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Short communication

Guanylhydrazones of 3-substituted 2-pyridinecarboxaldehyde and of (2-substituted 3-pyridinyloxy) acetaldehyde as prostanoid biosynthesis and platelet aggregation inhibitors

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Summary — Some guanylhydrazones of (3-benzyloxy)-2-pyridinecarboxaldehyde and of (2-substituted 3-pyridinyloxy)acetaldehyde were prepared in order to evaluate their possible activity as inhibitors of prostanoid biosynthesis in human serum. Only those products of the second group without the carboxylic function reduced prostanoid generation *in vitro* at the highest concentration; they also inhibited platelet aggregation induced by arachidonic acid and U-46619. The results suggest that these compounds are both inhibitors of cyclooxygenase and cyclic endoperoxides/TxA_2 platelet receptor antagonists.

Résumé — Guanylhydrazones de 2-pyridinecarboxaldéhyde substitués en 3 et de 3-pyridyloxyacétaldéhyde substitué en 2 comme inhibiteurs de la biosynthèse des prostanoïdes et de l'agrégation plaquettaire. *Une série de guanylhydrazones des (3-benzyloxy)-2-pyridinecarboxaldéhyde et 3-pyridyloxyacétaldéhyde substitué en position 2 a été préparée pour évaluer leur possible activité comme inhibiteurs de la biosynthèse des prostanoïdes dans le sérum humain. Seuls les produits du second groupe dépourvus de fonction carboxylique réduisent la génération des prostanoïdes* in vitro *aux concentrations les plus élevées; ils inhibent aussi l'agrégation plaquettaire induite par l'acide arachidonique et U-46619. Les résultats suggèrent que ces composés sont des inhibiteurs de la cyclooxygénase et antagonistes du récepteur plaquettaire des endopéroxydes cycliques/TxA_2.*

guanylhydrazones / cyclooxygenase inhibitors / platelet aggregation inhibitors / TxA_2 receptor antagonists

Introduction

In previous papers [1–3] we have described the synthesis of 3-pyridinol ethers substituted in position 2 or 4 with side chains similar to those of prostaglandins (PGs); we have also reported the results of the studies of their inhibitory activity on thromboxane-(Tx)-synthase, first demonstrated for simple pyridine derivatives [4]. Measurements in human serum of TxB_2, the stable metabolite of TxA_2, confirmed the higher activity of 4-alkylpyridines in comparison to the corresponding 2-alkylpyridines [3], but also suggested that a basic group introduced in the chain in the 2 position could preserve in part the inhibitory activity, conferring at the same time a good selectivity of the compounds [2].

Moreover, guanylhydrazones of substituted phenoxyacetaldehydes inhibit platelet aggregation *in vitro* [5]. Whatever the mechanism of action, it was interesting to substitute the 3-(ω-carboxypentyloxy) with a 3-(*p*-carboxybenzyloxy) group in 2-pyridinecarboxaldehyde guanylhydrazone [2]; this kind of substitution, as in **IIIc**, maintains the distance between the pyridine nitrogen and the carboxylic function favourable for Tx-synthase inhibition and could improve it as reported for other substituted pyridines [6]. The carboxy group was eliminated (**IIIa**) or substituted with a methyl group (**IIIb**) in order to evaluate its importance for activity.

The simple guanylhydrazone of (3-pyridinyloxy)-acetaldehyde has been studied in a previous work and a good and selective activity was found [2]. In order to verify the influence on the activity of a phenyl-containing side chain in 2 position of pyridine, we synthesized the analogues **VIIa–c**.

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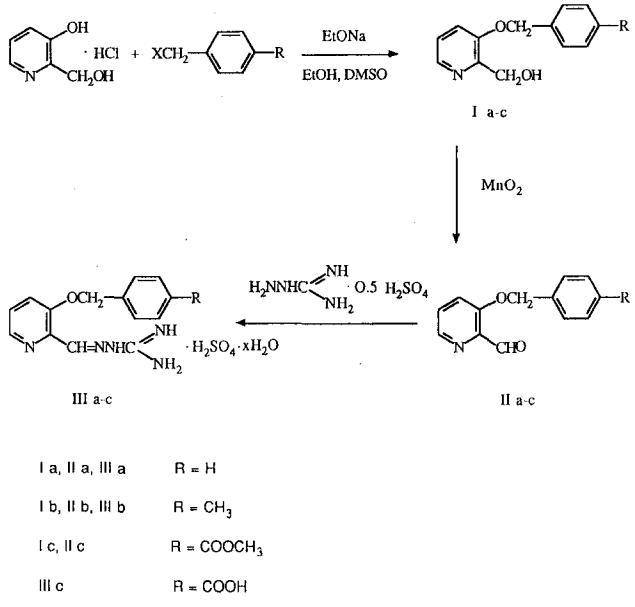
Chemistry

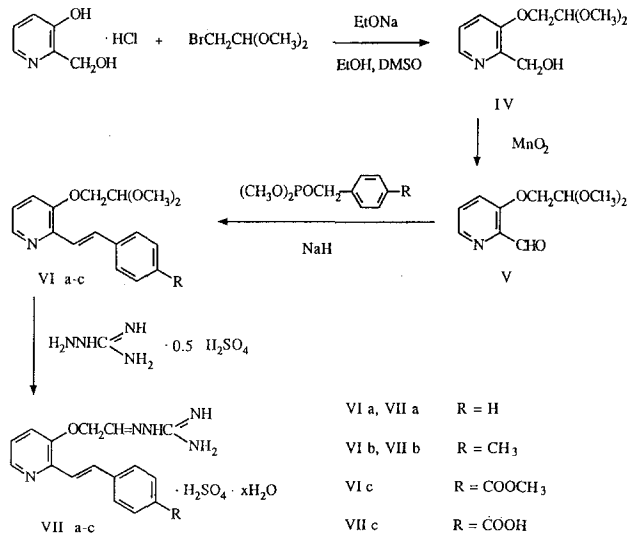
The synthesis of compounds **III** was performed starting from 3-hydroxy-2-pyridinemethanol, which was converted to its sodium salt with sodium ethylate in ethanol; this salt reacted at room temperature in dimethylsulfoxide (DMSO) with the proper benzyl halide. (3-Benzyloxy)-2-pyridinemethanol (**Ia**) was previously synthesized by Sheehan [7] starting from the same reagents, but using potassium hydroxide as a base; our method gave a higher yield.

The manganese dioxide oxidation of the ethers (**Ia–c**) gave excellent yields of the corresponding aldehydes (**IIa–c**), which were condensed with aminoguanidine sulphate at 80°C in aqueous acid. Under this condition the ester group of **IIc** was hydrolyzed and the desired acid (**IIIc**) was obtained (scheme 1).



Scheme 1.

In order to synthesize compounds **VIIa–c**, the first step was the preparation of (2-hydroxymethyl-3-pyridinyloxy)acetaldehyde dimethylacetal (**IV**), obtained by alkylation of 3-hydroxy-2-pyridinemethanol with bromoacetaldehyde dimethylacetal; in this case it was necessary to heat the reaction mixture, and the yield did not exceed 50%. The following reaction with manganese dioxide gave the aldehyde **V** in an almost quantitative yield. Wadsworth–Emmons reaction of the proper phosphonates with **V** yielded compounds **VIa–c** in the *trans* form, as demonstrated by the high coupling constants in NMR spectra. The condensation with aminoguanidine was accomplished under the already described conditions (scheme 2).



Scheme 2.

Biological results and discussion

All the new guanylhydrazones (**IIIa–c** and **VIIa–c**) were tested for their effect on serum TxB_2 and PGE_2 generation with specific radioimmunoassays (RIA), as previously described [2].

No significant reduction of prostanoids generation in serum occurred in human blood incubated in the presence of compounds **IIIa–c** and **VIIc** (data not shown).

The inactivity of the first series of guanylhydrazones (**IIIa–c**) on Tx-synthase is disappointing, especially for the acid **IIIc**. Indeed, the only difference between this compound and the active 3-(ω-carboxypentyloxy)-2-pyridinecarboxaldehyde guanylhydrazones [2] is a more constricted conformation of the chain in 3-position for the first acid.

Table I reports the inhibitory effect of compounds **VIIa** and **VIIb** on TxB_2 and PGE_2 production in serum obtained by whole blood, incubated at 37°C for 1 h. Generation of both TxB_2 and PGE_2 was significantly reduced by the highest tested concentration of the compounds.

The effect of compounds **VIIa** and **VIIb** was also studied on platelet aggregation on human platelet-rich plasma (PRP). Platelet aggregation induced by threshold aggregating concentrations of arachidonic acid

Table I. Effect of the guanidine derivatives, **VIIa** and **VIIb**, on human serum TxB_2 and PGE_2 production (% of control values). Figures are means ± SD (n = 3–6 samples). Control values (both in the presence and in absence of 5–10 µl/ml of dimethylsulphoxide) were 330 and 396 pmol/ml of serum for TxB_2 and PGE_2, respectively.

Concentration	***VIIa***		***VIIb***	
(µM)	*TxB_2*	*PGE_2*	*TXB_2*	*PGE_2*
50	96 ± 1	—	105 ± 3	—
100	59 ± 24*	46 ± 3*	80 ± 7	56 ± 6*
200	34 ± 2*	37 ± 7*	52 ± 13*	52 ± 6*

*$P < 0.01$ significantly different from control (ANOVA and Dunnet test).

was significantly suppressed by both compounds: the minimal inhibitory concentrations were 50 and 100 µM, respectively. However, the increase in concentration of arachidonic acid abolished the inhibitory effect of both compounds (fig 1).

Platelet aggregation induced by U-46619, a receptor agonist of cyclic endoperoxides/TxA_2 [8], was also reduced by compounds **VIIa** and **VIIb** (at similar concentration to arachidonic acid-induced platelet aggregation) (fig 2). The inhibitory effect of the compounds, also active when added simultaneously to U-46619, was reversed by increasing the stimulus concentration. The inhibition of U-46619-induced platelet aggregation was evident also in the presence of 100 µM aspirin, which completely prevented thromboxane synthesis, confirming a direct effect of the compounds on the receptor (data not shown).

The possible inhibitory activity on cyclic endoperoxides/TxA_2 receptor was also tested for those compounds that did not affect cyclooxygenase activity. Only compound **IIIa** (100–200 µM) showed a moderate inhibitory effect on U-46619-induced platelet aggregation (data not shown).

In conclusion, the newly synthesized guanylhydrazones, **VIIa** and **VIIb**, incubated *in vitro* with human whole blood, showed an inhibitory effect on cyclooxygenase at concentrations as low as 50 or 100 µM, respectively. The inhibitory effect on cyclooxygenase has been demonstrated by the reduction of 2 stable products of the arachidonic acid metabolism, TxB_2 and PGE_2, thus excluding the possibility of a selective inhibition of Tx-synthase. In fact, dazoxiben, a Tx-synthase inhibitor, suppressed TxB_2 generation, but induced a concomitant significant increase of PGE_2 synthesis [9]. It has been also reported that R68070 (Ridogrel), a Tx-synthase inhibitor and an antagonist of cyclic endoperoxides/TxA_2 receptor, induced inhibition of TxB_2 and increase of PGE_2 generation [10].

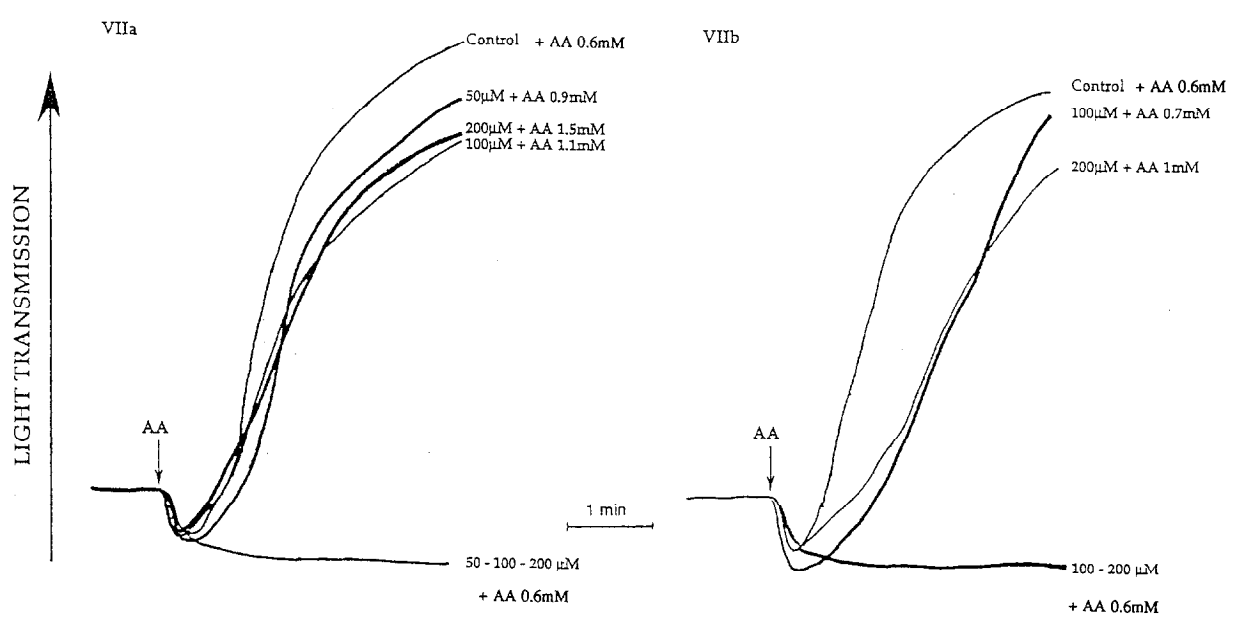


Fig 1. Inhibitory effect of different concentrations of compounds **VIIa** and **VIIb** on arachidonic acid-induced platelet aggregation and its reversal by increasing arachidonic acid concentrations. Representative curves of 3 different experiments performed on human PRP. The compounds (at the concentrations indicated) or their solvent (control) were added to PRP 3 min before the addition of arachidonic acid at the threshold aggregating concentrations (0.6 mM) or at gradually increased concentrations. For further details, see *Biological tests*.

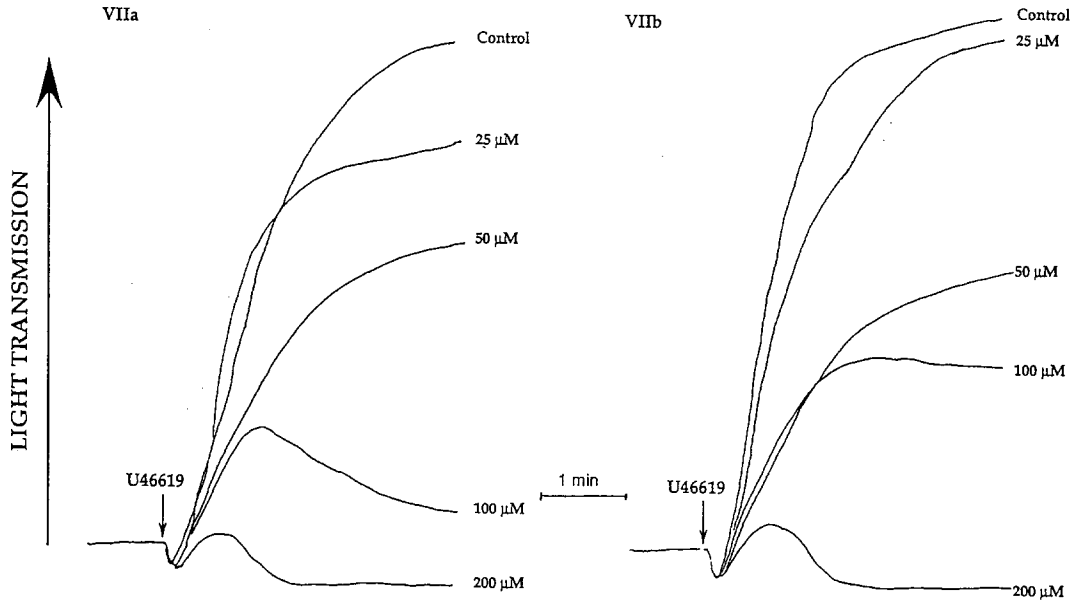


Fig 2. Inhibitory effect of different concentrations of compounds **VIIa** and **VIIb** on U-46619-induced platelet aggregation. Representative curves of 3 different experiments performed on human PRP. The compounds (at the concentrations indicated) or their solvent (control) were added to PRP 3 min before the addition of U-46619 at the threshold-aggregating concentrations (0.3 and 0.6 µM in the experiment with compounds **VIIa** and **VIIb**, respectively). Similar results were obtained when the compounds were added 1 min before or simultaneously to U-46619. For further details, see *Biological tests*.

At the tested concentrations of compounds, suppression of the arachidonic acid metabolism was not complete and was reversible.

The 2 compounds inhibited platelet aggregation induced by arachidonic acid and U-46619; this latter finding indicates that they also possess an antagonist activity on the cyclic endoperoxide/TxA_2 platelet receptor. With both agonists the inhibitory effect on platelet aggregation was reversed by increasing their concentrations. Indobufen, a reversible inhibitor of cyclooxygenase [11], at 3–8 µM completely suppressed platelet aggregation induced by threshold concentrations of arachidonic acid; this effect was reversed by doubling the arachidonic acid concentrations. On the other hand, U-46619-induced platelet aggregation was unaffected by indobufen. Cyclic endoperoxide/TxA_2 receptor antagonists, either selective or endowed with inhibitory activity on Tx-synthase, also prevented platelet aggregation induced by arachidonic acid and U-46619 [12, 10]. From our results it is suggested that the inhibitory effect of the compounds **VIIa** and **VIIb** on arachidonic acid-induced platelet aggregation is due to the combined inhibition of cyclooxygenase and antagonism of the cyclic endoperoxides/TxA_2 receptor. This original dual effect on platelet arachidonate metabolite synthesis and function is of interest, and could stimulate new research on this kind of chemical structure.

Experimental protocols

Chemistry

Melting points were determined in open glass capillaries on a Büchi SMP-20 apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 297 instrument; NMR spectra on a Varian EM-390 instrument, using TMS as internal standard. Only the NMR spectrum of the compound **VIc** was performed on a Varian XL-300 instrument. Elemental analyses were within ± 0.4% of theoretical value. Column chromatography was performed using silica gel RS, 0.05–0.20 mm (Carlo Erba).

*Synthesis of ethers **Ia–c**, and **IV***

3-Hydroxy-2-pyridinemethanol hydrochloride (0.1 mol) was added to a solution of sodium (0.2 mol) in absolute ethanol (80 ml). After 1 h at room temperature under stirring, DMSO (125 ml) was added and ethanol was completely removed under reduced pressure. The alkylating agent (0.1 mol of benzyl chloride, *p*-methylbenzyl bromide, methyl *p*-bromomethyl benzoate [13] or bromoacetaldehyde dimethylacetal) was added and the suspension was stirred overnight at room temperature; heating at 80°C for 3 h was necessary only to obtain **IV**. After dilution with water, ethers **Ia–c** were filtered, washed with water and crystallized from *n*-hexane. For the ether **IV**, the solution was acidified with 2 N hydrochloric acid, extracted with chloroform, alkalified with 2 N sodium hydroxide and again extracted with chloroform. The second extract was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure to give a colourless residue.

*(3-Benzyloxy)-2-pyridinemethanol **Ia**.*
Yield 99%, mp = 80–81°C as in [7].

3-(4-Methylbenzyloxy)-2-pyridinemethanol ***Ib***.
Yield 81%, mp = 96.5–97.5°C from n-hexane. IR (KBr): cm^{-1} 3400–3100. NMR ($CDCl_3$): ppm 8.15 (m, 1H, H_6); 7.35–7.05 (m, 6H, H_4, H_5, C_6H_4); 5.00 (s, 2H, OCH_2); 4.80 (s, 2H, CH_2O); 4.40 (s, 1H, OH); 2.25 (s, 3H, CH_3). Anal $C_{14}H_{15}NO_2$ (C, H, N).

Methyl 4-[(2-hydroxymethyl-3-pyridinyloxy)methyl]benzoate ***Ic***.
Yield 76%, mp = 109–109.5°C from n-hexane. IR (KBr): cm^{-1} 3350–3100, 1710. NMR ($CDCl_3$): ppm 8.20 (m, 1H, H_6); 8.10 (d, J = 9 Hz, 2H, $2H_\beta$); 7.45 (d, J = 9 Hz, 2H, $2H_\alpha$); 7.15 (m, 2H, H_4, H_5); 5.15 (s, 2H, OCH_2); 4.80 (s, 2H, CH_2O); 4.35–4.15 (bs, 1H, OH); 3.90 (s, 3H, CH_3). Anal $C_{15}H_{15}NO_4$ (C, H, N).

3-(2,2-Dimethoxyethoxy)-2-pyridinemethanol ***IV***. Yield 50%, oil, bp (0.06 mm Hg) = 134°C. IR (film): cm^{-1} 3600–3100. NMR ($CDCl_3$): ppm 8.20 (m, 1H, H_6); 7.20 (m, 2H, H_4, H_5); 4.75 (s, 2H, CH_2O); 4.70–4.45 (m, 2H, CH, OH); 4.00 (d, 2H, OCH_2); 3.40 (s, 6H, $2CH_3$). Anal $C_{10}H_{15}NO_4$ (C, H, N).

Synthesis of aldehydes ***IIa–c*** *and* ***V***

To a solution of the benzyl alcohol (**Ia–c** or **IV**) (0.1 mol) in 1,2-dimethoxyethane (150 ml) heated at 100°C, MnO_2 (0.9 mol), previously activated at 120°C for 2 h, was added with stirring over a period of 1 h; heating was continued for another hour. After cooling, the suspension was filtered and the filtrate evaporated under reduced pressure. The compounds were purified by crystallization or distillation.

(3-Benzyloxy)-2-pyridinecarboxaldehyde ***IIa***.
Yield 95%. Colourless oil. IR (film): cm^{-1} 2820, 2720, 1715. NMR ($CDCl_3$): ppm 10.45 (s, 1H, CHO); 8.35 (m, 1H, H_6); 7.35 (m, 7H, H_4, H_5, C_6H_5); 5.15 (s, 2H, CH_2). Anal $C_{13}H_{11}NO_2$ (C, H, N). The 2,4-dinitrophenylhydrazone crystallized from ethyl alcohol; mp = 202–204°C.

3-(4-Methylbenzyloxy)-2-pyridinecarboxaldehyde ***IIb***.
Yield 68%, mp = 62–63°C from tetrahydrofuran/petroleum ether. IR (KBr): cm^{-1} 2860, 2740, 1700. NMR ($CDCl_3$): ppm 10.40 (s, 1H, CH); 8.35 (m, 1H, H_6); 7.45–7.05 (m, 6H, H_4, H_5, $2H_\alpha$, $2H_\beta$); 5.10 (s, 2H, CH_2); 2.30 (s, 3H, CH_3). Anal $C_{14}H_{13}NO_2$ (C, H, N).

Methyl 4-[(2-formyl-3-pyridinyloxy)methyl]benzoate ***IIc***.
Yield 78%, mp = 131–132°C from ethyl acetate. IR (KBr): cm^{-1} 2820, 2720, 1730, 1700. NMR ($CDCl_3$): ppm 10.45 (s, 1H, CHO); 8.45 (m, 1H, H_6); 8.10 (d, J = 9 Hz, 2H, $2H_\beta$); 7.60 (d, J = 9 Hz, 2H, $2H_\alpha$); 7.50 (m, 2H, H_4, H_5); 5.30 (s, 2H, OCH_2); 3.90 (s, 3H, OCH_3). Anal $C_{15}H_{13}NO_4$ (C, H, N).

3-(2,2-Dimethoxyethoxy)-2-pyridinecarboxaldehyde ***V***.
Yield 82%. Oil. IR (film): cm^{-1} 2830, 2720, 1710. NMR ($CDCl_3$): ppm 10.40 (s, 1H, CHO); 8.40 (m, 1H, H_6); 7.60 (m, 2H, H_4, H_5); 4.80 (t, 1H, CH); 4.15 (d, 2H, CH_2); 3.45 (s, 6H, $2CH_3$). Anal $C_{10}H_{13}NO_4$ (C, H, N). The 2,4-dinitrophenylhydrazone crystallized from dimethyl formamide; mp = 230–236°C.

Reaction of Wadsworth–Emmons

To a solution of the convenient phosphonate (0.01 mol of diethyl benzylphosphonate [14], diethyl *p*-methylbenzylphosphonate [15] or dimethyl *p*-methylcarboxybenzylphosphonate [16]) in dry ethyl ether (35 ml) a suspension of NaH (0.01 mol) in dry ethyl ether (10 ml) was added and the mixture was stirred for 1 h at room temperature. Then the aldehyde **V** (0.01 mol) in dry ethyl ether (45 ml) was added dropwise at room temperature. The mixture was stirred overnight, washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The oil obtained was chromatographed on silica gel to give the *trans*-compounds (**VIa–c**).

(E)-[2-(2-Phenylethenyl)-3-pyridinyloxy]acetaldehyde dimethylacetal ***VIa***.
Ethyl ether/petroleum ether (3:1) was used as eluent. Oil. Yield 25%. NMR (CCl_4): ppm 8.10 (m, 1H, H_6); 7,90 (d, J = 18 Hz, 1H, PyCH=); 7.70–7.15 (m, 6H, =CHPh, C_6H_5); 6.95 (m, 2H, H_4, H_5); 4.65 (t, 1H, CH); 3.90 (d, 2H, CH_2); 3.35 (s, 6H, $2CH_3$). Anal $C_{17}H_{19}NO_3$ (C, H, N).

(E)-[2-[2-(4-Tolyl)ethenyl]-3-pyridinyloxy]acetaldehyde dimethylacetal ***VIb***
Ethyl ether/petroleum ether (5:1) was used as eluent. Oil. Yield 11%. NMR (CCl_4): ppm 8.20 (m, 1H, H_6); 7.85 (d, J = 16 Hz, 1H, PyCH=); 7.60–7.30 (m, 3H, =CHPh, $2H_\alpha$); 7.25–6.90 (m, 4H, H_4, H_5, $2H_\beta$); 4.70 (t, 1H, CH); 3.95 (d, 2H, CH_2); 3.35 (s, 6H, $2OCH_3$); 2.30 (s, 3H, CH_3). Anal $C_{18}H_{21}NO_3$ (C, H, N).

(E)-Methyl 4-[2-[3-(2,2-dimethoxyethoxy)-2-pyridinyl]-ethenyl]-benzoate ***VIc***
Ethyl ether was used as eluent. Yield 27%, mp = 90–91°C from ethyl ether. IR (KBr): cm^{-1} 1710. NMR ($CDCl_3$): ppm 8.25 (m, 1H, H_6); 8.05 (d, J = 9 Hz, 2H, $2H_\alpha$); 7.80 (d, J = 16 Hz, 1H, PyCH=); 7.70 (d, J = 16 Hz, 1H, =CHPh); 7.65 (d, J = 9 Hz, 2H, $2H_\beta$); 7.20 (m, 2H, H_4, H_5); 4.90 (t, 1H, CH); 4.10 (d, 2H, CH_2); 3.90 (s, 3H, $COOCH_3$); 3.45 (s, 6H, $2OCH_3$). Anal $C_{19}H_{21}NO_5$ (C, H, N).

Synthesis of guanylhydrazones ***IIIa, b*** *and* ***VIIa, b***

A solution of aminoguanidine sulphate (0.01 mol) in 2 N sulphuric acid (30 ml) was added to a solution of aldehyde **IIa, b** or of dimethylacetal **VIa, b** (0.01 mol) in ethyl alcohol (6 ml) and the mixture was heated at 80°C for 4 h while stirring. After cooling the precipitate was filtered, washed with water and ethyl alcohol. The guanylhydrazones **IIIa, b** were purified by crystallization; **VIIa, b** were directly analyzed.

(3-Benzyloxy)-2-pyridinecarboxaldehyde guanylhydrazone sulphate•1.5 H_2O ***IIIa***
Yield 54%, mp = 171–173°C from water/ethyl alcohol. IR (KBr): cm^{-1} 3400–2700, 1670. NMR (DMSO–d_6): ppm 8.65 (s, 1H, CH=N); 8.50 (d, 1H, H_6); 8.20–7.35 (m, 11H, H_4, H_5, C_6H_5, 2NH, NH_2); 7.20–6.90 (bs, 5H, H_2SO_4, 1.5 H_2O); 5.35 (s, 2H, CH_2). Anal $C_{14}H_{15}N_5O{\cdot}H_2SO_4{\cdot}1.5\ H_2O$ (C, H, N, S).

3-(4-Methylbenzyloxy)-2-pyridinecarboxaldehyde guanylhydrazone sulphate•1 H_2O ***IIIb***
Yield 69%, mp = 208–209°C dec from ethyl alcohol. IR (KBr): cm^{-1} 3500–2400, 1680. NMR (DMSO–d_6): ppm 8.65 (s, 1H, CH=N); 8.35 (d, 1H, H_6); 7.95–7.20 (m, 10H, H_4, H_5, $2H_\alpha$, $2H_\beta$, 2NH, NH_2); 5.25 (s, 2H, CH_2); 4.60–3.60 (bs, 4H, H_2SO_4, H_2O); 2.35 (s, 3H, CH_3). Anal $C_{15}H_{17}N_5O{\cdot}H_2SO_4{\cdot}H_2O$ (C, H, N, S).

(E)-[2-(2-Phenylethenyl)-3-pyridinyloxy]acetaldehyde guanylhydrazone sulphate•0.5 H_2O ***VIIa***
Yield 46%, mp = 208–210°C dec, IR (KBr): cm^{-1} 3600–2700, 1670. NMR (DMSO–d_6): ppm 8.35–7.15 (m, 15H, H_4, H_5, H_6, C_6H_5, CH=N, CH=CH, 2NH, NH_2); 5.60–5.00 (bs, 3H, H_2SO_4, 0.5 H_2O); 4.90 (d, 2H, CH_2). Anal $C_{16}H_{17}N_5O{\cdot}H_2SO_4{\cdot}0.5\ H_2O$ (C, H, N, S).

(E)-[2-[2-(4-Tolyl)ethenyl]-3-pyridinyloxy]acetaldehyde guanylhydrazone sulphate•0.5 H_2O ***VIIb***
Yield 63%, mp = 198–202°C dec, IR (KBr): cm^{-1} 3600–2700, 1670. NMR (DMSO–d_6): ppm 8.40–7.15 (m, 14H, H_4, H_5, H_6, $2H_\alpha$, $2H_\beta$, CH=N, CH=CH, 2NH, NH_2); 5.90–5.10 (bs, 3H, H_2SO_4, 0.5 H_2O); 4.90 (d, 2H, CH_2); 2.35 (s, 3H, CH_3). Anal $C_{17}H_{19}N_5O•H_2SO_4•0.5\ H_2O$ (C, H, N, S).

Synthesis of guanylhydrazones ***IIIc*** *and* ***VIIc***

A solution of aminoguanidine sulphate (0.01 mol) and aldehyde **IIc** or **VIIc** (0.01 mol) in diluted sulphuric acid (1 N 150 ml or 2 N 370 ml, respectively) was heated at 80°C for 4 h. After cooling, the precipitate was filtered, washed with water and crystallized.

4-[[2-[[(aminoiminomethyl)hydrazono]methyl]-3-pyridinyloxy]methyl]benzoic acid sulphate•1 H_2O ***IIIc***
Yield 43%, mp = 224–227°C dec from ethyl alcohol. IR (KBr): cm^{-1} 3500–2600, 1710, 1680. NMR (DMSO–d_6): ppm 11.90–11.70 (bs, 1H, COOH); 8.65–7.60 (m, 12H, H_4, H_5, H_6, $2H_\alpha$, $2H_\beta$, CH=N, 2NH, NH_2); 6.00–5.40 (m, 4H, H_2SO_4, H_2O); 5.50 (s, 2H, CH_2). Anal $C_{15}H_{15}N_5O_3•H_2SO_4•H_2O$ (C, H, N, S).

(E)-4-[2-[3-[[(aminoiminomethyl)hydrazono]ethoxy]-2-pyridinyl]ethenyl]benzoic acid sulphate•1.5 H_2O ***VIIc***
Yield 70%, mp = 220–224°C dec from water. IR (KBr): cm^{-1} 3600–2700, 1710, 1680. NMR (DMSO–d_6): ppm 11.75–11.50 (bs, 1H, COOH); 8.35–7.15 (m, 14H, H_4, H_5, H_6, $2H_\alpha$, $2H_\beta$, CH=N, CH=CH, 2NH, NH_2); 5.00–4.30 (m, 7H, CH_2, H_2SO_4, 1.5 H_2O). Anal $C_{17}H_{17}N_5O_3•H_2SO_4•1.5\ H_2O$ (C, H, N, S).

Biological tests

Venous blood from healthy volunteers was collected in glass tubes without anticoagulant, immediately mixed with microliter amounts (5–10 µl/ml of blood) of the solutions to be tested or the solvent (dimethylsulphoxide) and incubated at 37°C for 1 h. Serum was separated by centrifugation and stored at –20°C until assayed for thromboxane (TxB_2, the stable metabolite of TxA_2), and PGE_2 by specific radioimmunoassays. For TxB_2 determination was used a specific antiserum provided by Professor C Patrono, as previously described [2]; for PGE_2 a commercial kit was used (NEN, Du Pont). No relevant difference was observed in prostanoid synthesis between native and dimethylsulphoxide-added controls.

Aggregation studies were performed on citrated (3.8%; 1/10, v/v) human PRP: 250 µl of PRP were preincubated for 3 min at 37°C in a Born aggregometer (Elvi 840, Elvi Logos, Milan, Italy), aggregation recorded under continuous stirring (1000 rpm) for 3 min after addition of the stimulus. Arachidonic acid (Na salt, 99% pure, Sigma) and the compound U-46619 (Upjohn, Kalamazoo, Mo, USA) were used as platelet activators at the minimal concentration inducing an irreversible aggregation, with more than 50% increase of light transmission [17]. The inhibitory activity of the compounds was tested by adding their solutions or the solvent to PRP at different times (3–0 min), before addition of the stimulus.

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