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Bioorganic & Medicinal Chemistry
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Bioorganic & Medicinal Chemistry 12 (2004) 553–561

Synthesis and pharmacological evaluation of 5*H*-[1]benzopyrano[4,3-d]pyrimidines effective as antiplatelet/analgesic agents

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Received 7 July 2003; accepted 18 November 2003

Abstract—Synthesis and pharmacological screening of new 2-methylthio/2-methanesulfonyl/2-methoxy-5*H*-[1]benzopyrano[4,3-d]pyrimidines were planned in order to study the effects of the 5-substitution with alkoxy/phenoxy/alkylthio and phenylthio groups both on in vitro antiplatelet and in vivo antinociceptive activities. Antiplatelet activity was assessed in vitro against ADP, Arachidonic acid and U46619 induced aggregation, in rabbit plasma. Anti-inflammatory, analgesic and antipyretic activities were tested in rat paw edema, mouse writhing test and LPS induced rat fever, respectively. Amongst test compounds, 2-methylthio derivatives displayed an ASA-like antiplatelet activity whereas 2-methoxy and, particularly, 2-methanesulfonyl derivatives showed a broad spectrum of antiplatelet action, inhibiting both the ADP- and the AA- and U46619-induced aggregation. With regard to the in vivo pharmacological activities, mainly the 2-methoxy derivatives showed a significant analgesic effect comparable to that of indomethacin. SAR considerations, also in comparison with a number of previously described compounds, were performed.

1. Introduction

Thrombotic disorders such as myocardial infarction, stroke and cerebral ischemia are the main causes of death worldwide accounting for about 20% of fatalities.[1] Since the anomalous formation of intravascular occlusions is the common cause of these diseases, the prevention of thrombogenesis has become one of the most important targets in the prophylaxis and therapy of cardiocirculatory disorders with thromboembolic complications.[2] Nowadays different antiplatelet agents such as ticlopidine, clopidogrel, glycoprotein IIb/IIIa inhibitors and acetylsalicylic acid (ASA) are available for clinical use. Clinical studies have demonstrated their efficacy in the prevention of thromboembolic disease but they are not free from side effects such as gastric erosion (ASA) and agranulocytosis (ticlopidine). Oral platelet glycoprotein IIb/IIIa receptor inhibitors have failed to find their way into clinical implementation due to an unfavorable balance between therapeutic efficacy and haemorrhagic complications, drawbacks shared by many of the antiplatelet drugs currently used.[3] As concerns the platelet aggregation, ADP represents the primary endogenous platelet aggregant and it is maximally released from dense granules once platelets are activated. There are three different ADP receptors on platelet membrane, two of them coupled to G proteins, $P2Y_1$ and $P2Y_{12}$, and the third is a ligand-gated ion channel, $P2X_1$.[4–9] Due to these facts, aggregation induced by ADP is hard to be controlled and ASA has a very poor effect on it. A combination of drugs causing haemostasis with different mechanisms seems to improve clinical efficiency and safety. For instance, ASA plus Dipyridamole[10] or ASA plus glycoprotein IIb/IIIa-receptor inhibitors[11] are successfully used in stroke prevention, while ASA plus Warfarin,[12] and

Keywords: 2-Methylthio/2-methanesulfonyl/2-methoxy-5-alkoxy/phenoxy/alkylthio and phenylthio-5*H*-[1]benzopyrano[4,3-d]-pyrimidines; Antiplatelet activity; U46619-Antagonists; Analgesic activity.
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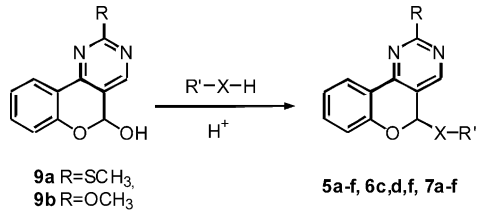
doi:10.1016/j.bmc.2003.11.018



Scheme 1.

ASA plus Amiodarone[13] are explored in patients with atrial fibrillation. Moreover, enhanced antiplatelet activity has been described for the combined administration of TXA_2 synthase inhibitor (Dazoxiben) plus TXA_2 receptor antagonist (Sulotroban) in experimental assays.[14] For these reasons, the new goal in the antiplatelet research is to combine, in the same molecule, different structures involved in alternative pathways of aggregation process.

In our previous works,[15–18] dealing with the development of novel antiplatelet agents, we documented the in vitro antiplatelet activity of several 5*H*-[1]benzopyrano[4,3-d]pyrimidin-5-amines (compounds **1**–**4**) (see Fig. 1)

Particularly, the introduction of the different substituents in position 2 generates molecules with an ASA like (series **1**[15] and **4**[18]) or with a broader spectrum antiplatelet activity (series **2**[16] and **3**[17]). An interesting analgesic activity emerged for compounds **1** and some of compounds **4**, in acetic acid writhing test. On the basis of these findings, we hypothesised that the substituent in position 2 plays a fundamental, but not exclusive, role in addressing the pharmacological activities and that the nature of 5-substitution provides a further contribution to the potency and efficacy.

Now, looking for further confirmation of our hypothesis and in the hope of obtaining compounds with a broader spectrum of antiplatelet activity, we planned the synthesis of new series of *5H*-[1]benzopyrano[4,3-d]pyrimidines **5a**–**g**, **6c**,**d**,**f**,**g**, **7a**–**g** and **8a**–**g**. In these molecules the amine substituents in position 5 were replaced by oxygen or sulfur functions such as alkoxy, phenoxy, alkylthio or phenylthio; in position 2 of compounds **5**, **6** and **7** we maintained the substituents which resulted more interesting in the previous series (see Fig. 2). In addition, in compounds **8** we considered the introduction, in position 2, of a methanesulphonyl group, as a possible improvement for antiplatelet activity because a number of molecules with sulfonamide or sulfone function, active as TXA_2 receptor antagonists,[19] dual TXA_2 synthase inhibitor/TXA_2-receptor antagonist[14] or $P2Y_{12}$ ADP receptor antagonists,[20] have been recently reported as antiplatelet agents.

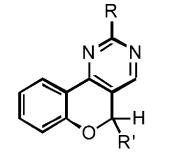
Hence, in this work we describe the synthesis of compounds **5**–**8** and the study of their antiplatelet activity towards AA-, ADP- and the stable TXA_2-receptor agonist U46619-induced aggregation in rabbit platelet rich plasma. In vivo antiphlogistic, antipyretic, and analgesic properties were also evaluated. Moreover, SAR considerations, also in comparison with a number of previously described compounds, were reported.

2. Chemistry

In a previous paper[16] we noticed, during the recrystallization, from absolute ethanol, of some 2-methylthio-5*H*-[1]benzopyrano[4,3 - d]pyrimidin - 5-amines hydrochlorides, the formation of a little amount of 2-methylthio-5-ethoxy-5*H*-[1]benzopyrano[4,3-d]pyrimidine **5b**.[16] Subsequent studies suggested this finding as a general behaviour for all the 5-amino derivatives, but only as hydrochlorides, because no transformation occurred for the free basic forms by refluxing with ethanol or sodium ethoxide in ethanol. Further inquiries made clear that it is possible to obtain the same compound **5b** starting from the 2-methylthio - 5 - hydroxy - *5H*-[1]benzopyrano[4,3-d]pyrimidine **9a** (Scheme 1) by refluxing in ethanol in the presence of a suitable strong acid as catalytic agent. Thus, considering the above reaction as a useful general method, we took advantage of this and prepared compounds **5a**–**f**, **6c,d,f** and **7a**–**f** starting from 5-OH derivatives **9a,b**[16,18] which were reacted with the proper alcohol or thioalcohol in the presence of a suitable acid agent (HCl, H_2SO_4 or p-toluenesulfonic acid) (see Scheme 1).

However, the reaction was not successful on compounds **9a**,**b** with phenol or thiophenol; thus, in order to obtain compounds **5g**, **6g** and **7g**, it was necessary to start from the 2-methylthio or 2-methoxy-5*H*-[1]benzopyrano[4,3-d]pyrimidin-5-piperidine hydrochlorides **10a**[16] or **10b**,[18] respectively (see Scheme 2).

Finally, compounds **8a**–**g** were easily prepared oxidizing the 2-methylthio function of compounds **5a**–**g** with *m*-chloroperbenzoic acid, as reported in Scheme 3.

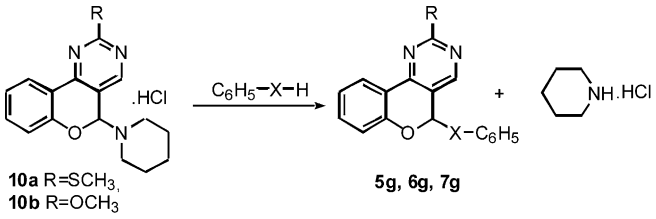


1a-c, e-g R = NH_2
2a-c, e-g R = SCH_3
4a-h R = OCH_3
3a-i

1, 2, 4	R'
a	isopropylamino
b	t-butylamino
c	cyclopropylamino
d	diethylamino
e	pyrrolidino
f	piperidino
g	morpholino
h	benzylamino

3	R	R'
a	pyrrolidino	pyrrolidino
b	piperidino	pyrrolidino
c	morpholino	pyrrolidino
d	pyrrolidino	piperidino
e	piperidino	piperidino
f	morpholino	piperidino
g	pyrrolidino	morpholino
h	piperidino	morpholino
i	morpholino	morpholino

Figure 1. Compounds **1**–**4**.



Scheme 2.

All compounds were characterized by IR and ^{1}H NMR spectroscopic methods (analytical data were reported in Tables 1–3). Microanalyses for C, H, N were performed in order to verify their purity, and data always resulted within ±0.4% of theoretical value (see Table 4).

3. Results

3.1. In vitro antiplatelet activity

Some of the compounds screened in this study display a complete antiplatelet activity towards ADP in addition to AA and the TXA_2-receptor agonist U46619. In particular, 2-methanesulfonyl derivatives show the highest antiplatelet activity towards aggregation induced by ADP, in terms of efficacy and potency. Derivatives **8a** and **8c** are the most potent while **8b**, **8e** and **8f** possess moderate activity, their IC_{50} being between 249 and 470 μM (Table 5). As concerns 2-methoxy-benzopyrano[4,3-d]pyrimidine derivatives, three compounds (**7b**,**f**,**g**) out of seven prove to be effective, although with lower potency than the previous molecules (IC_{50} between 604 and 841 μM).

Amongst 2-methylthio substituted compounds (series **5** and **6**) only **5a** and **5g** are able to completely prevent ADP-induced aggregation, while the remaining derivatives, like ASA, are quite inactive up to 1 mM concentration.

AA-induced aggregation is inhibited by all compounds, except for **5d**,**g**, **6d** and **7d**. In particular derivatives **5b**, **8c** and **e** are as potent as ASA.

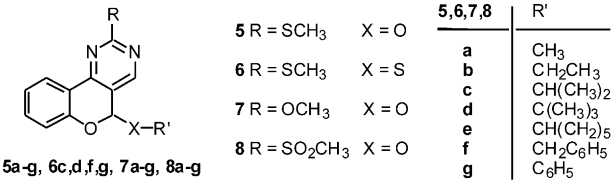
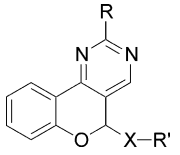
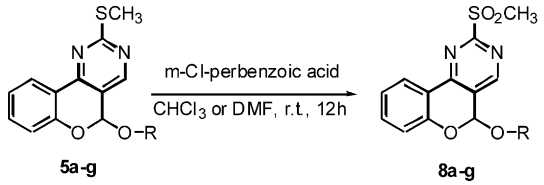


Figure 2. Compounds **5–8**.

Table 1. Analytical data of compounds **5–8**



Compd	R	X	R'	Yields (%)	Recryst. solvents	mp (°C)
5a	SCH_3	O	CH_3	68	An.methanol	112–113
5b	SCH_3	O	CH_2CH_3	69	Abs. ethanol	111–112
5c	SCH_3	O	$CH(CH_3)_2$	78	Isopropanol	150–151
5d	SCH_3	O	$C(CH_3)_3$	60	Isopropanol	152–153
5e	SCH_3	O	$CH(CH_2)_5$	40	Isopropanol	114–115
5f	SCH_3	O	$CH_2C_6H_5$	76	Abs. ethanol	137–138
5g	SCH_3	O	C_6H_5	76	Abs. ethanol	205–206
6c	SCH_3	S	$CH(CH_3)_2$	76	Isopropanol	154–155
6d	SCH_3	S	$C(CH_3)_3$	82	Isopropanol	155–156
6f	SCH_3	S	$CH_2C_6H_5$	92	Abs. Ethanol	132–133
6g	SCH_3	S	C_6H_5	30	Abs. ethanol	111–112
7a	OCH_3	O	CH_3	72	An.methanol	114–116
7b	OCH_3	O	CH_2CH_3	77	Abs. ethanol	98–99
7c	OCH_3	O	$CH(CH_3)_2$	87	Isopropanol	120–121
7d	OCH_3	O	$C(CH_3)_3$	50	Abs. ethanol	143–145
7e	OCH_3	O	$CH(CH_2)_5$	62	Isopropanol	122–123
7f	OCH_3	O	$CH_2C_6H_5$	40	Abs. ethanol	102–103
7g	OCH_3	O	C_6H_5	30	Abs. ethanol	219–210
8a	SO_2CH_3	O	CH_3	83	An.methanol	152–153
8b	SO_2CH_3	O	CH_2CH_3	69	Abs. ethanol	156–157
8c	SO_2CH_3	O	$CH(CH_3)_2$	74	Abs. ethanol	164–165
8d	SO_2CH_3	O	$C(CH_3)_3$	53	Ethanol/$CHCl_3$ (6:1)	207–208
8e	SO_2CH_3	O	$CH(CH_2)_5$	70	Isopropanol	181–182
8f	SO_2CH_3	O	$CH_2C_6H_5$	76	Ethanol/$CHCl_3$ (6:1)	155–156
8g	SO_2CH_3	O	C_6H_5	60	Abs. ethanol	120–121



Scheme 3.

This reference drug, as well as all the 2-methylthio derivatives **5** and **6** fail to prevent the aggregation induced by U46619, while the majority of both 2-methanesulfonyl and 2-methoxy derivatives demonstrate inhibitory activity against this aggregatory agent. In particular, compounds **8a**, **c** and **f** display the highest potency (Table 5).

3.2. In vivo experiments

Compounds **7a**,**b**,**c** and **e**, belonging to 2-methoxybenzopyrano[4,3-d]pyrimidine series, are able to significantly reduce rat paw edema induced by carrageenan subplantar injection, whereas all other compounds do not show antiphlogistic properties. Moreover, no compounds exhibited any significant effect in preventing rat LPS induced fever (Table 6).

Remarkable analgesic activity is displayed by many compounds under study in acetic acid writhing test. Molecules with 2-methoxy moiety show the highest analgesic potency and efficacy. In particular, compounds **7a**,**b**,**c**,**d** and **7e** reduce writhing episodes of 66–92% at 6–16-fold higher doses than indomethacin.

Table 2. ^{1}H NMR spectral data of compounds **5**–**7**

	^{1}H NMR ($CDCl_3$)
5a	2.65 (s, 3H, SCH_3), 3.55 (s, 3H, OCH_3), 6.02 (s, 1H, H_5), 6.96–7.58 (m, 3H, $H_7+H_8+H_9$), 8.17–8.57 (m, 2H, $H_{10}+H_4$).
5b	1.20 (t, $J=5.6$, 3H, CH_3), 2.64 (s, 3H, SCH_3), 3.62–4.10 (m, 2H, OCH_2), 6.18 (s, 1H, H_5), 6.98–7.60 (m, 3H, $H_7+H_8+H_9$), 8.27–8.60 (m, 2H, $H_{10}+H_4$).
5c	1.12 (d, $J=5.4$, 3H, CH_3), 1.23 (d, $J=5.4$, 3H, CH_3), 2.63 (s, 3H, SCH_3), 4.08–4.44 (m, 1H, OCH), 6.23 (s, 1H, H_5), 7.04–7.57 (m, 3H, $H_7+H_8+H_9$), 8.22–8.46 (m, 2H, $H_{10}+H_4$).
5d	1.34 (s, 9H, $3CH_3$), 2.63 (s, 3H, SCH_3), 6.42 (s, 1H, H_5), 6.93–7.57 (m, 3H, $H_7+H_8+H_9$), 8.22–8.53 (m, 2H, $H_{10}H_4$).
5e	1.00–2.58 (m, 10H, $5CH_2$ cyclohex), 2.62 (s, 3H, SCH_3), 3.60–4.10 (m, 1H, OCH), 6.26 (s, 1H, H_5), 6.97–7.54 (m, 3H, $H_7+H_8+H_9$), 8.22–8.52 (m, 2H, $H_{10}+H_4$).
5f	2.63 (s, 3H, SCH_3), 4.84 and 4.89 (q AB, $J=12$, 2H, OCH_2), 6.19 (s, 1H, H_5), 7.08 (d, $J=6$, 1H, H_7), 7.20 (t, $J=6$, 1H, H_8), 7.26–7.36 (m, 5H, Ar), 7.48 (t, $J=6$, 1H, H_9), 8.35 (d, $J=6$, 1H, H_{10}), 8.38 (s, 1H, H_4).
5g	2.64 (s, 3H, SCH_3), 6.51 (s, 1H, H_5), 6.79–7.59 (m, 8H, $H_7+H_8+H_9+5H$ Ar), 8.12–8.33 (m, 2H, $H_{10}+H_4$).[a]
6c	1.32 (d, $J=4.2$, 3H, CH_3), 1.46 (d, $J=4.2$, 3H, CH_3), 2.62 (s, 3H, SCH_3), 3.16–3.48 (m, 1H, SCH), 6.76 (s, 1H, H_5), 7.05–7.65 (m, 3H, $H_7+H_8+H_9$), 8.09–8.48 (m, 2H, $H_{10}+H_4$).
6d	1.49 (s, 9H, $3CH_3$), 2.61 (s, 3H, SCH_3), 6.90 (s, 1H, H_5), 7.02–7.60 (m, 3H, $H_7+H_8+H_9$), 8.22–8.43 (m, 2H, $H_{10}+H_4$).
6f	2.63 (s, 3H, SCH_3), 3.82–4.04 (m, 2H, SCH_2), 6.47 (s, 1H, H_5), 7.07–7.67 (m, 8H, $H_7+H_8+H_9+5$HAr), 8.15–8.47 (m, 2H, $H_{10}+H_4$).
6g	2.65 (s, 3H, SCH_3), 6.82 (s, 1H, H_5), 7.11–7.66 (m, 8H, $H_7+H_8+H_9+5$HAr), 8.21–8.49 (m, 2H, $H_{10}+H_4$).
7a	3.01 (s, 3H, 5–OCH_3), 4.08 (s, 3H, 2–OCH_3), 6.03 (s, 1H, H_5), 7.01–7.67 (m, 3H, $H_7+H_8+H_9$), 8.24–8.56 (m, 2H, $H_{10}+H_4$).
7b	1.21 (t, $J=7$, 3H, CH_3), 3.60–3.98 (m, 2H, OCH_2), 4.13 (s, 3H, OCH_3), 6.21 (s, 1H, H_5), 7.05–7.59 (m, 3H, $H_7+H_8+H_9$), 8.28–8.58 (m, 2H, $H_{10}+H_4$).
7c	1.17 (d, $J=4.2$, 3H, CH_3), 1.28 (d, $J=4.2$, 3H, CH_3), 4.09 (s, 3H, OCH_3), 4.18–4.46 (m, 1H, OCH), 6.27 (s, 1H, H_5), 7.11–7.57 (m, 3H, $H_7+H_8+H_9$), 8.28–8.52 (m, 2H, $H_{10}+H_4$).
7d	1.48 (s, 9H, $3CH_3$), 4.09 (s, 3H, OCH_3), 6.48 (s, 1H, H_5), 6.99–7.50 (m, 3H, $H_7+H_8+H_9$), 8.22–8.49 (m, 2H, $H_{10}+H_4$).
7e	1.05–2.20 (m, 10H, $5CH_2$ cyclohex), 3.71–3.94 (m, 1H, OCH), 4.08 (s, 3H, OCH_3), 6.29 (s, 1H, H_5), 6.96–7.55 (m, 3H, $H_7+H_8+H_9$), 8.22–8.49 (m, 2H, $H_{10}+H_4$).
7f	4.10 (s, 3H, OCH_3), 4.83–4.96 (m, 2H, OCH_2), 6.24 (s, 1H, H_5), 6.82–7.44 (m, 8H, $H_7+H_8+H_9+5$HAr), 8.22–8.49 (m, 2H, $H_{10}+H_4$).
7g	4.01 (s, 3H, OCH_3), 6.76 (s, 1H, H_5), 6.85–7.62 (m, 8H, $H_7+H_8+H_9+5$HAr), 8.02–8.39 (m, 2H, $H_{10}+H_4$).[a]

[a] Recorded in DMSO-d_6.

Table 3. IR and ^{1}H NMR spectral data of compounds **8**

Compd	IR cm^{-1} ($CHCl_3$)	^{1}H NMR δ ($CDCl_3$)
8a	1323, 1129 (SO_2)	3.42 (s, 3H, SO_2CH_3), 3.61 (s, 3H, OCH_3), 6.15 (s, 1H, H_5), 7.13–7.30 (m, 2H, H_7+H_8), 7.58 (t, $J=6$, 1H, H_9), 8.40 (d, $J=6$, 1H, H_{10}), 8.80 (s, 1H, H_4).
8b	1323, 1129 (SO_2)	1.22 (t, $J=7$, 3H, CH_3), 3.42 (s, 3H, SO_2CH_3), 3.79–4.12 (m, 2H, OCH_2), 6.27 (s, 1H, H_5), 7.16–7.29 (m, 2H, H_7+H_8), 7.57 (t, $J=6$, 1H, H_9), 8.37 (d, $J=6$, 1H, H_{10}), 8.81 (s, 1H, H_4).
8c	1323, 1129 (SO_2)	1.21 (d, $J=5.8$, 3H, CH_3), 1.34 (d, $J=5.8$, 3H, CH_3), 3.45 (s, 3H, SO_2CH_3), 4.13–4.53 (m, 1H, OCH), 6.39 (s, 1H, H_5), 7.03–7.69 (m, 3H, $H_7+H_8+H_9$), 8.48 (d, $J=6$, 1H, H_{10}), 8.80 (s, 1H, H_4).
8d	1323, 1129 (SO_2)	1.88 (s, 9H, $3CH_3$), 3.43 (s, 3H, SO_2CH_3), 6.58 (s, 1H, H_5), 6.98–7.71 (m, 3H, $H_7+H_8+H_9$), 8.44 (d, $J=6$, 1H, H_{10}), 8.74 (s, 1H, H_4).
8e	1323, 1129 (SO_2)	1.20–2.00 (m, 10H, $5CH_2$ cyclohex), 3.43 (s, 3H, SO_2CH_3), 3.94–4.10 (m, 1H, OCH), 6.39 (s, 1H, H_5), 7.10–7.27 (m, 2H, H_7+H_8), 7.56 (t, $J=6$, 1H, H_9), 8.41 (d, $J=6$, 1H, H_{10}), 8.75 (s, 1H, H_4).
8f	1323, 1130 (SO_2)	3.42 (s, 3H, SO_2CH_3), 4.88 and 4.94 (q AB, $J=12$, 2H, OCH_2), 6.31 (s, 1H, H_5), 7.10–7.27 (m, 2H, H_7+H_8), 7.32–7.39 (m, 5HAr), 7.58 (t, $J=6$, 1H, H_9), 8.40 (d, $J=6$, 1H, H_{10}), 8.71 (s, 1H, H_4).
8g	1305, 1122[a] (SO_2)	3.51 (s, 3H, SO_2CH_3), 6.71 (s, 1H, H_5), 6.81 (d, $J=6$, 1H, H_7), 7.08–7.30 (m, 6H, H_8+5HAr), 7.56 (t, $J=6$, 1H, H_9), 8.25 (d, $J=6$, 1H, H_{10}), 8.65 (s, 1H, H_4).[b]

[a] Recorded in KBr.
[b] Recorded in DMSO-d_6.

Amongst the remaining compounds only **5a**,**b**,**g**, **6f** and **8a** demonstrate analgesic action, inhibiting writhing events of about 70% (Table 7).

4. Conclusions

Comparing the results obtained for the new benzopyrano[4,3-d]pyrimidine series (**5–7**) with the pharmacological effects showed by the previous reported compounds (**2–4**), we can affirm that a remarkable difference of activity is obtained. In the ADP-induced aggregation the 2-methylthio-5-alkoxy/phenoxy derivatives **5,** as well as 2-methylthio-5-alkylthio/phenylthio derivatives **6**, are inactive, unlike the 2-methylthio-5-amino analogues **2** (the most active being **2e** with an $IC_{50} = 180$ μM). Thus the replacement of the basic substituent in position 5 seems to be detrimental to antiplatelet activity against ADP when in position 2 is present a methylthio function, but not when is present a methoxy group. In fact the 2-methoxy-5-alkoxy/phenoxy derivatives **7** are quite active in the ADP-induced aggregation, while the analogues 2-methoxy-5-amino **4** were inactive.[18] On the contrary, this chemical modification has no influence on AA-induced aggregation because most tested compounds are active, as well as the previous reported series.

Moreover, the oxidation of the 2-methylthio function gives a new series of compounds **8** active both in ADP-, AA- and U46619-induced aggregation. In particular, 5-methoxy, 5-ethoxy, 5-isopropoxy and 5-benzyloxy substituted show a remarkable antiplatelet activity of broad spectrum. Interestingly, the presence of *t*-butyl moiety hinders any interaction between molecules and platelets, within all the series here studied. On the contrary, the insertion of 5-substituents with lower steric hindrance (methyl, ethyl, isopropyl) positively influences antiaggregatory activity in all the series herein reported. In addition, we can observe that, in the previous series, only compounds **3** showed a large field of action, inhibiting both the ADP-, AA- and collagen-induced aggregation. However, it is noteworthy that compound **3g** (the most active in this series) is inactive in the U46619-induced aggregation (unpublished data). Thus, we can state that the new series **7** and **8** show a different antiplatelet action in comparison with all the other compounds synthesized.

On the basis of these results the most promising compounds (**7a,b,c,f,g** and **8a,b,c,f**) are currently under further pharmacological investigation in order to assess their potential antithrombotic activity in vivo. Moreover, more careful studies will be performed to identify their molecular targets.

Another contribution of this work is the observation that 2-methoxy - *5H* - [1]benzopyrano[4,3-*d*]pyrimidine scaffold plays a favourable role to produce also analgesic agents. Indeed many compounds, belonging to 2-methoxy series described here, possess remarkable antinociceptive activity coupled with a certain antiphlogistic action in models of peripheral pain and acute inflammation. These findings are consistent with previous observations reported for 2-methoxy *5H*-[1]benzopyrano[4,3-*d*]pyrimidino-5-amines **4**.[18] Hence, all of these data lead us to hypothesize that the insertion of alkyl groups with low steric hindrance in position 5 of this benzocondensed system is essential to confer analgesic activity, regardless of the nature of the bridging heteroatom (O or N).

Interestingly, to confer antinociceptive activity to 2-methylthio-benzopyranopyrimidines, alkyloxy or alkylthio groups must replace alkylamino substituents in position 5.[16]

Finally, we can conclude that the pharmacological profile of these benzofused derivatives always seems to

Table 4. Microanalysis of compounds **5–8**

Compd	Formula	Calculated/found%		
		C	H	N
5a	$C_{13}H_{12}N_2O_2S$	59.98	4.65	10.76
		59.99	4.5	10.79
5b	$C_{14}H_{14}N_2O_2S$	61.29	5.14	10.21
		61.12	5.05	10.42
5c	$C_{15}H_{16}N_2O_2S$	62.48	5.59	9.71
		62.16	5.62	9.76
5d	$C_{16}H_{18}N_2O_2S$	63.55	6	9.26
		63.29	6.01	9.27
5e	$C_{18}H_{20}N_2O_2S$	65.83	6.19	8.53
		66.06	6.29	8.63
5f	$C_{19}H_{16}N_2O_2S$	67.84	4.79	8.33
		67.5	4.89	8.22
5g	$C_{18}H_{14}N_2O_2S$	67.06	4.38	8.69
		67.06	4.22	8.65
6c	$C_{15}H_{16}N_2OS_2$	59.18	5.3	9.2
		58.91	5.1	9.1
6d	$C_{16}H_{18}N_2OS_2$	60.35	5.7	8.8
		60.41	5.7	8.91
6f	$C_{19}H_{16}N_2OS_2.\ 0.5H_2O$	64.09	4.64	7.87
		64.35	4.43	7.82
6g	$C_{18}H_{14}N_2OS_2$	63.88	4.17	8.28
		63.89	4.01	8.05
7a	$C_{13}H_{12}N_2O_3$	63.93	4.95	11.47
		63.69	5.1	11.51
7b	$C_{14}H_{14}N_2O_3$	65.11	5.46	10.85
		64.89	5.66	10.87
7c	$C_{15}H_{16}N_2O_3$	66.16	5.92	10.29
		66.24	5.8	10.31
7d	$C_{16}H_{18}N_2O_3$	67.12	6.34	9.78
		67.21	6.43	9.77
7e	$C_{18}H_{20}N_2O_3$	69.21	6.45	8.97
		68.95	6.34	8.88
7f	$C_{19}H_{16}N_2O_3$	71.24	5.03	8.74
		71.19	5.22	8.9
7g	$C_{18}H_{14}N_2O_3$	70.58	4.61	9.15
		70.2	4.8	9.11
8a	$C_{13}H_{12}N_2O_4S$	53.42	4.14	9.58
		53.65	4.35	9.36
8b	$C_{14}H_{14}N_2O_4S$	54.89	4.61	9.14
		54.66	4.41	9.09
8c	$C_{15}H_{16}N_2O_4S$	56.24	5.03	8.74
		56.29	5.06	8.75
8d	$C_{16}H_{18}N_2O_4S$	57.47	5.43	8.38
		57.33	5.15	8.52
8e	$C_{18}H_{20}N_2O_4S$	59.98	5.59	7.77
		59.81	5.55	7.75
8f	$C_{19}H_{16}N_2O_4S$	61.94	4.38	7.6
		61.65	4.17	7.51
8g	$C_{18}H_{14}N_2O_4S$	61.01	3.98	7.9
		61.28	4.07	7.92

Table 5. In vitro antiplatelet activity, expressed as efficacy (maximal inhibition%) and potency (IC_{50}) of ASA and compounds **5**–**8** on rabbit platelet rich plasma against ADP, Arachidonic acid (AA) or U46619 induced aggregation (mean of five experiments)

Compd	ADP 5 μM		AA 100 μM		U46619 2 μM	
	Maximal inhibition (%)	IC_{50} (μM)	Maximal inhibition (%)	IC_{50} (μM)	Maximal inhibition (%)	IC_{50} (μM)
5a	100	858	100	129	[a]	
5b	[a]		100	90	[a]	
5c	[a]		100	520	[a]	
5d	[a]		[a]		[a]	
5e	[a]		100	750	[a]	
5f	[a]		100	666	[a]	
5g	100	303	[a]		[a]	
6c	[a]		100	750	[a]	
6d	[a]		[a]		[a]	
6f	[a]		100	328	[a]	
6g	[a]		100	716	[a]	
7a	48	> 1000	100	396	100	790
7b	100	841	100	160	100	480
7c	47	> 1000	100	375	100	372
7d	46	> 1000	[a]		[a]	
7e	39	> 1000	100	768	[a]	
7f	100	722	100	300	100	827
7g	100	604	100	300	100	666
8a	100	174	100	256	100	113
8b	100	249	100	116	100	286
8c	100	194	100	72	100	73
8d	[a]		100	450	[a]	
8e	100	470	100	39	[a]	
8f	100	344	100	247	100	127
8g	[a]		100	721	[a]	
ASA	45	> 1000	100	55	[a]	

[a] Ineffective up to 1000μM.

Table 6. Anti-inflammatory and antipyretic activity of compounds **5**–**8** (100 mg/kg os) and indomethacin (10 mg/kg os) on carrageenan rat paw edema and LPS induced rat fever. Data are expressed as% of inhibition at the peak effect

Compd	Edema	Fever
5a	0	22
5b	3	0
5c	0	23
5d	0	37
5e	12	0
5f	0	26
6f	13	0
6g	15	0
7a	32**	0
7b	28**	0
7c	28*	22
7d	5	0
7e	23*	18
7f	5	0
5g, **6c**, **6d**, **7g**, **8a**–**g**	0	0
Indomethacin	45**	93**

Student's *t*-test with $*P<0.05$ and $**P<0.01$ versus vehicle treated rats (mean of eight experiments).

Table 7. Analgesic activity of compounds **5**–**8** on acetic acid induced writhing test in mice expressed in terms of efficacy (% of writhing inhibition) and potency (ID_{50})

Compd	Maximal inhibition (%)	ID_{50} (mg/kg os)
5a	72*	73
5b	63*	89
5f	46*	nc
5g	66*	88
6c	47*	nc
6d	30	nc
6g	78**	81
7a	77**	35
7b	92**	31
7c	66*	58
7d	71*	79
7e	80**	38
8a	70**	42
8d	39	nc
8e	47*	nc
5c, **5d**, **5e**, **6f**, **7f**, **7g**, **8b**, **8c**, **8f**, **8g**	0	
Indomethacin	81**	5

Student's *t*-test with $*P<0.05$ and $**P<0.01$ versus vehicle treated rats (mean of eight experiments).

derive from the concomitant contribution of both functions inserted in position 2 and 5 of the tricyclic system.

5. Experimental

5.1. Materials and chemical methods

All chemicals were obtained from Sigma-Aldrich s.r.l. (Milan, Italy). Melting points are uncorrected and were measured with a Büchi 540 instrument. IR spectra were recorded with a Perkin–Elmer 398 spectrophotometer. ^1H NMR were recorded on a Varian Gemini 200 (200MHz) instrument; chemical shifts are reported as δ (ppm) relative to tetramethylsilane (TMS) as internal stardard; signals were characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad signal); J in Hz. Reactions were followed by TLC on Kieselgel $60F_{254}$ (DC-Alufolien, E. Merck, Darmstadt, Germany). Analyses for C, H, N

(±0.4% of the theoretical value), were determined with an Elemental Analyzer EA 1110 (Fison-Instruments, Milan, Italy).

5.2. Preparation of 5-alkoxy-and 5-phenoxy-2-methylthio-5*H*-[1]benzopyrano[4,3-d]pyrimidines (5a–g)

5.2.1. Compounds 5a,b. To a suspension of compound **9a** (10 mmol, 2.46 g) in methanol or ethanol (25 mL) was added ethyl ether saturated with hydrochloric acid (2.5 mL) and the mixture was refluxed for 6 h. The solvents were evaporated under reduced pressure and the crude solids were solved in $CHCl_3$ (20 mL). The organic solution was washed once with a 1M solution of Na_2CO_3 (25 mL), once with water (25 mL), then was dried ($MgSO_4$) and evaporated under reduced pressure. The white solids obtained were recrystallized by the suitable solvent (Table 1).

5.2.2. Compounds 5c,d. To a suspension of compound **9a** (10 mmol, 2.46 g) in isopropyl or *tert*-buthyl alcohol (25 mL) was added concentrated sulfuric acid (10 drops) and the mixture was refluxed for 6 h. The treatment was the same as the previous preparation.

5.2.3. Compound 5e. To a suspension of compound **9a** (10 mmol, 2.46 g) in cyclohexyl alcohol (20 mL) was added p-toluenesulfonic acid (400 mg) and the mixture was stirred at 140 °C for 6 h. After cooling the reaction mixture was poured into water (200 mL) and extracted twice with $CHCl_3$ (25 mL). The organic phase was washed once with a 1M solution of Na_2CO_3 (25 mL), once with water (25 mL), then dried ($MgSO_4$) and evaporated under reduced pressure. The excess cyclohexanol was distilled in high vacuo and the white solid obtained was recrystallized by isopropanol.

5.2.4. Compound 5f. To a suspension of compound **9a** (10 mmol, 2.46 g) in anhydrous Dimethylformamide (DMF) (25 mL) were added benzyl alcohol (1 mL) and ethyl ether saturated with hydrochloric acid (2.5 mL) and the mixture was heated at 40–50 °C for 6 h. Then, the reaction mixture was worked up as compound **5e**.

5.2.5. Compound 5g. 2-Methylthio-5*H*-[1]benzopyrano[4.3-d]pyrimidin-5-piperidine hydrochloride **10a** (5 mmol, 1.75 g) was dissolved in freshly distilled phenol (20 mL) and heated at 70 °C for 6 h. The reaction mixture was poured into a 1M solution of NaOH (100 mL) and extracted three times with $CHCl_3$ (20 mL). The organic phase was washed once with a 1M solution of HCl (25 mL), once with water (25 mL) and dried ($MgSO_4$). The crude oil, obtained after solvent evaporation, was crystallized by ethyl ether and recrystallized by absolute ethanol.

5.3. Preparation of 5-alkylthio-2-methylthio-5*H*-[1]benzopyrano[4,3-d]pyrimidines (6c,d,f)

To a suspension of compound **9a** (10 mmol, 2.46 g) in anhydrous DMF (25 mL) were added the suitable thioalcohol (2 mL) and concentrated sulfuric acid (10 drops); the reaction mixture was heated at 70–80 °C for 12 h, under pressure and, after cooling, was poured into water (100 mL) and extracted twice with $CHCl_3$ (25 mL). The organic phase was washed once with a 1M solution of Na_2CO_3 (25 mL), once with water (25 mL), then dried ($MgSO_4$) and evaporated under reduced pressure giving white solids which were recrystallized by a suitable solvent (Table 1).

5.4. Preparation of 5-phenylthio-2-methylthio-5*H*-[1]benzopyrano[4,3-d]pyrimidines (6g)

To a solution of 2-methylthio-5*H*-[1]benzopyrano[4,3-d]pyrimidin-5-piperidine hydrochloride **10a** (5 mmol, 1.75 g) in anhydrous DMF (30 mL) was added thiophenol (5 mmol, 0.55 g) and the mixture was heated at 80 °C for 12 h. After cooling, the reaction mixture was poured into ice-water (200 mL) and stirred for 2 h. The white solid precipitated was filtered and recrystallized by absolute ethanol.

5.5. Preparation of 5-alkoxy- and 5-phenoxy-2-methoxy-5*H*-[1]benzopyrano[4,3-d]pyrimidines (7a–g)

5.5.1. Compounds 7a–d. A solution of compound **9b** (10 mmol, 2.30 g) and *p*-toluenesulfonic acid (400 mg) in the suitable alcohol (25 mL) was refluxed for 1–4 h, controlling by TLC the reaction progress. Then, the reaction mixture was worked up as compound **5a–d**. The yellow oils obatined were crystallized by ethyl ether and recrystallized by a suitable solvent (Table 1).

5.5.2. Compound 7e. A mixture of compound **9b** (10 mmol, 2.30 g), *p*-toluenesulfonic acid (400 mg) and cyclohexanol (25 mL) was heated at 110 °C for 2 h. Then, the reaction mixture was worked up as compound **5e**. The yellow oil obtained was crystallized by ethyl ether and recrystallized by absolute ethanol.

5.5.3. Compound 7f. A solution of compound **9b** (10 mmol, 2.30 g), benzyl alcohol (2 mL) and p-toluenesulfonic acid (400 mg) in anhydrous DMF (20 mL) was heated at 80 °C for 4 h and, after cooling, was poured into a 1M solution of Na_2CO_3 (100 mL). The suspension was extracted three times with $CHCl_3$ (25 mL); the organic phases were washed once with water (25 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The yellow oil obtained was crystallized by ethyl ether and recrystallized by absolute ethanol.

5.5.4. Compound 7g. A solution of 2-methoxy-5*H*-[1]benzopyrano[4,3-d]yirimidin-5-piperidine hydrochloride **10b** (10 mmol, 3.33 g) in freshly distilled phenol (20 mL) was heated at 70 °C for 10 h. After cooling, the red solution was poured into a 1M solution of NaOH (100 mL) and the red oil obtained was extracted three times with $CHCl_3$ (20 mL). The organic phases were washed once with a 1M solution of HCl (25 mL), once with water (25 mL), then dried ($MgSO_4$) and evaporated under reduced pressure. The yellow oil obtained was crystallized by ethyl ether and recrystallized by absolute ethanol.

5.6. Preparation of 5-alkoxy- and 5-phenoxy-2-methanesulphonyl-5*H*-[1]benzopyrano[4,3-*d*]pyrimidines (8a–g)

5.6.1. Compounds 8a–f. To a cooled solution of the appropriate 5-alkoxy - 2 - methylthio - 5*H* - [1]benzopyrano[4,3-d]pyrimidine **5** (5 mmol) in $CHCl_3$ (20 mL) was added, in small portions, m-chloroperbenzoic acid (70–75%) (10 mmol, 2.5g) and the mixture was stirred at room temperature for 12 h. Then, it was diluted with $CHCl_3$ (25 mL) and washed once with a saturated solution of $Na_2S_2O_5$ (25 mL), once with a saturated solution of $NaHCO_3$ (25 mL), once with water (25 mL) and, finally, dried ($MgSO_4$) and evaporated under reduced pressure. The white solids obtained were recrystallized by a suitable solvent (Table 1).

5.6.2. Compound 8g. To a cooled solution of 5-phenoxy-2-methylthio-5*H*-[1]benzopyrano[4,3-*d*]pyrimidine **5g** (5 mmol, 1.61 g) in anhydrous DMF (20 mL) was added, in small portions, *m*-chloroperbenzoic acid (70–75%) (10 mmol, 2.5 g) and the mixture was stirred at room temperature for 12 h. Then, it was poured into a saturated solution of $NaHCO_3$ (100 mL) to give a pale yellow solid which was filtered and recrystallized by absolute ethanol.

6. Materials and pharmacological methods

6.1. Animals

Swiss mice, Wistar rats and rabbits were used for the experiments. The animals were starved at 20 °C and fasted 24 h with free access to water. All the experiments were performed according to ethical standard guidelines and were approved by Italian Ministry of Health.

6.2. In vitro antiplatelet activity

Rabbit blood, anticoagulated with sodium citrate 3.8% 1 part citrate:9 part blood, was obtained by cardiac puncture after CO_2 euthanasia and collected in plastic tubes.

After centrifugation for 15 mins at 180*g* to obtain platelet rich plasma (PRP), the remaining blood was spinned 10 min at 2000*g* to obtain platelet poor plasma (PPP). Platelet aggregation was performed in the Aggrecorder PA 3220 (Menarini, Firenze) at 37 °C and continuous stirring (1000 rpm) following Born's turbidimetric method.[21] Aggregation was recorded as the percent change in light transmission: the baseline was set using PRP and maximal transmission using PPP. PRP was preincubated at 37 °C for 5 min with solvent (dimethyl sulfoxide, at the maximal final concentration 0.5%), the compounds under study or the reference drug (ASA) before addition of platelet aggregatory agents. Maximal aggregation was induced stimulating platelets with 5 μM ADP, 100 μM AA or 2 μM U46619. Tests were performed within 3 h to avoid platelet inactivation. The effects of test compounds and ASA were assessed as percent inhibition compared with control sample. DMSO 0.5% did not interfere with platelet aggregation.

6.3. In vivo experiments

Test compounds were suspended in 0.5% methoxycellulose and orally administered at 100 mg/kg to animals simultaneously with the phlogogen agent or 1 h before the application of algogen or pyretogen agents. Indomethacin 10 mg/kg os was used as reference drug in all the tests while control animals received the vehicle alone. Antiphlogistic, analgesic and antipyretic activities were evaluated as previously described.[15] Briefly, antiphlogistic activity was studied in rats inducing paw edema by mean of carrageenan subplantar injection. Analgesic activity was evaluated in mice using acetic acid writhing test and antipyretic activity was determined in rats with *E. coli* LPS induced fever. Pharmacological activities were expressed as percentage of inhibition calculated from the difference in the response between treated and control group at the time of maximum noxious effect. Dose–response curves were constructed to evaluate antinociceptive effect. Analgesic potency of compounds was expressed as ID_{50}, the dose that produced 50% of antinociception (50% reduction of control whrites).

6.4. Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using two-tailed Student's *t*-test for paired or unpaired data. $p < 0.05$ was considered significant, $P < 0.01$ was considered very significant.

Acknowledgements

The authors wish to thank Dr. R. Raggio, Dr. C. Rossi, Mr. F. Tuberoni and Mr. O. Gagliardo for spectral recording, Mr G. Domenichini for skillful help in the experimental work. Financial support from MIUR (Cofinanziamento Nazionale) is gratefully acknowledged.

References and notes

1. Murray, J. L. C.; Lopez, D. A. *The Lancet* **1997**, *349*, 1269.
2. Fitzgerald, D. J. *Neurology* **2001**, *57*, S1.
3. Van De Graaff, E.; Steinhubl, S. R. *Curr. Cardiol. Rep.* **2001**, *3*, 371.
4. Daniel, J. L.; Dangelmaier, C.; Jin, J.; Ashby, B.; Smith, J. B.; Kunapuli, S. P. *J. Biol. Chem.* **1998**, *273*, 2024.
5. Guile, S. D.; Ince, F.; Ingall, A. H.; Kindon, N. D.; Meghani, P.; Mortimore, M. P. *Progr. Med. Chem.* **2001**, *38*, 115.
6. Eckly, A.; Gendrault, J. L.; Hechler, B.; Cazenave, J. P.; Gachet, C. *Thromb. Haemost.* **2001**, *85*, 694.
7. Turner, N. A.; Moake, J. L.; McIntire, L. V. *Blood* **2001**, *98*, 3340.
8. Jin, J.; Quinton, T. M.; Zhang, J.; Rittenhouse, S. E.; Kunapuli, S. P. *Blood* **2002**, *99*, 193.
9. Remijn, J. A.; Wu, J. P.; Jeninga, E. H.; Ijsseldijk, M. J.; van Willingen, G.; de Groot, P. G.; Sixma, J. J.; Nurden, A. T. *Art. Thromb. Vasc. Biol.* **2002**, *22*, 686.
10. Paciaroni, M.; Gallai, V. *Cerebrovasc. Dis.* **2000**, *10*, 36.
11. Weksler, B. B. *Cerebrovasc. Dis.* **2000**, *10*, 41.

12. Knottenbelt, C.; Brennan, P. J.; Meade, T. W. *Arch. Int. Med.* **2002**, *162*, 881.
13. Naganuma, M.; Shiga, T.; Nishikata, K.; Tsuchiya, T.; Kasanuki, H.; Fujii, E. *J. Card. Pharm. Ther.* **2001**, *6*, 363.
14. Dickinson, R. P.; Dack, K. N.; Long, C. G.; Steele, J. *J. Med. Chem.* **1997**, *40*, 3442.
15. Bruno, O.; Schenone, S.; Ranise, A.; Barocelli, E.; Chiavarini, M.; Ballabeni, V.; Bertoni, S. *Arzn. Forsch. Drug Res.* **2000**, *50*, 1 140.
16. Bruno, O.; Schenone, S.; Ranise, A.; Bondavalli, F.; Barocelli, E.; Ballabeni, V.; Chiavarini, M.; Bertoni, S.; Tognolini, M.; Impicciatore, M. *Bioorg. Med. Chem.* **2001**, *9*, 629.
17. Bruno, O.; Brullo, C.; Ranise, A.; Schenone, S.; Bondavalli, F.; Barocelli, E.; Ballabeni, V.; Chiavarini, M.; Tognolini, M.; Impicciatore, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*/*11*, 1397.
18. Bruno, O.; Brullo, C.; Schenone, S.; Ranise, A.; Bondavalli, F.; Barocelli, E.; Tognolini, M.; Magnanini, F.; Ballabeni, V. *Il Farmaco* **2002**, *57*, 753.
19. Campillo, N.; Garcia, C.; Goya, P.; Paez, J. A.; Carrascoo, E.; Grau, M. *J. Med. Chem.* **1999**, *42*, 1698.
20. Scarborough, R. M.; Laibelman, A. M.; Clizbe, L. A.; Fretto, L. J.; Conley, P. B.; Reinolds, E. E.; Sedlock, D. M.; Jantzen, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1805.
21. Born, G. *Nature* **1962**, *194*, 927.