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**Short communication**

# New pyridazinone derivatives as inhibitors of platelet aggregation

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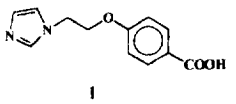
**Summary** — The synthesis and evaluation of the biological activity of a series of 3(2*H*)-pyridazinone derivatives is reported. We assessed the *in vitro* activity of these compounds on aggregation and production of thromboxane $A_2$ and prostaglandin $E_2$ of human platelets. In compounds **11** and **14** the 3-phenylpropyl group is *N*-linked to the 2 position of the pyridazinone ring of 6-(1*H*-imidazole-1-yl)-3(2*H*)-pyridazinone **3** or 6-[4-(1*H*-imidazole-1-yl)-phenyl]-3(2*H*)-pyridazinone **4**, respectively. These compounds inhibited platelet aggregation induced by arachidonic acid, ADP and collagen, and simultaneously suppressed the synthesis of $TxA_2$ and increased the production of $PGE_2$. These results characterize compounds **11** and **14** as thromboxane synthase inhibitors. However, the inhibition of platelet aggregation induced by U46619 and of the first wave of ADP-induced aggregation, which is not normally observed with thromboxane synthase inhibitors, suggests additional mechanisms of action for our compounds. On the basis of structural similarities with compounds described previously, these are possibly related to a phosphodiesterase inhibitory activity.

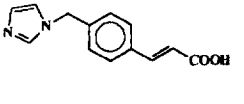
**antiplatelet agent / human blood platelets / 3(2*H*)-pyridazinone derivative / thromboxane synthase inhibition**

## Introduction

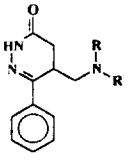
Thromboxane $A_2$ ($TxA_2$) is a powerful platelet aggregating and vasoconstricting agent [1]. Therefore, drugs suppressing the synthesis and/or the activity of $TxA_2$ have been developed as inhibitors of platelet aggregation and potential antithrombotic agents [2]. Compounds with an imidazole ring are platelet aggregation inhibitors [3, 4], *eg*, Dazoxiben **1** and Ozagrel **1a** which inhibit thromboxane synthase (the enzyme synthesizing $TxA_2$ in human platelets), and compounds with 4,5-dihydropyridazinone ring **2** show a similar action [5]. We were therefore interested in the synthesis of compounds carrying both an imidazole ring and a pyridazinone ring with the aim of obtaining new platelet aggregation inhibitors.




**1**



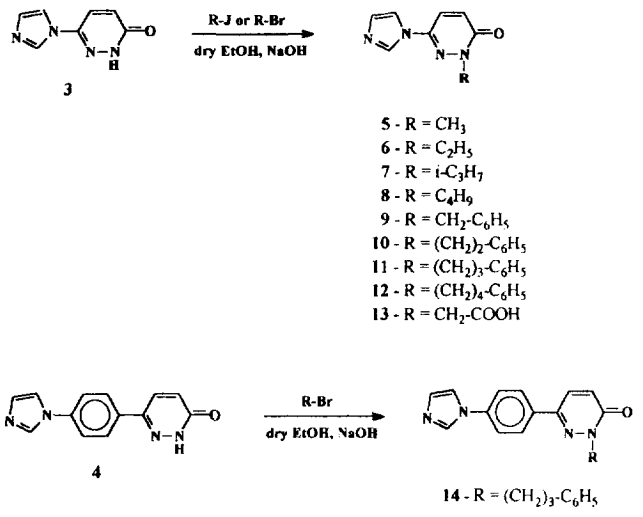

**1a**




## Chemistry

The compounds reported in this work were prepared as reported in scheme 1. The *N*-alkylation of 6-(1*H*-imidazole-1-yl)-3(2*H*)-pyridazinone **3**, prepared using the method of Steiner *et al* [6] and Sircar *et al* [7], with the alkyl halide in dry ethanol and sodium hydroxide pellets in equimolar ratio gave compounds **5–13**. According to the same procedure, compound **14** was prepared by *N*-alkylation of 6-[4-(1*H*-imidazole-1-yl)phenyl]-3(2*H*)-pyridazinone **4** [8, 9] with 1-chloro-3-phenylpropane.

*Abbreviations*: AA, arachidonic acid; ADP, adenosine diphosphate; cAMP, cyclic adenosine monophosphate; $IC_{50}$, concentration of the tested drug giving 50% inhibition of the control response; $PGD_2$, prostaglandin $D_2$; $PGE_2$, prostaglandin $E_2$; PPP, platelet-poor plasma; PRP, platelet-rich plasma; $TxA_2$, thromboxane $A_2$; $TxB_2$, thromboxane $B_2$; U46619, 9,11-dideoxy-11α,9α-epoxymethano prostaglandin $F_{2\alpha}$.




**Scheme 1.**

## Results

The inhibitory activities of compounds **5–14** on platelet aggregation induced by arachidonic acid (AA), adenosine diphosphate (ADP), collagen and the stable endoperoxide analogue 9,11-dideoxy-11α,9α-epoxymethano prostaglandin $F_{2\alpha}$ (U46619) are reported in table I.

For compounds **11** and **14**, which were the most active at inhibiting platelet aggregation, the concentrations inhibiting the control response by 50% ($IC_{50}$) were calculated, as described in the *Experimental protocols*. The $IC_{50}$ values are reported in table II, together with those of some reference drugs acting at different levels of arachidonate metabolism. As reference compounds we used aspirin, a cyclooxygenase inhibitor [10] widely used as antithrombotic agent, a thromboxane synthase inhibitor (OKY1581) [11], a thromboxane receptor antagonist (BM13.177) [12] and a dual thromboxane synthase inhibitor/receptor antagonist (Ridogrel) [13].

Compounds **11** and **14** displayed an inhibitory activity against platelet aggregation induced by all the agonists tested (AA, ADP, collagen and U46619) and also suppressed the first wave of ADP-induced platelet aggregation, which is independent of AA-metabolism (table II). The reference compounds OKY1581, BM13.177, aspirin and Ridogrel displayed a different pattern of inhibitory activity (table II). Indeed, while all of them suppressed, with different potencies, the aggregation induced by AA, collagen and the second wave of ADP-induced aggregation, only the $TxA_2$-receptor antagonist BM13.177 blocked U46619-induced aggregation. None of the reference compounds affected the first wave of aggregation induced by ADP (table II).

In terms of potency, compounds **11** and **14** displayed an inhibitory activity that compared well with that of aspirin on AA-induced aggregation while they were stronger than the latter on ADP- and collagen-induced aggregation. In addition, they inhibited U46619-induced aggregation, a property they share only with the thromboxane receptor antagonist BM13.177.

The effects of compounds **11** and **14** were also tested on the production of two eicosanoids, thromboxane $B_2$ ($TxB_2$), the stable metabolite of $TxA_2$, and prostaglandin $E_2$ ($PGE_2$), in serum. Both compounds reduced the synthesis of $TxB_2$ dose-dependently (compound **11**: $IC_{50}$ = 67.5 μM; compound **14**: $IC_{50}$ = 60 μM) but with a lower potency than aspirin, the

**Table I.** Effect of 2-substituted-3(2*H*)-pyridazinone on platelet aggregation.

| *Compound* | *Dose (μM)* | *% Inhibition of platelet aggregation* | | | |
|---|---|---|---|---|---|
| | | *AA (1 mM)* | *ADP (1.2 μM)* | *Collagen (1.5 μg/ml)* | *U46619 (0.6 μM)* |
| **11** | 100 | 100 ± 0.0[a] | 70.3 ± 17.7 | 97.0 ± 7.3 | 97.0 ± 6.7 |
| | 50 | 64.3 ± 33.0 | 47.9 ± 24.6 | 82.1 ± 18.6 | 42.0 ± 27.6 |
| | 20 | 11.1 ± 18.7 | 22.8 ± 11.8 | 23.3 ± 13.2 | 2.5 ± 18.2 |
| | 10 | –6.0 ± 8.7 | 20.1 ± 8.8 | 20.9 ± 22.6 | 17.5 ± 24.8 |
| **14** | 100 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| | 50 | 78.9 ± 38.0 | 92.3 ± 15.6 | 100 ± 0.0 | 97.4 ± 5.8 |
| | 25 | 6.5 ± 11.7 | 29.8 ± 26.8 | 82.6 ± 21.8 | 62.6 ± 46.2 |
| | 12.5 | 2.0 ± 13.0 | 3.3 ± 21.3 | 8.9 ± 20.9 | 5.9 ± 12.5 |

[a]Values represent the means ± SD of two to eleven separate experiments. Compounds **5–10**, **12** and **13** were substantially ineffective on platelet aggregation at 100 μM.

**Table II.** $IC_{50}$ values (μM) for compounds **11** and **14** and for some reference drugs on platelet aggregation induced by different agonists.

| *Compound* | *AA* | *ADP* | | *Collagen* | *U46619* |
|---|---|---|---|---|---|
| | | *2nd wave* | *1st wave* | | |
| **11** | 51.5 | 57.1[a] | 71.3[b] | 36.3 | 59.6 |
| **14** | 39.0 | 17.6[a] | 36.3[b] | 26.5 | 28 |
| Aspirin | 35.8 | 79.0[a] | > 1000[b] | 41 | > 1000 |
| BM 13.177 | 7[c] | 130[a] | > 300[b] | 4.7[c] | 4[c] |
| OKY 1581 | < 0.01[d] | 180[a,d] | > 1000[b,d] | nd | > 3000[d] |
| Ridogrel | 0.03[e] | 3.1 > 50 | 6.1 | 18.6 | |

The reference drugs used were: OKY 1581 (thromboxane synthase inhibitor); BM 13.177 (thromboxane receptor antagonist); aspirin (cyclooxygenase inhibitor); and ridogrel (dual thromboxane synthase inhibitor/receptor antagonist). [a]$IC_{50}$ values were calculated on the aggregation at 3 min after the addition of the inducer; [b]$IC_{50}$ values were calculated on the maximal amplitude of aggregation; [c]from ref [12]; [d]from ref [13]; [e]these results refer to one responder to thromboxane synthase inhibition [10]; in three other patients tested, and found to be non-responders, the $IC_{50}$ values were as follow: AA: > 3000; ADP: 270[a] and > 1000[b]; collagen: > 3000; U46619: > 3000; nd: not determined.

thromboxane synthase inhibitors OKY1581 ($IC_{50}$ = 0.29 μM) [11] and Dazoxiben ($IC_{50}$ = 0.9 μM) [14] and the dual thromboxane synthase inhibitor/receptor antagonist Ridogrel ($IC_{50}$ = 0.1 μM) [13].

Compounds **11** and **14** also increased $PGE_2$ synthesis in serum dose-dependently, with maximal rises in the tested concentration range (12.5–300 μM) of 3.7- and 13.4-fold, respectively. These compounds were more active at increasing $PGE_2$ synthesis than at inhibiting thromboxane synthesis. Similar findings were previously observed with Picotamide, a drug that possesses both thromboxane synthase inhibitory and thromboxane receptor antagonistic properties [14], and with other pure thromboxane synthase inhibitors [15].

Aspirin, on the other hand, an inhibitor of cyclooxygenase, suppresses both $TxB_2$ and $PGE_2$ synthesis (table III).

As expected, the pure thromboxane receptor antagonist BM13.177 did not show any inhibitory action on either $TxA_2$ or $PGE_2$ synthesis (table III).

## Discussion

The newly synthesized compounds were tested for their activity on the aggregation of human blood platelets induced *in vitro* by various agonists. Only compounds **11** and **14**, which contain a 3-phenylpropyl group *N*-linked to the 2-position of the pyridazinone ring, have shown an interesting activity as antiplatelet agents. This activity disappeared when the chain linked to the 2-position of the pyridazinone contained one, two or four carbon atoms (compounds **9**, **10** and**12**). Similarly, compounds **5–8**, in which the phenyl group has been eliminated, and compound **13**, which contains an acetic group in 2-position are not active.

Our data show that only pyridazinone derivatives **11** and **14**, containing the 3-phenylpropyl group linked at the 2-position of the pyridazinone ring, inhibit the aggregation of human blood platelets. They also decrease $TxA_2$ and simultaneously increase $PGE_2$ synthesis in serum. The effects of our compounds on $TxA_2$ and $PGE_2$ synthesis are reminiscent of the activity of thromboxane synthase inhibitors [11]. However, the pattern of inhibition of platelet aggregation is different from any of the reference drugs acting on AA metabolism: aspirin, a cyclooxygenase inhibitor; OKY1581, a thromboxane synthase inhibitor; BM13.177, a thromboxane receptor antagonist; and Ridogrel, a dual thromboxane synthase inhibitor/thromboxane receptor antagonist. Indeed, in contrast to thromboxane synthase inhibitors, compounds **11** and **14** also reduced the first wave of ADP-induced platelet aggregation (which occurs independently of arachidonate metabolism) and inhibited the aggregation induced by U46619, an endoperoxide analogue, the effect of which is not blocked by thromboxane synthase inhibitors. In addition, all the

**Table III.** Effect of compounds **11** and **14** on $TxB_2$ and $PGE_2$ production in human serum.

| *Compound* | *Dose (μM)* | *$TxB_2$ (% of control)* | *$PGE_2$ (% of control)* |
|---|---|---|---|
| **11** | 50 | 57.9 ± 31.8 | 243.9 ± 37.4 |
| | 100 | 42.7 ± 14.9 | 307.4 ± 40.8 |
| | 300 | 23.1 ± 13.9 | 366.6 ± 42.3 |
| **14** | 12.5 | 85.9 ± 33.6 | 1105.0 ± 379.6 |
| | 25 | 79.8 ± 24.2 | 1164.2 ± 720.8 |
| | 50 | 53.1 ± 19.1 | 1396.4 ± 572.3 |
| | 100 | 39.3 ± 14.0 | 1344.7 ± 431.9 |
| | 300 | 13.3 ± 3.5 | 578.0 ± 483.2 |
| Aspirin | 100 | 5.2 ± 1.2 | < 4 |
| BM 13.177 | 100 | 101.6 ± 10.7 | 95.5 ± 6.6 |
| OKY 1581 | 100 | < 5[a] | 823 ± 78[a] |
| Ridogrel | 1 | < 5[b] | 2349 ± 514[b] |

For reference drugs, see table II. [a]From ref [11]; [b]from ref [13]. Control values for serum $TxB_2$ and $PGE_2$ were 566.9 ± 247.8 ng/ml ($n$ = 23) and 8.7 ± 6.2 ng/ml ($n$ = 27), respectively (mean ± SD).

**Table IV.** Analytical data of new 3(2*H*)-pyridazinone derivatives.

| *Compound* | *Molecular formula (MW)* | *Yield (%)* | *Mp (°C)* | *$^1$H NMR[a] (ppm)* |
|---|---|---|---|---|
| **5** | $C_8H_8N_4O$ (176) | 35 | 140–143 | δ: 3.75 (3H, s, N-$CH_3$), 7.05 (1H, d, $J$ = 9.5 Hz, C*H*=CH), 7.1–7.45 (3H, m, imidazol 2H, CH=C*H*), 7.95 (1H, s, imidazol H) |
| **6** | $C_9H_{10}N_4O$ (190) | 35 | 63–66 | δ: 1.35 (3H, t, $J$ = 6 Hz, $CH_3$), 4.2 (2H, q, $CH_2$), 7.0 (1H, d, $J$ = 9.5 Hz, C*H*=CH), 7.05 (2H, m, imidazol H), 7.5 (1H, d, $J$ = 9.5 Hz, CH=C*H*), 8.0 (1H, s imidazol H) |
| **7** | $C_{10}H_{12}N_4O$ (204) | 55 | 38–41 | δ: 1.45 (6H, d, $2CH_3$), 5.3 (1H, q, CH), 7.05 (1H, d, $J$ = 9.5 Hz, C*H*=CH), 7.1–7.2 (2H, m, imidazol H), 7.55 (1H, d, $J$ = 9.5 Hz, CH = C*H*), 8.0 (1H, s, imidazol H) |
| **8** | $C_{11}H_{14}N_4O$ (218) | 60 | 71–73 | δ: 1.0 (3H, t, $J$ = 6 Hz, $CH_3$), 1.45 (2H, q, $CH_2$), 1.85 (2H, t, $J$ = 6 Hz, $CH_2$), 4.2 (2H, t, $J$ = 6 Hz, $CH_2$), 7.05 (1H, d, $J$ = 9.5 Hz, C*H*=CH), 7.1–7.2 (2H, m, imidazol H), 7.5 (1H, d, $J$ = 9.5 Hz, CH=C*H*), 8.0 (1H, imidazol H) |
| **9** | $C_{14}H_{12}N_4O$ (252) | 60 | 110–114 | δ: 5.3 (2H, s, $CH_2$), 7.1–7.45 (9H, m, imidazol 2H, pyridazinonic 2H, aromatic 5H), 7.95 (1H, s, imidazol H) |
| **10** | $C_{15}H_{14}N_4O$ (266) | 55 | 70–73 | δ: 3.1 (2H, t, $J$ = 6 Hz, $CH_2$), 4.4 (2H, t, $J$ = 6 Hz, $CH_2$), 6.95 (1H, d, $J$ = 9.5 Hz, C*H*=CH), 7.05–7.35 (8H, m, imidazol 2H, CH=C*H* aromatic 5H), 7.8 (1H, s, imidazol H) |
| **11** | $C_{16}H_{16}N_4O$ (280) | 70 | Oil | δ: 2.2 (2H, q, $CH_2$), 2.7 (2H, t, $J$ = 6 Hz, $CH_2$), 4.2 (2H, t, $J$ = 6 Hz, $CH_2$), 6.95 (1H, d, $J$ = 9.5 Hz, C*H*=CH), 7.05–7.4 (8H, m, imidazol 2H, CH=C*H* aromatic 5H, 7.95 (1H, s, imidazol H) |
| **12** | $C_{17}H_{18}N_4O$ (294) | 50 | Oil | δ: 1.6–2.05 (4H, m, $2CH_2$), 2.7 (2H, t, $J$ = 6 Hz, $CH_2$), 4.15 (2H, t, $J$ = 6 Hz, $CH_2$), 6.95–7.2 (8H, m, imidazol 2H, CH=C*H* aromatic 5H), 7.3 (1H, d, $J$ = 9.5 Hz, CH=C*H*), 7.95 (1H, s, imidazol H) |
| **13** | $C_9H_8N_4O_3$ (220) | 40 | 230–235 | δ: 3.3 (2H, s, $CH_2$), 7.2 (1H, d, CH=C*H*), 7.7 (1H, s, imidazol H), 8.15–8.3 (3H, m, imidazol 2H, CH=C*H*), 9.6 (1H, s, COOH) |
| **14** | $C_{22}H_{20}N_4O$ (356) | 35 | 111–115 | δ: 2.2–2.4 (2H, m, $CH_2$), 2.8 (2H, t, $J$ = 6 Hz, $CH_2$), 4.25 (2H, t, $J$ = 6 Hz, $CH_2$), 6.9 (1H, d, C*H*=CH), 7.05–7.15 (9H, m, aromatic H), 7.65 (1H, d, $J$ = 9.5 Hz, CH=C*H*), 7.7–7.8 (3H, m, imidazol H) |

[a]For compounds **1–12** and **14** in $CDCl_3$, for compound **13** in MeOD.

blood donors we studied behaved as responders to the *in vitro* effects of compounds **11** and **14** on AA-induced aggregation, in contrast to what is usually observed with thromboxane synthase inhibitors [11].

The broad range of the antiplatelet activity of compounds **11** and **14** might be explained by considering that molecules containing the pyridazinone group are potent antiplatelet agents acting as selective inhibitors of phosphodiesterase III [16], the isoenzyme responsible for the cAMP degradation within the human platelets [17]. The combination of a thromboxane synthase inhibitor with a phosphodiesterase inhibitor leads to a synergistic antiplatelet effect. The stimulation of cAMP synthesis by prostacyclin or $PGD_2$ (synthesized from the endoperoxides when the enzyme thromboxane synthase is blocked) and the inhibition of cAMP breakdown lead to an enhanced accumulation of this second messenger and to a strong

platelet suppression [18]. Similarly, dual inhibitors/receptor antagonists/Tx-synthase may increase intraplatelet cAMP in stimulated platelets [2], although less than compounds acting directly on phosphodiesterase [2, 17, 19].

Our data on the effects of compounds **11** and **14** on platelet aggregation and on eicosanoid measurement in serum, and the structural analogies between our compounds and other substances described previously [16], suggest that the pyridazinone derivatives described in this paper may possess both a thromboxane synthase inhibitory activity and a depressing effect on platelet phosphodiesterase.

Thus, molecules **11** and **14** may open new perspectives for the development of new and, possibly, more powerful drugs for the treatment of thrombotic disorders [2, 18].

## Experimental protocols

*Biological methods*

*Platelet aggregation studies*

Blood was collected from healthy, drug-free volunteers and placed in sodium citrate 3.8% (1:10 v/v). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained by centrifugation at 150 *g* for 15 min, as previously described [20], and the platelet count was adjusted to 250 x $10^6$/ml. Platelet aggregation was studied by the photometric method with an automated platelet aggregometer analyzer (PA-3220 Aggrecorder II, Kagatu Co Ltd, Kyoto, Japan).

All the compounds were tested at a fixed dose of 100 μM. For the most active compounds, dose–response curves were drawn in order to calculate the $IC_{50}$. Aliquots of PRP (250 μl) were stimulated with microliter amounts of different inducers (AA, ADP, collagen and the stable endoperoxide analogue U46619) after preincubation with the tested compounds or with their solvents for 10 min at 37°C. $IC_{50}$ values were calculated by linear regression analysis of the aggregation values (as a percentage of the theoretical maximal amplitude) plotted against the drug concentration.

Compounds acting at different levels of AA metabolism were used as reference drugs: the cyclooxygenase inhibitor aspirin [10]; the thromboxane synthase inhibitor OKY1581 [11]; the thromboxane receptor antagonist BM13.177 [12]; and the dual thromboxane synthase inhibitor/receptor antagonist ridogrel [13].

*Measurement of eicosanoids*

Compounds active on platelet aggregation were also tested for their activity on the synthesis of $TxB_2$ and $PGE_2$ in clotting whole blood. The levels of $TxB_2$ (the stable metabolite of $TxA_2$) and $PGE_2$ in human serum were measured as previously described [11] by specific radioimmunoassays. The same reference drugs used for platelet aggregation experiments (see above) were tested in this experimental system.

*Chemistry*

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. The NMR spectra were recorded with a Varian EM-390 (90 MHz) instrument in the solvents indicated below. The chemical shift values (ppm) are relative to tetramethylsilane as the internal standard. Elemental analyses are within ±0.4% of the theoretical values. Precoated Keisegel 60 $F_{254}$ plates (Merck) were used for TLC.

*General method for the preparation of compounds 5–12 and 14*

Alkyl halide (4.5 x $10^{-3}$ mol) was added to a mixture of 4.5 x $10^{-3}$ mol of NaOH pellets and 4.5 x $10^{-3}$ mol of 6-(1*H*-imidazole-1-yl)-3(2*H*)-pyridazinone **3** or 6-[4-(1*H*-imidazole-1-yl)phenyl]-3(2*H*)-pyridazinone **4** in 20 ml dry EtOH. The mixture was refluxed for 4–8 h. The solution was evaporated under reduced pressure and the residue digested with hot EtOAc. The organic phase was dried and further evaporated and the residue was purified by flash chromatography using as eluent a stepwise gradient of EtOH (0–7%) in $CH_2Cl_2$. The analytical data are reported in table IV.

*2-[6-(1H-Imidazole-1-yl)-3(2H)-pyridazinonyl]acetic acid*

This compound was prepared by alkylation of 3 with bromoacetic acid and NaOH pellets in dry EtOH; time of reaction = 8 h. The mixture was filtered and the solid was treated with HCl 6 N, the solution was evaporated under reduced pressure. The residue was digested several times with hot dry EtOH. The organic phase was evaporated *in vacuo* and the residue was crystallized from EtOH. The analytical data are reported in table IV.

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