Synthesis of Some Guanylhydrazones and Imidazolinylhydrazones as Thromboxane-Synthase and Platelet Aggregation Inhibitors

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The imidazolinylhydrazones of (3-pyridinyloxy)-acetaldehyde and of 6-[3-(2-formyl-pyridinyl)oxy]hexanoic acid were synthesized as cyclic analogues of the corresponding guanylhydrazones which were found to be selective inhibitors of human thromboxane-synthase. The benzene isosters were also prepared in order to define the importance of the ring nitrogen for the activity. - Moreover, the guanyl- and imidazolinyl-hydrazones of two 6-[(3-pyridinyl)oxy]hexanoic acids showing in the 2 position an alkyl chain with an α,β-unsaturated ketonic function were prepared. - Imidazolinylhydrazones **7** and **18** are selective inhibitors of thromboxane-synthase, while the two guanylhydrazones **14** and **15** which do not affect prostanoid biosynthesis seemed to be antagonists at the thromboxane receptor.

Synthese einiger Guanyl- und Imidazolinyl-Hydrazone als Thromboxan-Synthase- und Plättchenaggregation-Hemmstoffe

(3-Pyridinyloxy)acetaldehyd- und 6-[3-(2-Formyl-pyridinyl)oxy]hexansäure-Imidazolinylhydrazone wurden dargestellt als zyklische Analoga der Guanylhydrazone, die sich als selektive Inhibitoren der menschlichen Thromboxan-Synthase erwiesen haben. Die Benzol-Isosteren wurden auch synthetisiert, um die Bedeutung des Ring-Stickstoffs für die Aktivität zu definieren. Die Guanyl- und Imidazolinyl-Hydrazone der 6-[(3-Pyridinyl)oxy]hexansäuren mit einer α,β-ungesättigen Keto-Funktion in der Seitenkette in 2-Stellung wurden auch dargestellt. Die Imidazolinyl-Hydrazone **7** und **18** erwiesen sich Thromboxan-Synthase Inhibitoren, während die Guanyl-Hydrazone **14** und **15** Antagonisten der Thromboxan-Rezeptoren zu sein scheinen.

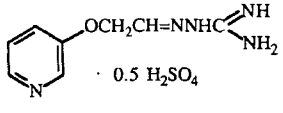
We have reported[1] that the guanylhydrazone of (3-pyridinyloxy)-acetaldehyde (**1**) selectively inhibits human thromboxane (Tx) A_2-synthase, and that the 6-[(3-pyridinyl)oxy]hexanoic acids substituted in 2 (**2**) or in 4 (**3**) by the carboxaldehyde guanylhydrazone residue are interesting inhibitors of thromboxane synthesis (IC_{50} values 1.3, 2.0 and 0.2 μmol/l, respectively). However, compound **2** is a Tx-synthase inhibitor that acts on cyclooxygenase at higher concentrations, while compound **3** is an inhibitor of cyclooxygenase, since it blocks both TxB_2 and prostaglandin (PG) E_2 serum production.

Here we describe the analogues **7** and **16** in which the amidino group in **1** and **2** was replaced by the 2-imidazoline residue. Moreover, in order to evaluate the influence of structural features on the inhibitory potency, we distanced the basic group in the 2-substituent from the pyridine nitrogen, and modified the length of the alkyl chain in the same position (**14**, **15**, **18**, and **19**).

2-Substituted pyridines normally are inactive on Tx-synthase[2,3], probably because the N-atom of the pyridine ring must be unhindered for binding the heme Fe-atom of the enzyme. Compound **2** was the first example of a 2-substituted pyridine that affects Tx-synthase. We hypothesized that a N-atom of the guanylhydrazone residue could act as substitutive binding site. In order to confirm this assumption, we prepared the benzene isosters **6**, **8**, **13**, and **17** of the pyridine derivatives; they should show comparable activities, if our hypothesis were correct.

The new compounds were studied as TxA_2-synthase inhibitors by evaluating human serum TxB_2 production as an index of platelet TxA_2-synthase activity, and PGE_2 production in order to ascertain an action on cyclooxygenase.

The combination of a Tx-synthase inhibitor and a receptor antagonist has been proposed for the treatment of a variety of circulatory deseases, in



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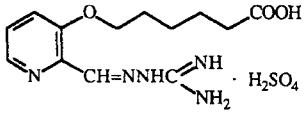

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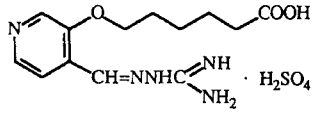

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the pathogenesis of which TxA_2 is implicated[4-6]. Theoretical arguments support the potentially superior efficacy of the association over either agent alone. In fact, Tx-synthase inhibitors prevent the biosynthesis of platelet aggregating TxA_2 and induce an overproduction of antiaggregatory prostanoids, like PGI_2 and PGD_2[7-9]. However, they also lead to the accumulation of prostaglandin endoperoxides, which exert TxA_2 like activity *via* the common receptor[6-10]. On the other hand, receptor antagonists neutralize the actions of TxA_2 and cyclic endoperoxides, but they do not increase the biosynthesis of beneficial prostaglandins.

The results obtained in animals[11,12] and human volunteers[13] showed the greater efficacy of combined therapy with Tx-synthase inhibitors and receptor blockers.

Recently compounds with both activities in the same molecule have been reported[14-18], among them R 68070 (Ridogrel)[19].

In a previous paper[20] we have reported that guanylhydrazones of [(2-substituted 3-pyridinyl)oxy]-acetaldehyde were inhibitors of cyclooxygenase as well as antagonists of cyclic endoperoxide/TxA_2 platelet receptor; therefore, we assayed also the new compounds and the guanylhydrazones **1-3** for the effect on this receptor.

Chemistry

The guanylhydrazone **6** was prepared from phenoxyacetaldehyde dimethylacetal as in ref.[21]. In a similar manner all the other hydrazones were obtained by condensation of the proper carbonyl compound with aminoguanidine sulphate or 2-hydrazino-2-imidazoline · HBr in water or water/ethanol solution, acidified with H_2SO_4 or HBr (schemes 1, 2). Under these conditions, hydrolysis of an ester group, if present, took place. All the compounds crystallized from the reaction mixture as sulphates or hydrobromides. Only the hydrazone **8** was separated as free base.

Concerning the carbonyl compounds, we have described (3-pyridinyloxy)-acetaldehyde dimethylacetal[1], the aldehyde **9** and the ketone **11**[22], while the analogue of **11** with a shorter side chain (**12**) was synthesized by *Wadsworth-Emmons* reaction of dimethyl (2-oxopropyl)-phosphate with aldehyde **9**. Only the *trans* form was separated, as demonstrated by the high coupling constant in its ^{1}H-NMR spectrum. The aldehyde **10** was obtained by reaction of 2-hydroxybenzaldehyde with ethyl bromohexanoate in the presence of K_2CO_3.

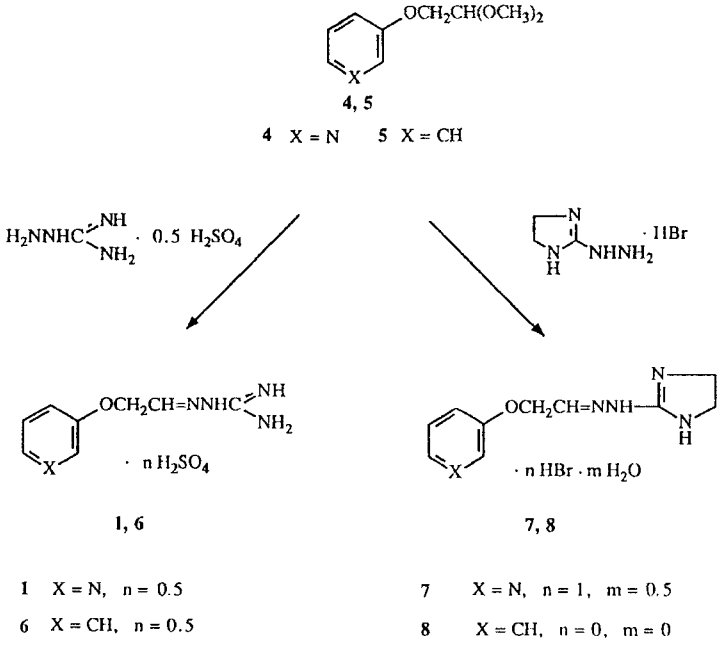
Biological results and discussion

All new compounds were tested for their effect on TxB_2 and PGE_2 generation in serum obtained from human whole blood, incubated at 37 °C for 1 h. TxB_2 and PGE_2 were measured by specific radioimmunoassays (RIA) as described[1].

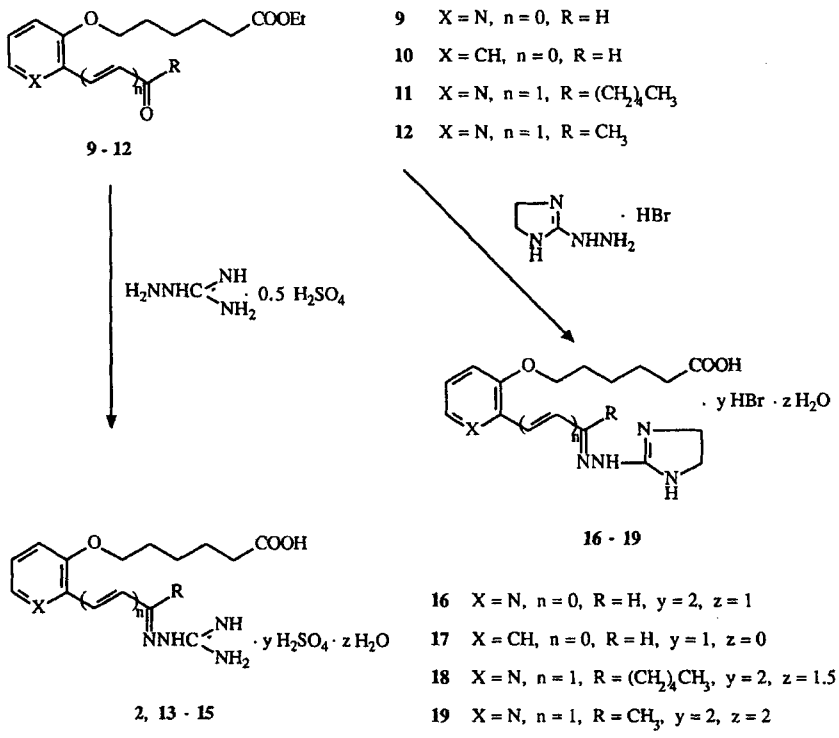
Only the imidazolinylhydrazones **7** and **18** significantly affected TxB_2 generation at the highest tested concentration. The IC_{50}s calculated from the dose-response plots were 92.7 and 93.1 μM, respectively. The reduction of TxB_2 synthesis was accompanied by an increase of PGE_2 production (5.7 and 4.7 times control values, at 100 μM concentration). These results indicate a selective inhibition of Tx-synthase by hydrazones **7** and **18**, since cyclic endoperoxides are diverted from Tx-synthase to PGE isomerase.

Compounds **7** and **18**, which were able to inhibit platelet TxA_2 formation, were tested on human platelet aggregation induced by threshold concentrations (0.4-0.7 mM) of arachidonic acid. Inhibition was induced by incubation for 3 min with the compounds at concentrations ranging between 50 and 200 μM, but was overcome by increasing arachidonic acid concentrations.

In consideration of the inactivity on Tx-synthase of all the benzene derivatives (**6**, **8**, **13**, and **17**), the hypothesis of



Scheme 1



Scheme 2

binding of the heme Fe by the N-atom of the hydrazone was not confirmed.

The new hydrazones and the guanylhydrazones **1-3**, previously described[1], were also tested for inhibitory effects on U-46619-induced platelet aggregation. U-46619, a stable analogue of cyclic endoperoxides, is a cyclic endoperoxide/TxA_2 receptor agonist[23] and was also used at threshold aggregating concentrations (200-600 nM).

Only the guanylhydrazones **14** and **15** completely suppressed U-46619-induced platelet aggregation at concentrations between 50 and 200 µM, suggesting an antagonistic activity on the cyclic endoperoxide/TxA_2 platelet receptor. The direct effect of the compounds on the receptor was confirmed by the permanence of the inhibition in presence of 100 µM aspirin, which completely prevented Tx biosynthesis.

Therefore, the increase of the distance between the guanylhydrazone residue and the N-containing ring (compounds **14** and **15** with respect to **2**) cancelled the activity on Tx-synthase and led to antagonism on the receptor. This last activity disappeared in the corresponding imidazolinylhydrazones **18, 19**; only the most lipophilic compound **18** is a Tx-synthase inhibitor. The other imidazolinylhydrazones were also inactive on the receptor.

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Experimental Part

Chemistry

Melting points: Büchi SMP-20 (uncorrected).- IR: Perkin-Elmer 1310.- NMR: Varian EM-390 (90 MHz), TMS as internal standard.- Elemental Analyses: Laboratorio di Microanalisi, Dipartimento di Scienze farmaceutiche, Univ. di Padova.- Column chromatography: silica gel RS, 0.05-0.20 mm (Carlo Erba). The guanylhydrazone **6** was prepared as in ref.[21] and (3-pyridinyloxy)-acetaldehyde dimethylacetal, the aldehyde **9** and the ketone **11** were obtained by the methods described[1,22].- Temp. in °C.- Chem. shifts in δ (ppm).

Imidazolinylhydrazones 7 and 8

A solution of 2-hydrazino-2-imidazoline · HBr (0.01 moles) in 2N HBr (40 ml) was added to a solution of (3-pyridinyloxy)-acetaldehyde dimethylacetal[1] or phenoxyacetaldehyde dimethylacetal[21] (0.01 moles) in ethanol (6 ml) and the mixture was heated at 100° for 4 h under stirring. After cooling, ethanol was evaporated *in vacuo* and the solution was worked up as described below.

3-[2-[(4,5-Dihydro-1H-imidazol-2-yl)hydrazono]ethoxy]pyridine hydrobromide, hemihydrate (7)

The solution was neutralized with 2N NaOH. The precipitate was filtered, washed with water and crystallized from ethanol.- Yield 20%.- M.p. 132-134 °.- IR (KBr): cm^{-1} 3540-3400; 3400-3000; 3000-2700; 1660.- ^1H-NMR (DMSO-d_6): 9.10-8.50 (bs, 2H, NH_2^+); 8.50-8.25 (m, 2H, 2-H, 6-H);

7.85 (t, 1H, CH=N); 7.70-6.90 (m, 4H, 4-H, 5-H, NH, 0.5 H_2O); 4.85 (d, J = 6 Hz, 2H, OCH_2); 3.70 (s, 4H, 2 CH_2 Imid.).- $C_{10}H_{13}N_5O \cdot HBr \cdot 0.5\ H_2O$ (309.2) Calcd. C 38.8 H 4.89 N 22.6 Br 25.8 Found C 39.0 H 4.74 N 22.9 Br 25.9.

2-[[2-(Phenoxy)ethyl]hydrazono]-4,5-dihydro-1H-imidazole (8)

The solution was alkalinized with 2N NaOH. The precipitate was filtered, washed with water and crystallized from acetone.

Yield 40%.- M.p. 152-154 °.- IR (KBr): cm^{-1} 3400-3300; 3300-3000; 1640.- ^{1}H-NMR (DMSO-d_6): 7.60-6.80 (m, 6H, 2-H - 6-H, CH=N); 6.70-6.25 (bs, 2H, 2NH); 4.65 (d, J = 6 Hz, 2H, OCH_2); 3.35 (s, 4H, 2 CH_2 Imid.).- $C_{11}H_{14}N_4O$ (218.3) Calcd. C 60.5 H 6.47 N 25.7 Found C 60.3 H 6.69 N 26.1.

Ethyl 6-[2-(formyl)phenoxy]hexanoate (10)

A suspension of 2-hydroxybenzaldehyde (0.1 moles), ethyl ω-bromohexanoate (0.1 moles) and K_2CO_3 (0.11 moles) in dry acetone (130 ml) was heated at 80° for 3 h under stirring. After cooling, the suspension was filtered and the filtrate was evaporated *in vacuo*. The residue was taken up with 2N NaOH and extracted with ethyl acetate. The org. solution was washed with water, dried (Na_2SO_4), and evaporated *in vacuo*. The obtained oil was purified by column chromatography on silical gel (ethyl acetate/petroleum ether 1:5).- Yield 22%.- IR (film): cm^{-1} 2820; 2720; 1720; 1685.- ^{1}H-NMR ($CDCl_3$): ppm 10.30 (s, 1H, CHO); 7.85 (dd, $J_{3,4}$ = 8 Hz, $J_{3,5}$ = 2 Hz, 1H, 3-H); 7.70-7.40 (m, 1H, 5-H); 7.15-6.90 (m, 2H, 4-H, 6-H); 4.30-4.00 (m, 4H, OCH_2, $COOCH_2$); 2.30 (t, J = 7 Hz, 2H, CH_2COO); 2.05-1.40 (m, 6H, 3 CH_2); 1.20 (t, J = 7 Hz, 1H, CH_3).- $C_{15}H_{20}O_4$ (264.3) Calcd. C 68.2 H 7.63 Found C 68.2 H 7.7.

Hydrazones 13 and 17

Aldehyde **10** (0.01 moles) was added to a solution of aminoguanidine sulphate (0.01 moles) in 2N H_2SO_4 (15 ml) or of 2-hydrazino-2-imidazoline · HBr (0.01 moles) in 2N HBr (15 ml). The mixture was heated at 80° for 4 h under stirring. After cooling the precipitate was filtered, washed with water and crystallized.

6-[2-[[(Aminoiminomethyl)hydrazono]methyl]phenoxy]hexanoic acid hemisulfate (13)

Yield 65%.- M.p. 209-211° from water.- IR (KBr): cm^{-1} 3500-3000; 3000-2700; 1725; 1680.- ^{1}H-NMR (DMSO-d_6): 8.50 (s, 1H, CH=N); 8.10 (dd, $J_{3,4}$ = 8 Hz, $J_{3,5}$ = 2 Hz, 1H, 3-H); 8.00-7.55 (m, 6H, COOH, 2NH, NH_3^+); 7.55-7.25 (m, 1H, 5-H); 7.15-6.90 (m, 2H, 4-H, 6-H); 4.00 (t, J = 7 Hz, 2H, OCH_2); 2.35 (t, J = 7 Hz, 2H, CH_2COO); 2.05-1.30 (m, 6H, 3 CH_2).- $C_{14}H_{20}N_4O_3 \cdot 0.5\ H_2SO_4$ (341.4) Calcd. C 49.3 H 6.20 N 16.4 S 4.7 Found C 49.4 H 6.40 N 16.3 S 4.9.

6-[2-[[(4,5-Dihydro-1H-imidazol-2-yl)hydrazono]methyl]phenoxy]hexanoic acid hydrobromide (17)

Yield 35%.- M.p. 172-175° from acetone.- IR (KBr): cm^{-1} 3450-3300; 3300-2700; 1710; 1650.- ^{1}H-NMR (DMSO-d_6): 12.45-12.25 (bs, 1H, COOH); 8.85-8.65 (m, 2H, NH_2^+); 8.60 (s, 1H, CH=N); 8.10 (dd, $J_{3,4}$ = 8 Hz, $J_{3,5}$ = 2 Hz, 1H, 3-H); 7.60-7.35 (m, 1H, 5-H); 7.25-6.95 (m, 2H, 4-H, 6-H); 4.10 (t, J = 7 Hz, 2H, OCH_2); 3.75 (s, 4H, 2 CH_2 Imid.); 2.25 (t, J = 7 Hz, 2H, CH_2COO); 2.00-1.30 (m, 6H, 3 CH_2).- $C_{16}H_{23}N_4O_3 \cdot HBr$ (400.3) Calcd. C 48.1 H 5.80 N 14.0 Br 20.0 Found C 48.0 H 5.99 N 14.2 Br 20.1.

(E)-Ethyl 6-[[2-(3-oxo-1-butenyl)-3-pyridinyl]oxy]hexanoate (12)

A suspension of NaH (0.01 moles) in dry ethyl ether (10 ml) was added to a solution of dimethyl (2-oxopropyl)-phosphonate (0.01 moles) in dry ethyl ether (35 ml) and the mixture was stirred for 1 h at room temp. A solution of ethyl 6-[(2-formyl-3-pyridinyl)oxy]hexanoate (0.01 moles) (**9**)[6] in dry ethyl ether (45 ml) was added dropwise with stirring at room temp. The mixture was stirred overnight, washed with water, dried (Na_2SO_4), and evaporated *in vacuo*. The *trans*-enone was obtained as a colourless oil and purified by column chromatography on silical gel (ethyl ether/petroleum ether 5:1).- Yield 33%.- M.p. 40-42° from n-hexane.- IR (KBr): cm^{-1} 1730; 1665.- ^{1}H-NMR ($CDCl_3$): 8.30 (m, 1H, 6-H); 8.00 (d, J = 16 Hz, 1H, PyCH=); 7.50-7.20 (m, 3H, 4-H, 5-H, =CHCO); 4.30-4.00 (m, 4H, OCH_2, $COOCH_2$); 2.50-2.20 (m, 5H, $COCH_3$, CH_2COO); 2.10-1.40 (m, 6H, 3 CH_2); 1.25 (t, J = 7 Hz, 3H, CH_3).- $C_{17}H_{23}NO_4$ (305.4) Calcd. C 66.9 H 7.59 N 4.6 Found C 67.0 H 7.36 N 4.4.

(E)-6-[[2-[3-(Aminoiminomethyl)hydrazono]octen-1-yl]pyridinyl]oxy]-hexanoic acid sulfate, dihydrate (14)

A solution of **11**[22] (0.01 moles) and aminoguanidine sulphate (0.01 moles) in N H_2SO_4 (100 ml) was heated at 80° for 4 h under stirring. After cooling, the precipitate was filtered, washed with water, acetone and crystallized from 2N H_2SO_4.- Yield 39%.- M.p. 204-210° dec.- IR (KBr): cm^{-1} 3600-2700; 1720; 1670.- ^{1}H-NMR (DMSO-d_6): 10.95-10.70 (bs, 1H, COOH); 8.30 (d, J = 5 Hz, 1H, 6-H); 8.10-7.30 (m, 14 H, 4-H, 5-H, PyCH=CH, 2NH, NH_3^+, NH^+, $2H_2O$); 4.20 (t, J = 7 Hz, 2H, OCH_2); 2.65 (t, J = 8 Hz, 2H, N=CCH_2); 2.30 (t, J = 7 Hz, 2H, CH_2COO); 2.00-1.10 (m, 12 H, 6 CH_2); 0.90 (t, J = 7 Hz, 3H, CH_3).- $C_{20}H_{31}N_5O_3 \cdot H_2SO_4 \cdot 2\ H_2O$ (523.6) Calcd. C 45.9 H 7.12 N 13.4 S 6.1 Found C 45.5 H 6.76 N 13.8 S 6.1.

(E)-6-[[2-[3-(Aminoiminomethyl)hydrazono]buten-1-yl]pyridinyl]oxy]-hexanoic acid sulfate (15)

A solution of aminoguanidine sulphate (0.01 moles) in 2N H_2SO_4 (250 ml) was added to a solution of **12** (0.01 moles) in ethanol (100 ml) and the mixture was heated at 100° for 4 h under stirring. After cooling the ethanol was evaporated *in vacuo* and the solution was brought to pH ≅ 5 with 2N NaOH. The precipitate was filtered, washed with water, and crystallized from water.- Yield 57%.- M.p. 228-231° dec.- IR (KBr): cm^{-1} 3600-2500; 1720; 1670.- ^{1}H-NMR (DMSO-d_6): 11.10-10.30 (bs, 1H, COOH); 8.50 (d, J = 5 Hz, 1H, 6-H); 8.30-7.30 (m, 10 H, 4-H, 5-H, PyCH=CH, 2NH, NH_3^+, NH^+); 4.35 (t, J = 7 Hz, 2H, OCH_2); 2.65-2.20 (m, 5H, CH_2COO, CH_3); 2.20-1.50 (m, 6H, 3 CH_2).- $C_{16}H_{23}N_5O_3 \cdot H_2SO_4$ (431.5) Calcd. C 44.5 H 5.83 N 16.2 S 7.5 Found C 44.2 H 6.01 N 16.2 S 7.6.

Imidazolinylhydrazones 16, 18, and 19

The carbonyl compound {ethyl 6-[(2-formyl-3-pyridinyl)oxy]hexanoate (**9**)[1], ethyl 6-[[2-(3-oxo-1-octenyl)-3-pyridinyl]oxy]hexanoate (**11**)[22] or ethyl 6-[[2-(3-oxo-1-butenyl)-3-pyridinyl]oxy]hexanoate (**12**)} (0.01 moles) was added to a solution of 2-hydrazino-2-imidazoline · HBr (0.01 moles) in 2N HBr (15 ml). The mixture was heated at 80° for 2 h under stirring. After cooling, the precipitate was filtered, washed with acetone and crystallized.

6-[[2-[[(4,5-Dihydro-1H-imidazol-2-yl)hydrazono]methyl]-3-pyridinyl]-oxy]hexanoic acid dihydrobromide, hydrate (16)

Yield 67%.- M.p. 210-213° from water/acetone.- IR (KBr): cm^{-1} 3600-3340; 3340-2500; 1720; 1640.- ^{1}H-NMR (DMSO-d_6): 9.25 (s, 2H, NH_2^+); 8.70 (s, 1H, CH=N); 8.60 (d, J = 5 Hz, 1H, 6-H); 8.45 (d, J = 9 Hz, 1H, 4-H); 8.05 (dd, $J_{4,5}$ = 9 Hz, $J_{5,6}$ = 5 Hz, 1H, 5-H); 8.00-6.40 (bs, 5H, COOH, NH, NH^+, H_2O); 4.35 (t, J = 7 Hz, 2H, OCH_2); 3.85 (s, 4H, 2 CH_2 Imid.);

2.25 (t, J = 7 Hz, 2H, CH_2COO); 2.00-1.30 (m, 6H, 3 CH_2).- $C_{15}H_{21}N_5O_3 \cdot 2\ HBr \cdot H_2O$ (499.2) Calcd. C 36.1 H 5.05 N 14.0 Br 32.0 Found C 36.1 H 5.09 N 14.2 Br 31.8.

(E)-6-[[2-[3-[(4,5-Dihydro-1H-imidazol-2-yl)hydrazono]octen-1-yl]pyridinyl]oxy]hexanoic acid dihydrobromide, 1.5 H_2O (18)

Yield 50%.- M.p. 166-169° from water.- IR (KBr): cm^{-1} 3550-3400; 3400-3000; 3000-2800; 1710; 1640.- ^{1}H-NMR (DMSO-d_6): 11.80-11.50 (bs, 1H, COOH); 8.60-8.30 (m, 3H, 6-H, NH_2^+); 8.10 (d, J = 9 Hz, 1H, 4-H); 7.70 (dd, $J_{4,5}$ = 9 Hz, $J_{5,6}$ = 5 Hz, 1H, 5-H); 7.60 (d, J = 16 Hz, 1H, PyCH=); 7.40 (d, J = 16 Hz, 1H, CH=); 7.00-6.45 (bs, 5H, NH, NH^+, 1.5 H_2O); 4.30 (t, J = 7 Hz, 2H, OCH_2); 3.80 (s, 4H, 2 CH_2 Imid.); 2.70 (t, J = 8 Hz, 2H, $N{=}CCH_2$); 2.25 (t, J = 7 Hz, 2H, CH_2COO); 2.00-1.20 (m, 12 H, 6 CH_2); 0.85 (t, J = 7 Hz, 3H, CH_3).- $C_{22}H_{33}N_5O_3 \cdot 2\ HBr \cdot 1.5\ H_2O$ (604.4) Calcd. C 43.7 H 6.34 N 11.6 Br 26.4 Found C 43.7 H 6.04 N 11.8 Br 26.4.

(E)-6-[[2-[3-[(4,5-Dihydro-1H-imidazol-2-yl)hydrazono]buten-1-yl]pyridinyl]oxy]hexanoic acid dihydrobromide, dihydrate (19)

Yield 73%.- M.p. 203-206° from water/acetone.- IR (KBr): cm^{-1} 3500-3300; 3300-3100; 3100-2800; 1720; 1640.- ^{1}H-NMR (DMSO-d_6): 11.70-11.40 (bs, 1H, COOH); 8.60-8.35 (m, 3H, 6-H, NH_2^+); 8.10 (d, J = 9 Hz, 1H, 4-H); 7.75 (dd, $J_{4,5}$ = 9 Hz, $J_{5,6}$ = 5 Hz, 1H, H_5); 7.65 (d, J = 16 Hz, 1H, PyCH=); 7.40 (d, J = 16 Hz, 1H, CH=); 6.70-6.05 (bs, 6H, NH, NH^+, $2H_2O$); 4.30 (t, J = 7 Hz, 2H, OCH_2); 3.80 (s, 4H, 2 CH_2 Imid.); 2.35-2.05 (m, 5H, CH_2COO, CH_3); 2.00-1.25 (m, 6H, 3 CH_2).- $C_{18}H_{25}N_5O_3 \cdot 2\ HBr \cdot 2\ H_2O$ (557.3) Calcd. C 38.8 H 5.61 N 12.6 Br 29.7 Found C 38.9 H 5.55 N 12.9 Br 29.3.

Biological tests

Venous blood from healthy volunteers, who had not received any medication for at least two weeks, 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 of the solvent (dimethylsulphoxide) and incubated at 37° for 1 h. Serum was separated by centrifugation and stored at -20° until assayed for TxB_2, the stable metabolite of TxA_2, and for PGE_2, by specific radioimmunoassays. A specific antiserum provided by Professor *C. Patrono* (Catholic University, Rome, Italy) was used for TxB_2 determination, as described[1]; for PGE_2 a commercial kit (NEN, Du Pont, Florence, Italy) was used. No relevant difference was observed in prostanoids synthesis between native and dimethylsulphoxide-added controls.

Aggregation studies were performed on citrated (3.8%, 1/10 v/v) human platelet-rich plasma (PRP): 250 µl of PRP were preincubated for 3 min at 37° in a Born aggregometer (Elvi 840, Elvi Logos, Milan, Italy), and 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, MI USA) were used as platelet activators at the minimal concentration inducing an irreversible aggregation, with more than 50% increase of light transmission[24]. The compounds were tested by adding their solutions to PRP 3 min before addition of the stimulus, in comparison with the solvent.

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