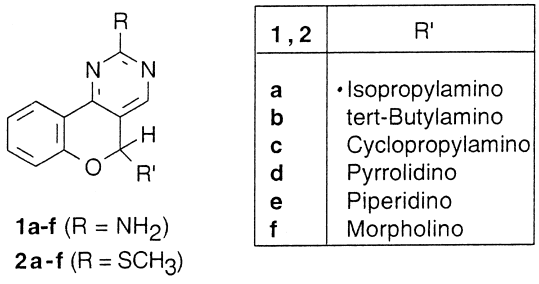
Compounds **3a–i** have been prepared following the previous synthetic method used for compounds **1** and **2a–f** [1,2] with some proper modifications.

3-Formylchromone **4**, which was obtained from 2-hydroxyacetophenone by a Vilsmeier reaction,[3] has been condensed with pyrrolidine-1-carboxamidine hydrochloride (**5a**) piperidine-1-carboxamidine sulfate (**5b**) or morpholine-4-carboxamidine hydrochloride (**5c**) (starting from *S*-methylisothiourea sulfate and the proper amines by well known reactions),[4,5] to give intermediates 2-cycloamino-5-hydroxy-5*H*-[1]benzopyrano[4,3-*d*]pyrimidines **6a–c**.[6,7] Then, the hemiacetalic hydroxy-group in position 5 of the benzopyrano[4,3-*d*]pyrimidine system has been replaced with the suitable amines in the presence of $TiCl_4$, according to a method already described[1] to give the desired **3a–i**[8] (Scheme 1).

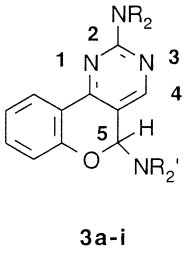
Results and Discussion

All the compounds under study failed to exhibit anti-inflammatory/analgesic/antipyretic activity when orally administered up to 100 mg/kg in rodents using the conventional experimental models described in details in a previous paper.[1] On the other hand, they showed an interesting in vitro antiplatelet effect. This activity was studied by measuring the aggregation of guinea-pig platelet-rich plasma (PRP) applying Born's turbidimetric method.[9] Platelet-rich plasma was incubated with the test compounds (10–500 μM) or with dimethylsulfoxide (0.5% maximal concentration, control) at 37 °C for 5 min and the aggregation was stimulated by adding ADP (3 μM), AA (50 μM) or collagen (5 μg/mL) at concentrations giving maximal aggregatory response, as previously described in detail.[1] The antiplatelet activity was expressed as percent inhibition with respect to control. Anti-aggregating potency of the test compounds was indicated by IC_{50} values which were calculated by linear regression analysis of the concentration–response curves obtained for each compound. Data are reported in Table 1.

Most compounds under study showed antiplatelet effect although with a different ability to prevent the aggregation induced by the three distinct aggregants. By comparing the IC_{50} values it emerges that only the 5-substituted pyrrolidino derivatives (**3a** and **3c**) possess inhibitory activity against all the inducers of aggregation, whilst the introduction of morpholino function in the same position confers to the molecules an ASA-like activity. Indeed, **3g–i** inhibited platelet aggregation

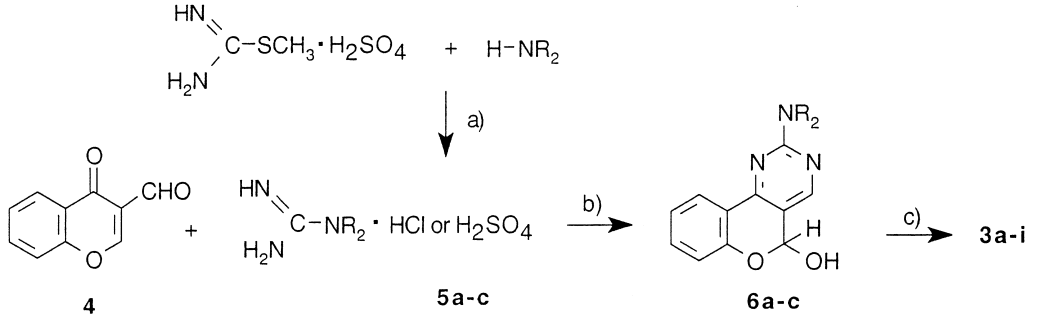


1,2	R'
a	Isopropylamino
b	tert-Butylamino
c	Cyclopropylamino
d	Pyrrolidino
e	Piperidino
f	Morpholino



3	NR_2	NR'_2
a	Pyrrol.	Pyrrol.
b	Piper.	Pyrrol.
c	Morph.	Pyrrol.
d	Pyrrol.	Piper.
e	Piper.	Piper.
f	Morph.	Piper.
g	Pyrrol.	Morph.
h	Piper.	Morph.
i	Morph.	Morph.

Figure 1. Compounds **1a–f**, **2a–f** and **3a–i**.



5,6	NR_2	Yields%
a	Pyrrolidinyl	100
b	Piperidinyl	62
c	Morpholinyl	100

Scheme 1. (a) **5a·HCl** and **5c·HCl**:H_2O, 100 °C for 30 min, then hot, satd $BaCl_2$ solution; **5b·H_2SO_4**: H_2O, 40 °C for 16 h, then reflux for 8 h; (b) **6a** and **6c**: EtONa, EtOH absolute, reflux for 3 h; **6b**: NaOH 1 M, H_2O, Et_3N, 70–80 °C for 3 h; (c) $TiCl_4$, toluene, anisole, HNR'_2, reflux for 6 h.

Table 1. In vitro antiplatelet activity of compounds **3a–i** and ASA in guinea-pig PRP

Compounds	Collagen-induced aggregation IC_{50} (μM)	ADP-induced aggregation IC_{50} (μM)	AA-induced aggregation IC_{50} (μM)
1d	[a]	750	400
2d	[a]	180	85
3a	170	200	80
3b	nc[b]	nc[b]	nc[b]
3c	80	250	430
3d	nc[b]	nc[b]	90
3e	nd[c]	nd[c]	nd[c]
3f	200	nc[b]	nc[b]
3g	50	nc[b]	75
3h	60	nc[b]	25
3i	60	nc[b]	95
ASA	48	nc[b]	61

[a]Data not available.
[b]Not calculable because maximal inhibition of aggregation is lower than 50%.
[c]Not determined because of its low solubility (inactive up 250 μM).

caused by collagen and AA at concentrations close to the IC_{50} value of ASA, which produced less than 50% inhibition of ADP aggregation.

The present results, compared to the previous findings obtained from the analogous series of benzopyrano[4,3-*d*]pyrimidine derivatives (**1a–f** and **2a–f**), strengthen the hypothesis that a free amino group in position 2 is essential to confer in vivo anti-inflammatory/analgesic/antipyretic activities. However, the replacement of the amino or methylthio groups with cyclic amines, produces a more potent in vitro antiplatelet activity. In particular, the 2-morpholino derivatives (**3c**, **3f** and **3i**) are effective against collagen-induced platelet aggregation, while the 2-pyrrolidino substituted compounds (**3a**, **3d** and **3g**) especially inhibit the AA-induced aggregation. Moreover, the piperidine moiety in position 2 generates only one compound (**3h**) endowed with antiplatelet activity.

A potent ASA-like antiplatelet activity emerges when a tertiary amino group in position 2 is coupled with a morpholine residue in position 5 (compounds **3g–i**). Finally, it is noteworthy that the presence of a pyrrolidino substituent in 5, apart from the group in 2, confers the capability of inhibiting the aggregation induced not only by AA or collagen, but also by ADP (compounds **1d**, **2d**, **3a** and **3c**).

Actually, compounds **3a**, **3c** and **3g–i**, endowed with a wide and powerful anti-aggregatory activity in vitro deserve additional in vivo investigations in order to define their pharmacological profile in comparison with ASA, whose useful antithrombotic effect[10] is frequently associated with gastrointestinal and hemorrhagic adverse side effects.[11]

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6. Preparation of 2-cycloamino-5-hydroxy-5*H*-[1]benzopyrano[4,3-*d*]pyrimidines **6a** and **6c**: To a solution of sodium ethoxide, obtained from Na (0.46 g, 20 mmol) and dry ethanol (40 mL), was added pyrrolidine-1-carboxamidine hydrochloride **5a** (2.99 g, 20 mmol) or morpholine-4-carboxamidine hydrochloride **5c** (3.31 g, 20 mmol). The mixture was stirred at room temperature for 20 min, then NaCl was removed by filtration in vacuo and, immediately, a solution of 3-formylchromone (3.48 g, 20 mmol) dissolved in dry ethanol (40 mL) was added. The red reaction mixture was refluxed for 3 h and concentrated by evaporation under reduced pressure at about 20 mL. The white solid, obtained after cooling at room temperature, was filtered and recrystallized from ethanol 95%. **6a**: Yield: 2.61 g, 97%; mp 194 °C; IR (KBr) cm^{-1} 3000–3170 (OH); ^1H NMR (DMSO-d_6) δ 1.70–2.20 (m, 4H, $2CH_2$ Pyrr), 3.30–3.80 (m, 4H, 2 CH_2N Pyrr), 6.45 (s, 1H, 5-H), 6.80–7.70 (m, 4H, 7,8,9-H, OH, 1H disappears with D_2O), 8.10–8.40 (m, 2H, 4-H + 10-H). Anal. calcd for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.62; N, 15.60; found: C, 66.64; H, 5.64; N, 15.62. **6c**: Yield: 2.76 g, 97%; mp 194–195 °C; IR (KBr) cm^{-1} 3300 (OH); ^1H NMR (DMSO-d_6) δ 3.85 (m, 9H, $4CH_2N$ Morph, OH, 1H disappears with D_2O), 6.55 (s, 1H, 5-H), 7.05–7.75 (m, 3H, 7,8,9-H), 8.15–8.40 (m, 1H, 10-H), 8.55 (s, 1H, 4-H). Anal. calcd for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73; found: C, 63.09; H, 5.33; N, 14.71.
7. Preparation of 5-hydroxy-2-piperidino-5*H*[1]benzopyrano-[4,3-*d*]pyrimidine **6b**: 3-Formylchrone (1.74 g, 10 mmol) was suspended in 1 M NaOH solution (10 mL), then, in close succession were added piperidine-1-carboxamidine sulfate (1.74 g, 10 mmol), water (40 mL) and Et_3N (1 mL). The mixture was refluxed for 3 h and after cooling the crude solid obtained was filtered and recrystallized from dry ethanol. **6b**: Yield: 2.69 g, 95%; mp 173–174 °C; IR ($CHCl_3$) cm^{-1} 3570 (OH); ^1H NMR (DMSO-d_6 + $CDCl_3$) δ 1.67 (m, 6H, 3 CH_2 Pip), 2.98 (s, 1H, OH, disappears with D_2O), 3.90 (m, 4H, 2 CH_2N Pip), 6.39 (d, J = 3 Hz, 1H, 5-H, became s after D_2O), 6.90–7.65 (m, 3H, 7,8,9-H), 8.15–8.45 (m, 1H, 10-H), 8.32 (s, 1H, 4-H). Anal. calcd for $C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N, 14.83; found: C, 67.59; H, 5.98; N, 15.00.
8. Physical properties, spectral and analytical data of compounds **3a–i**. **3a**: Yield: 2.45 g, 76%; mp 98–99 °C; ^1H NMR ($CDCl_3$) δ 1.74 (m, 4H, $2CH_2$ 5-Pyrr), 2.00 (m, 4H, 2 CH_2 2-Pyrr), 2.50–3.70 (m, 4H, 2 CH_2N 5-Pyrr), 3.70 (m, 4H, 2 CH_2N 2-Pyrr), 6.14 (s, 1H, 5-H), 6.80–7.60 (m, 3H, 7,8,9-H), 8.10–8.30 (m, 1H, 10-H), 8.30 (s, 1H, 4-H). Anal. calcd for $C_{19}H_{22}N_4O$: C, 70.78; H, 6.88; N, 17.38; found: C, 70.53; H, 6.87; N, 17.29. **3b**: Yield: 1.98 g, 59%; mp 110–111 °C; ^1H NMR ($CDCl_3$) δ 1.40–2.05 (m, 10H, 3 CH_2 Pip, 2 CH_2 Pyrr), 2.70–3.20 (m, 4H, 2 CH_2N Pyrr), 3.90 (m, 4H, 2 CH_2N Pip), 6.14 (s, 1H, 5-H), 6.80–7.50 (m, 3H, 7,8,9-H), 8.00–8.40 (m,

1H, 10-H), 8.30 (s, 1H, 4-H). Anal. calcd for $C_{20}H_{24}N_4O$: C, 71.40; H, 7.19; N, 16.65; found: C, 71.16; H, 7.16; N, 16.70. **3c**: Yield: 2,30 g, 68%; mp 87–88 °C; ^{1}H NMR ($CDCl_3$) δ 1.76 (m, 4H, 2CH_2 Pyrr), 2.55–3.20 (m, 4H, 2 CH_2N Pyrr), 3.89 (m, 8H, 4 CH_2 Morph), 6.16 (s, 1H, 5-H), 6.80–7.60 (m, 3H, 7,8,9-H), 8.00–8.40 (m, 1H, 10-H), 8.33 (s, 1H, 4-H). Anal. calcd for $C_{19}H_{22}N_4O_2$: C, 67.44; H, 6.55; N, 16,56; found: C, 67.44; H, 6.54; N, 16.52. **3d**: Yield: 2.39 g, 71%; mp 116–117 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (m, 6H, 3 CH_2 Pip), 1.97 (m, 4H, 2CH_2 Pyrr), 2.40–3.15 (m, 4H, 2 CH_2N Pip), 3.67 (m, 4H, 2 CH_2N Pyrr), 5.92 (s, 1H, 5-H), 6.80–7.60 (m, 3H, 7,8,9-H), 8.10–8.40 (m, 1H, 10-H), 8.27 (s, 1H, 4-H). Anal. calcd for $C_{20}H_{24}N_4O$: C, 71.40; H, 7.19; N, 16.65; found: C, 71.41; H, 7.20; N, 16.78. **3e**: Yield: 1.72 g, 49%; mp 113–114 °C; ^{1}H NMR ($CDCl_3$) δ 1.48 (m, 6H, 3 CH_2 5-Pip), 1.67 (m, 6H, 3 CH_2 2-Pip), 2.35–3.10 (m, 4H, 2 CH_2N 5-Pip), 3.90 (m, 4H, 2 CH_2N 2-Pip), 5.92 (s, 1H, 5-H), 6.65–7.50 (m, 3H, 7,8,9-H), 8.05–8.40 (m, 1H, 10-H), 8.26 (s, 1H, 4-H). Anal. calcd for $C_{21}H_{26}N_4O$: C, 71.97; H, 7.48; N, 15.99; found: C, 71.68; H, 7.34; N, 15.95. **3f**: Yield: 1.51 g, 43%; mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 1.49 (m, 6H, 3 CH_2 Pip), 2.40–3.15 (m, 4H, 2CH_2N Pip), 3.88 (m, 8H, 4 CH_2 Morph), 5.96 (s, 1H 5-H), 6.00–7.60 (m, 3H, 7,8,9-H), 8.15–8.40 (m, 1H, 10-H), 8.30 (s, 1H, 4-H). Anal. calcd for $C_{20}H_{24}N_4O_2$: C, 68.16; H, 6.86; N, 15.90; found: C, 67.85; H, 6.88; N, 15.96. **3g**: Yield: 2.23, 66%; mp 118–119 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (m, 4H, 2 CH_2 Pyrr), 2.40–3.00 (m, 4H, 2 CH_2N Morph), 3.40–3.90 (m, 8H, 2 CH_2O Morph, 2CH_2N Pyrr), 5,91 (s, 1H, 5-H), 6.80–7.60 (m, 3H, 7,8,9-H), 8.10–8.40 (m, 1H, 10-H), 8.30 (s, 1H, 4-H). Anal. calcd for $C_{19}H_{22}N_4O_2$: C, 67.44; H, 6.55; N, 16.56; found: C, 67.61; H, 6.76; N, 16.41. **3h**: Yield: 2.50 g, 71%; mp 108–109 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 (m, 6H, 3CH_2 Pip), 2.60–3.00 (m, 4H, 2 CH_2N Morph), 3.65 (m, 4H, 2 CH_2O Morph), 3.90 (m, 4H, 2 CH_2N Pip), 5.93 (s, 1H, 5-H), 6.80–7.60 (m, 3H, 7,8,9-H), 8.10–8.40 (m, 1H, 10-H), 8.30 (s, 1H, 4-H). Anal. calcd for $C_{20}H_{24}N_4O_2$: C, 68.16; H, 6.86; N, 15.90; found: C, 67.88; H, 6.94; N, 15.63. **3i**: Yield: 1.95 g, 55%; mp 148–149 °C; ^{1}H NMR ($CDCl_3$) δ 2.60–3.00 (m, 4H, 2CH_2N 5-Morph), 3.67 (m, 4H, 2 CH_2O 5-Morph), 3.88 (m, 8H, 4 CH_2 2-Morph), 5.93 (s, 1H, 5-H), 6.80–7.50 (m, 3H, 7,8,9-H), 8.10–8.40 (m, 1H, 10-H), 8.32 (s, 1H, 4H). Anal. calcd for $C_{19}H_{22}N_4O_3$: C, 64.39; H, 6.26; N, 15.80; found: C, 64.10; H, 6.33; N, 15.65.

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