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N-Imidazolyl derivatives of the napthalene and chroman rings as thromboxane A_2 synthase inhibitors

P Cozzi[1], U Branzoli[2*], G Carganico[1**], C Ferti[2], A Pillan[1], D Severino[1], R Tonani[3]

[1]*Cardiovascular Department, Laboratory of Chemistry;*
[2]*Cardiovascular Department, Laboratory of Pharmacology;*
[3]*Computer assisted molecular design (CAMD) Unit, Farmitalia Carlo Erba, Research & Development, Erbamont Group, Via C Imbonati 24, 20159 Milan, Italy*

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Summary — A series of *N*-imidazol-1-yl derivatives of 1,2-dihydronaphthalene, 1,2,3,4-tetrahydronaphthalene, 2H-1-benzopyran and some related compounds were synthesized and tested as inhibitors of thromboxane A_2 synthase in *ex vivo* experiments with orally treated rats. Some compounds showed good activity which was confirmed in experiments *in vitro* in rabbit whole blood. Some structural requirements for significant TxA_2 synthase inhibitory activity are discussed. The selected 5,6-Dihydro-7-(1H-imidazol-1-yl)-2-naphthalene-carboxylic acid (compound **7**) was conformationally analysed using the Sybyl molecular model system in comparison with dazoxiben. Compound **7** was further pharmacologically investigated and on the basis of its interesting activities *in vitro*, *ex vivo* and *in vivo* and its low toxicity was selected for clinical investigation.

Résumé — **Dérivés *N*-imidazolyl du naphthalène et du chromane, inhibiteurs de la thromboxane A_2 synthase.** Une série de dérivés de *N*-imidazol-1-yl 1,2-dihydronaphthalène, 1,2,3,4-tétrahydronaphthalène, 2H-1-benzopyrane et quelques composés apparentés ont été synthétisés et testés *ex vivo* comme inhibiteurs de la synthase du Thromboxane A_2 sur des rats traités par voie orale. Certains de ces composés ont montré une bonne activité, confirmée par des expérimentations *in vitro* sur le sang entier de lapin. Certaines exigences structurales nécessaires pour atteindre une activité élevée sont discutées. L'acide 5,6-dihydro-7-(1H-imidazol-1-yl)-2-naphthalène- carboxilique (composé **7**) a été analysé conformationellement, en utilisant le système de modèle moléculaire Sybyl en comparaison avec le dazoxibène. Le composé **7** a été ultérieurement examiné pharmacologiquement et en considération de ses intéressantes activités *in vitro*, *ex vivo* et *in vivo* et de sa basse toxicité il a été choisi pour l'investigation clinique.

thromboxane A_2 synthase inhibitors / *N*-imidazolyl derivatives / naphthalene derivatives / chroman derivatives / structure–activity relationship

Introduction

Since the discovery of the properties and the role of thromboxane A_2 (TxA_2) by Samuelsson *et al* [1, 2] and Prostacyclin (PGI_2) by Vane *et al* [3–5], the balance between TxA_2 and PGI_2 has been considered important for the maintenance of normal haemostatic function, which was found to be altered in many pathological conditions, such as myocardial infarction [6], unstable angina [7], diabetes [8], circulatory shock [9], and more recently nephropathies [10] including lupus nephritis [11], where the TxA_2 levels are abnormally high.

Moreover, a role for TxA_2 in asthma has been hypothesized on the basis of its bronchoconstrictory activity in experimental animal models [12]. It has been suggested that, since TxA_2 and PGI_2 have the same metabolic precursors, namely prostaglandin endoperoxides (PGG_2, PGH_2), the inhibition of TxA_2 synthase (TxAS) could not only lower elevated TxA_2 levels but also spare the endoperoxide substrate for redirection towards increased synthesis of PGI_2 [13–15].

These facts and hypotheses made us consider TxAS inhibition an important antithrombotic strategy [16] and, beginning with the first agents such as nictindole [17] and imidazole itself [18], a number of compounds, mainly containing the *N*-1-imidazolyl or the 3-pyridyl residue, have been described by various

*Present address: Italfarmaco SpA, Viale F Testi 330, Milan, Italy
**Present address: Laboratorios Menarini, Alfonso XII 587, Badalona, Spain

groups [19–39]. Compounds such as OKY-046, dazoxiben, dazmegrel, CGS-13080, furegrelate (fig 1) and others, have been thoroughly investigated.

More recently, the known fact that endoperoxides themselves show proaggregatory and vasoconstrictory activities similar to, albeit less potent than TxA_2 [40] has received deeper consideration and has been suggested as the main possible explanation of the poor antiplatelet activity shown by known TxA_2 synthase inhibitors (TxASI) [41].

PGH_2 and TxA_2 are thought to share a common receptor which could be more properly defined as the endoperoxide-thromboxane receptor. Therefore the blocking of the PGH_2/TxA_2 receptor can be considered an alternative approach to TxAS inhibition [42].

A definitive conclusion concerning the two approaches has not yet been established, although an association of the inhibitory and antagonistic activities, already proposed in the past [43], has been recently supported by experimental evidence [44]).

More recently, two agents, displaying a weak TxA_2 antagonism in addition to potent TxA_2 synthase inhibition have been reported to show significant antiplatelet activity [45, 46].

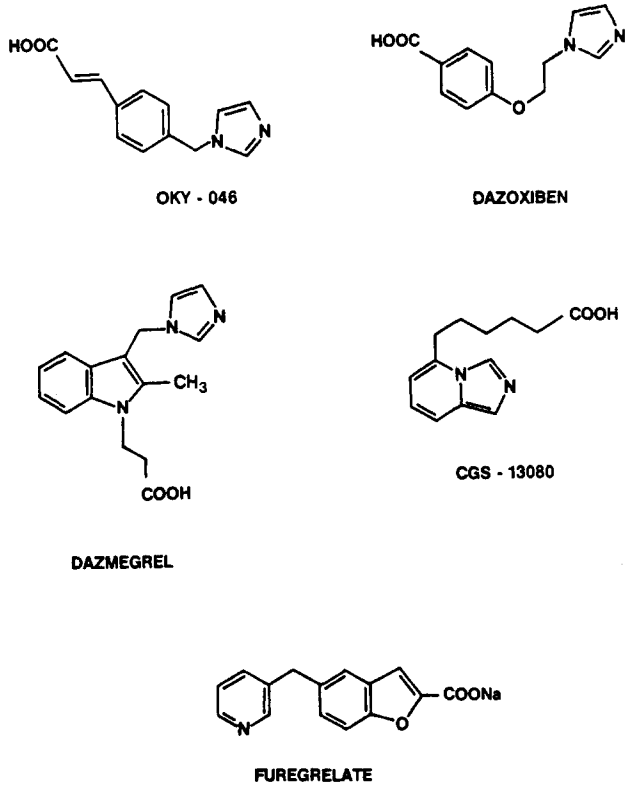


Fig 1.

This paper describes the synthesis and the activity of a series of *N*-1-imidazolyl derivatives of naphthalene and chroman rings [47] and of some related compounds and reports the selection of a compound for further pharmacological evaluation.

Chemistry

The compounds, synthesized mainly according to scheme 1 or from parent compounds prepared in turn following the same scheme, are reported in tables I and II which summarize their physico-chemical properties and their biological activity *ex vivo* in the rat. Some of the intermediate ketones of formula I are known compounds, described by us in previous papers [48, 49], others were prepared following the same procedure.

Ketones II were easily prepared *via* the Mannich bases, 2-dimethylaminomethyl-tetralones and 3-dimethylaminomethyl-chromanones, following a known method [50].

Tetralols and chromanols of formula **III**, some of which are known compounds, were obtained by reduction of Ketones **I** and **II** with $NaBH_4$.

Compounds **IV**, both in the naphthalene and in the chroman series, were prepared in good yield from the intermediate alcohols III by dehydration in a mixture of glacial acetic acid (AcOH) and concentrated sulphuric acid (method A) or in concentrated hydrobromic acid (method B).

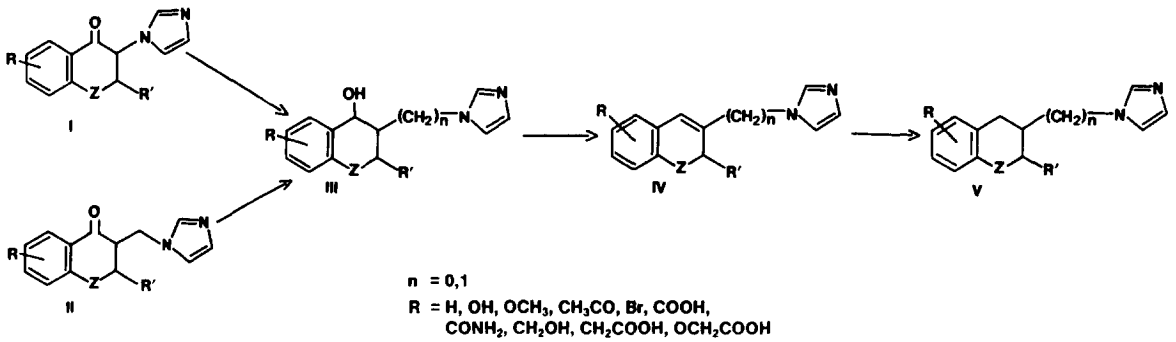
Finally, compounds **V** were obtained from **IV**, in good yield, by hydrogenation over a palladium catalyst (method C).

Some compounds of formula **IV** were prepared by modifying a substituent or by introducing a substituent on the phenyl ring of other compounds of formula **IV**.

Compound **3** was prepared from **2** by acetylation with acetic anhydride followed by Fries rearrangement (method D). The acid **7**, refluxed in ethanol in the presence of gaseous HCl, gave the ester **10** (method E), which in turn was reduced with $LiAlH_4$ to the carbinol **15** (method F). The same acid **7**, refluxed with thionyl chloride gave the corresponding acid chloride, which in turn, by reacting with gaseous ammonia, gave the amide **11** (method G).

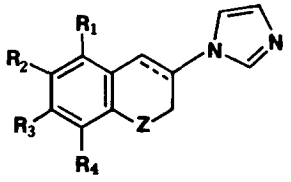
The acid **8** and its amide **12** were obtained by acidic hydrolysis of the corresponding nitrile, prepared in turn from 1,2-dihydro-3-(1H-imidazol-1-yl)-7-bromonaphthalene by bromo-cyanide exchange with CuCN in DMF (method H).

From the carbinol **15**, by reaction with thionyl chloride, subsequent reaction of the chloromethyl derivative with NaCN and acid hydrolysis of the cyanomethyl derivative, the acid **16** was obtained (method I).



Scheme 1.

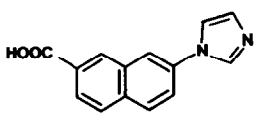
Table I. 1-Imidazolyl derivatives of 1,2-dihydronaphthalene, 1,2,3,4-tetrahydronaphthalene and 2H-1-benzopyran. Physical properties, methods, yields and *ex vivo* TxA_2 synthase inhibitory activity in orally treated rats.



Compd	Z	═	R_1	R_2	R_3	R_4	Formula [a]	mp [b]	Method	Yield [c]	Dose mg/kg p.o.	TxB_2 % change [d] Vs controls	LD_{50} mg/kg [e]
1	CH_2	double bond	H	H	H	H	$C_{13}H_{12}N_2$	oil	A	75	10	− 46 [f]	400-800
2	CH_2	" "	H	OH	H	H	$C_{13}H_{12}N_2O$	218-220	B	68	10	− 3	> 800
3	CH_2	" "	H	OH	CH_3CO	H	$C_{15}H_{14}N_2O_2$	136-140	D	58	10	+ 2	—
4	CH_2	" "	H	OCH_3	H	H	$C_{14}H_{14}N_2O$	63-65	A	80	10	− 53 [f]	> 800
5	CH_2	" "	H	H	OCH_3	H	$C_{14}H_{14}N_2O$	108-110	A	78	10	− 33 [f]	200-400
6	CH_2	" "	Br	OCH_3	H	H	$C_{14}H_{13}BrN_2O$	140-142	A	64	10	− 39 [f]	200-400
7	CH_2	" "	H	COOH	H	H	$C_{14}H_{12}N_2O_2$	323-325	A	71	10	− 75 [f]	> 800
8	CH_2	" "	H	H	COOH	H	$C_{14}H_{12}N_2O_2$	292-295	H	39	10	− 65 [f]	400-800
9	CH_2	" "	H	H	H	COOH	$C_{14}H_{12}N_2O_2$	218-220	A	58	10	− 37 [f]	> 800
10	CH_2	" "	H	COOEt	H	H	$C_{16}H_{16}N_2O_2$	117-119	G	72	50	− 63 [f]	> 800
11	CH_2	" "	H	$CONH_2$	H	H	$C_{14}H_{13}N_3O$	205-210	G	75	50	− 85 [f]	200-400
12	CH_2	" "	H	H	$CONH_2$	H	$C_{14}H_{13}N_3O$	217-219	H	33	10	− 65 [f]	200-400
13	CH_2	" "	H	COOH	OH	H	$C_{14}H_{12}N_2O_3$	265-268	A	77	50	− 27	—
14	CH_2	" "	H	COOH	H	Cl	$C_{14}H_{11}ClN_2O_2$	335-336	A	83	10	− 37 [f]	> 800
15	CH_2	" "	H	CH_2OH	H	H	$C_{14}H_{14}N_2O$	88-91	F	88	10	− 66 [f]	400-800
16	CH_2	" "	H	CH_2COOH	H	H	$C_{15}H_{14}N_2O_2$	155-158	I	50	10	− 46 [f]	> 800
17	CH_2	" "	H	OCH_2COOH	H	H	$C_{15}H_{16}N_2O_3$	206-208	J	70	10	− 30 [g]	> 800
18	CH_2	single bond	H	OCH_3	H	H	$C_{14}H_{16}N_2O$	40-42	C	85	10	− 19 [g]	> 800
19	CH_2	single bond	H	COOH	H	H	$C_{14}H_{14}N_2O_2$	275-276	C	66	10	− 28 [g]	> 800
20	CH_2	single bond	H	H	H	COOH	$C_{14}H_{14}N_2O_2$	262-264	C	75	10	− 26	> 800
21	O	double bond	H	OCH_3	H	H	$C_{13}H_{12}N_2O_2$	104-106	A	62	10	− 28 [g]	400-800
22	O	double bond	H	COOH	H	H	$C_{13}H_{10}N_2O_3$	> 290	A	64	10	− 17	> 800
Benzylimidazole		double bond									10	− 39 [f]	

[a]All compounds were analyzed for C, H, N and Br when present; analytical results were within ± 0.4% of the theoretical values; [b]Most of the compounds were purified by chromatographic column separation; compounds **5** and **10** were crystallized, respectively, from ethylacetate and cyclohexane; [c]No effort was made to optimize yields; [d]Groups of 10 rats were used to test all products; TxB_2 assayed on serum by RIA; [e]Groups of 4 mice for each dose were used; LD_{50} was calculated 7 days after dosing; [f]Statistically highly significant ($P \leq 0.01$) according to Dunnett's test; [g]Statistically significant ($P \leq 0.05$) according to Dunnett's test.

Table II. Analogues of 1-imidazolyl derivatives of table I substituted by carboxy group on the phenyl ring. Physical properties, methods, yields and *ex vivo* TxA_2 synthase inhibitory activity in orally treated rats.



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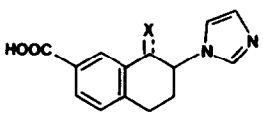

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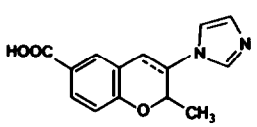

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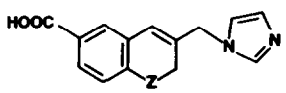

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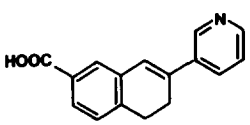

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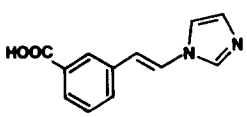

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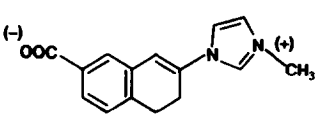

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Compd	Z	—	X	Formula [a]	mp [b]	Method	Yield [c]	Dose mg/kg p.o.	TxB_2 % change [d] Vs controls	LD_{50} mg/kg [e]
23				$C_{14}H_{10}N_2O_2$	292-295	K	48	50	− 77 [f]	> 400
24			C=O	$C_{14}H_{12}N_2O_3$	>290	j	78	10	+ 15	—
25			CHOH [h]	$C_{14}H_{14}N_2O_3 \cdot HCl$	240-242	j	87	50	− 28 [g]	> 800
26		double bond		$C_{14}H_{12}N_2O_3$	225-227	A	82	10	− 90 [f]	200-400
27		single bond [i]		$C_{14}H_{14}N_2O_3 \cdot HCl$	231-235	C	78	10	+ 2	> 800
28	CH_2	double bond		$C_{15}H_{16}N_2O_2$	155-158	A	83	50	− 80 [f]	> 800
29	O	double bond		$C_{14}H_{12}N_2O_3$	258-259	A	60	50	− 90 [f]	—
30				$C_{16}H_{13}NO_2$	255-257	A	70	50	− 77 [f]	25-50
31				$C_{13}H_{10}N_2O_2$	231-233	L	65	10	− 16	—
32				$C_{15}H_{16}N_2O_2$	267-269	M	51	50	+ 5	—
Benzylimidazole								10	− 39 [f]	

[a,c,d,e,f,g]See the corresponding footnotes in table I; [b]Most of the compounds were purified by chromatographic column separation; compounds **23** and **31** were crystallized from 95% ethanol; [h]*Cis-trans*-mixture; [i]*Cis*; [j]See [47].

The oxyacetic derivative **17** was obtained from compound **2** by reaction with ethyl bromoacetate and subsequent alkaline hydrolysis (method J).

Compounds **23**, **30**, **31** and **32** are not included in the formulae **I-IV** of scheme 1.

Compound **23** was obtained by aromatization of the ester **10** with elemental sulfur and subsequent alkaline hydrolysis of the ester group (method K). The 3-pyridyl analogue **30** was prepared by modification of a sequence of synthesis described for a parent compound [51], starting from β-bromoethylbenzene and ethyl 3-pyridylacetate and through the final dehydration of the intermediate 5,6,7,8-tetrahydro-8-hydroxy-7-(3-pyridyl)-2-naphthalenecarboxylic acid.

The styryl analogue **31** was prepared by dehydration of the corresponding benzylic alcohol with thionyl chloride and KOH (method L). The betaine **32** was obtained from **7** by exhaustive methylation with methyl iodide (method M).

Results and discussion

The compounds reported in tables I and II were evaluated as regards serum TxB_2, the stable metabolite of TxA_2, in rats orally treated as described in *Experimental Protocols*.

The compounds are structurally related to previously reported [49] hypolipidaemic *N*-imidazolyltetralones, *N*-imidazolylchromanones and corresponding alcohols, but did not show any activity on blood lipids.

Most of the compounds bear a carboxy group, an ester or an amide of it, on the phenyl moiety of the condensed rings.

This stems from the fact that our compounds were explicitly designed as possible conformationally restricted analogues of known TxAS inhibitors (TxASI) and among the latter a particular role for a terminal carboxy group, both as regards the potency [(20, 27] and the selectivity [29, 30], was reported.

As an example, our selected compound **7** (FCE 22178) can be considered a possible conformationally restricted analogue of compound **33** described by Iizuka *et al* (21) or, more faintly, of *N*-carboxyeptylimidazole **34** described by Yoshimoto *et al* [20] (fig 2).

The activities reported in tables I and II, as variations of TxB_2, even though expressed by single dose experiments, enable us to make some preliminary observations about the possible structure–activity relationship. If we first consider the compounds reported in table I, the chroman derivatives unexpectedly fail to show the activity of corresponding naphthalene analogues (**21** vs **4**, **22** vs **7**). It must be noted that compound **22** apparently presents the same kind of conformational analogy towards dazoxiben [29] as that of compound **7** towards compound **33** (fig 2). In the naphthalene series, esters and amides maintain the activity of the corresponding acids (**11**, **10** and **7**; **12** and **8**).

The presence of a phenolic hydroxy group seems to play a negative role (**2** *vs* **1**, **4** and **7**, **13** *vs* **7**). On the other hand the carbinol **15** maintains an activity similar to that of acid **7** and its carbonyl derivatives.

The distance between the carboxy group or its equivalent groups and imidazole seems to play a role, since compounds where the two radicals are closer (**9**) or farther apart (**16, 17**) are less active than those in which the distance is intermediate (**7** and its derivatives).

The saturation of vinylic 1,2 double bond of the naphthalene ring, giving racemic compounds, significantly lowers the activity (**18** *vs* **4**, **19** *vs* **7**).

The compounds of table II constitute a heterogeneous group having in common a carboxy group in the same or equivalent position on the phenyl ring.

Naphthyl derivative **23**, fully aromatic analogue of **7**, shows good activity. Compounds **24** and **25**, with an oxygenated function in the benzylic position adjacent to imidazole, have no activity. Compound **26** shows significant activity in contrast to the activity of its chromene analogue **22** and of the corresponding saturated derivative **27**. Compounds **28** and **29**, homologues of **7** and **22**, both show good activity, irrespective of the chroman or naphthalene nature of the condensed rings.

As could be expected, compound **30**, a close 3-pyridyl analogue of **7**, shows significant activity, associated on the other hand with marked toxicity.

The open styryl derivative **31** is inactive despite the fact that its *trans* configuration makes it a relatively close analogue of the cyclic compound **7**, thus underlying the role of the substantially rigid and planar naphthalene frame. Not surprisingly, the betaine **32**, where N-3 lone pair is no more available, is fully inactive.

Some of the compounds most active *ex vivo*, together with two close analogues of compound **7** which are practically inactive (**13, 22**), were tested *in vitro* in rabbit whole blood (table III) as reported in *Experimental Protocols*.

On the whole, the trend of activity which emerged *ex vivo* was confirmed and acids **7** and **8** and the carbinol **15** resulted the best of the series.

Moreover, table III shows that the inactivity of **13** and **22** is a matter of pharmacodynamics and not simply of pharmacokinetics as could be hypothesized from *ex vivo* data.

Data of *ex vivo* experiments (table I) suggest a relationship between the activity and the distance imidazole-carboxylic group as reported for other TxASI [20, 21, 29].

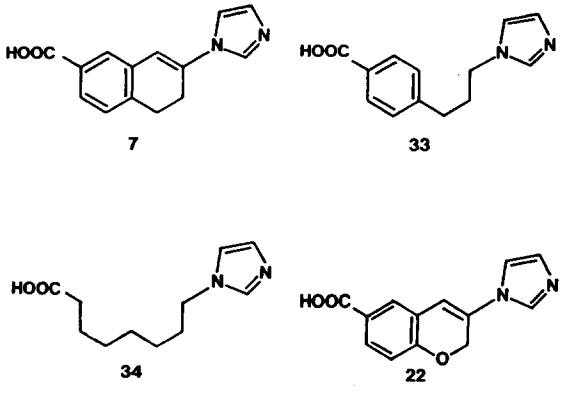

Fig 2.

Table III. *In vitro* TxA_2 synthase inhibitory activity in rabbit whole blood.

No.	Activity: % change vs controls[a]	
	0.36 μg/ml	0.067 μg/ml
7	− 76.5[b]	− 46.2[b]
8	− 52.3[b]	− 41.8[c]
11	− 52.2[b]	− 29.1
13	− 16.1	− 8.4
14	− 42.9[c]	− 24.3
15	− 56.7[b]	− 44.1[c]
16	− 27.3	− 17.7
19	− 50.6[b]	− 26.2
22	− 3.7	+ 12.7
28	− 30.3	− 29.4
Dazoxiben	− 69.9[b]	− 33.5[c]

[a]Each value is the mean of six experiments; TxB_2 was assayed on plasma by RIA; [b]Statistically highly significant ($P \leq 0.01$) according to Dunnett's test; [c]Statistically significant ($P \leq 0.05$) according to Dunnett's test.

The hypothesis currently accepted is that TxSI can interfere, by the lone pair either of pyridine nitrogen or of imidazole N-3 nitrogen, with the heme moiety of the cytochrome P-450 that constitutes the enzyme TxA_2 synthase [52], thus competing at this site with the oxygenated ring of the endoperoxides. Analogously the terminal carboxy group of many TxSI could mimic that of the acidic chain of endoperoxides [28, 29].

Preferred conformations for compound **7** were calculated using the SYBYL molecular modelling system [53, 54] and the distances between the N-3 and the carbon of carboxy group were measured for conformers within 15 Kcal mol^{-1} of the minimum.

A range of 8.95–9.45 Å was found, which falls within the broader range 8.5–10 Å calculated by different authors for the same distance in potent TxASI.

The superimposition of the conformations of our compound **7** with dazoxiben was made by using the SYBYL software. The molecules were built with a dummy atom, mimicking the heme iron, at a distance of 2.8 Å along the direction of the lone pair of imidazole N-3 nitrogen. The datum-point constituted by the dummy atom allows a better definition of the spatial reciprocal positions of the two anchoring groups, N-3 and carboxy. We found two different ways of superimposing N-3, carboxy and dummy atom which are reported in figure 3a.

The distance between N-3 and the carboxy atom are of 9.0 and 9.4 Å for the two models and the corresponding distances between the dummy atom and the carboxy carbon are 10.8 and 12.1 Å respectively.

Figure 3a shows that, if the two anchoring groups of the two products must be recognised on the basis of their 3-dimensional arrangement, the other parts of their molecules must be in different orientation in the enzyme active site pocket. This is confirmed by the fact that the best superimposition (RMS = 0.29) of the heavy atoms (C, N, O) of the two compounds, calculated by us, corresponds to an unfavourable conformation of dazoxiben, resulting in 42,6 kcal/mole above the minimum found after a complete conformational analysis.

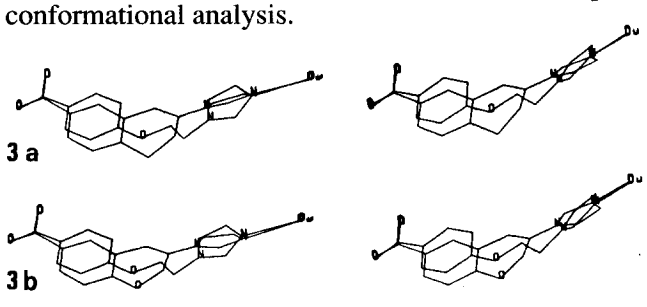

Fig 3. a: Superimposition of molecules of compound **7** and dazoxiben; b: superimposition of molecules of compound **22** and dazoxiben.

Analogously we compared compound **22**, the inactive chromene analogue of **7**, with dazoxiben, by the method of N-3, carboxy and dummy atom superimposition (fig 3b). As could be expected, we found for **22** the same two conformations reported in figure 3a for compound **7**. In these two models the distances between the oxygen of chromene ring of **22** and the oxygen on the phenyl ring of dazoxiben were found to be 1.2 and 1.3 Å respectively.

Therefore, a plausible reason for the inactivity of **22** could be that the oxygen of the chroman ring alters the lipophylicity of the moiety of the molecule (position 3, 4, 5 and 6 in the naphthalene ring of compound **7**) deputated [28] to non bonded hydrophobic binding with a specific region inside the active site of the enzyme. This would not be the case for dazoxiben due to the remarkably different positioning of its oxygen.

Interestingly, compound **26**, where a methyl group α to the oxygen of the chroman ring can restore the local hydrophobicity, shows significant inhibitory activity.

Compounds **7** and **15** were tested in the rabbit as regards the prevention of the mortality induced by arachidonic acid (AA) (table IV) as reported in *Experimental Protocols*. Both compounds showed significant prevention of the mortality.

Compound **7** (FCE 22178), whose selectivity for TxA_2 synthase as against cyclooxygenase can be inferred from the increase of production of PGE_2 along with the decrease of that of TxA_2 (fig 4), was selected for further pharmacological investigation on the basis of its activities and low toxicity (LD_{50} > 5 000 mg/kg, single oral dose in the mouse).

FCE 22178 showed in normal rats and in two models of glomerular disease, characterized by enhanced renal TxB_2 levels, an inhibitory effect on glomerular TxB_2 production markedly greater than on platelets [55].

The compound is now undergoing phase II clinical trials in patients with diabetic nephropathy [56] and will be the subject of future papers.

Table IV. Prevention of the mortality induced by *iv* administration of arachidonic acid in the rabbit.

No.	ED_{50} [a] (mg/kg)
7	2.4 (1.4 - 4.1)
15	0.66 (0.40 - 1.07)
ASA	8 (7.0 - 9.2)
Dazoxiben	1.5 (1.0 - 2.2)

[a]Groups of 12 animals treated by gavage.

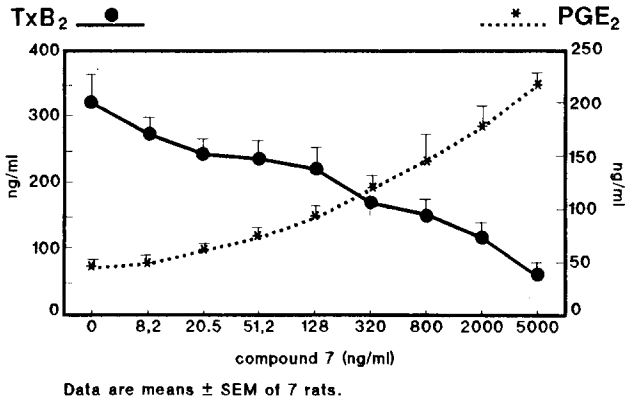

Fig 4. Effect of compound **7** on thromboxane B_2 and prostaglandin E_2 synthesis *in vitro* in MNS rats. Data are means ± SEM of 7 rats.

Experimental protocols

Chemistry

Melting points were determined in open glass capillaries with a Buchi melting point apparatus and are uncorrected. Elemental analyses were performed on a Carlo Erba 1106 instrument and where analytical results are indicated only by symbols of the elements, they were within ± 0.4% of theoretical values. ^1H NMR spectra for all the compounds described were recorded on a Bruker HX 90 instrument with tetramethylsilane as the internal standard and chemical shifts are expressed in parts per million (δ). IR spectra were recorded on a Perkin–Elmer 683 instrument and frequencies are expressed in reciprocal cm. Column Chromatographic separations were performed by the flash technique on 40.60 μm silica gel (Merck No 9385). Of the compounds used as references in pharmacological tests, dazoxiben was prepared according to the literature [29], *N*-benzylimidazole was purchased from Janssen Chimica, Beerse (Belgium) and ASA was furnished by Carlo Erba Reagenti, Milan (Italy).

Method A
5,6-Dihydro-7-(1H-imidazol-1-yl)-2-naphthalenecarboxylic acid 7
A solution of 5,6,7,8-tetrahydro-8-hydroxy-7-(1H-imidazol-1-yl)-2-naphthalenecarboxylic acid; *cis-trans* mixture **25** [47] (43.17 g, 0.167 mol), glacial AcOH (400 ml) and concentrated H_2SO_4 (50 ml) was heated at 100°C for 4 hours, under stirring. The reaction mixture was poured into ice–water (500 ml) and the pH was adjusted to neutrality by adding 35% NaOH. The precipitate was collected, filtered and washed with water, giving 28.49 g (71%) of **7**; mp = 323–325°C, NMR (DMSO–d_6) δ 2.97 (4H, m, CH_2 CH_2), 6.96 (1H, br s, **CH**=C-N), 7.07 (1H, br s, imidazole H^4), 7.28 (1H, d, phenyl H^4), 7.70 (3H, m, imidazole H^5 + phenyl H^1 and H^3), 8.19 (1H, br s, imidazole H^2); $IR_{(C=O)}$ (KBr) 1685 cm^{-1}. Anal $C_{14}H_{12}N_2O_2$ (C, H, N).

Method B
5,6-Dihydro-7-(1H-imidazol-1-yl)-2-naphthalenol 2
A solution of trans 1,2,3,4-tetrahydro-2-(1H-imidazol-1-yl)-7-methoxy-1-naphthalenol [49] (5 g, 0.02 mol) and concentrated HBr (100 ml) was refluxed for 8 h. The solution was poured into ice-water and the pH was made alcaline with $NaHCO_3$. The solid precipitated was filtered, washed with water and dried under vacuum. Purification of the crude product by flash chromatography on silica gel (eluant $CHCl_3$:CH_3OH = 180:20) furnished 2.89 g (68%) of **2**; mp = 218–220°C, NMR (DMSO–d_6) δ 2.82 (4H, m, CH_2 -CH_2), 6.61 (1H, br s, CH=C-N), 6.54–8.13 (6H, m, aromatics). Anal $C_{13}H_{12}N_2O$ (C, H, N).

Method C
(±)5,6,7,8-Tetrahydro-7-(1H-imidazol-1-yl)-2-naphthalene-carboxilic acid 19
A mixture of **7** (13 g, 0.054 mol), palladium 10% on activated carbon (2.2 g), 95% ethanol (300 ml), glacial AcOH (100 ml) and concentrated HCl (15 ml) was hydrogenated for 12 h at room temperature in a Parr-Burgess apparatus at an initial pressure of 50 psi. The catalyst was filtered off and the solution was evaporated to half the volume and neutralized with 20% NaOH. The precipitate was filtered, washed with water and then dried under vacuum. Flash-chromatography on silica gel, eluant $CHCl_3$-CH_3OH-AcOH (100:20:1), furnished 8.63 g (66%) of **19**; mp = 274–275°C; NMR (DMSO–d_6) δ 2.00–2.30 (2H, m, CH_2-CH_2-CH), 2.75–3.30 (4H, m, CH_2 benzylic), 4.30–4.80 (1H, m, CH_2-*CH*-CH_2), 6.92–7.90 (6H, m, aromatics). Anal $C_{14}H_{14}N_2O_2$ (C, H, N).

Method D
3-Acetyl-5,6-dihydro-7-(1H-imidazol-1-yl)-2-naphthalenol 3
Acetic anhydride (10 ml, 0.106 mol) was cooled in an ice-bath, with stirring, and **2** (2.6 g, 0.0122 mol) was added portionwise. The reaction mixture was stirred at room temperature for 2 h. The excess of AC_2O was evaporated under vacuum and the residue taken up with H_2O and AcOEt. The aqueous solution was extracted with AcOEt and the combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated, giving 2.4 g of acetyloxy derivative, as an oil. The latter was treated, without further purification, with $AlCl_3$ (2.76 g, 0.02 mol) and the mixture was heated at 165°C, under vigorous stirring, for 5 h. The reaction product was carefully decomposed with ice (50 g) and concentrated HCl (2 ml) and the resulting solution was neutralized with 20% NaOH and extracted with $CHCl_3$. The organic layer was separated, washed with brine, dried over $CaCl_2$ and evaporated under vacuum, giving 2.31 g of crude product. Flash chromatography purification, eluant $CHCl_3$–CH_3OH (100:3), furnished 1.8 g (58% from **2**) of **3**; mp = 136–140°C; NMR ($CDCl_3$) δ 2.60 (3H, s, $CH_3$3CO), 2.93 (4H, m, CH_2 -CH_2), 6.51 (1H, br s, *CH*=C-N), 6.70 (1H, s, phenyl H^1), 7.16 and 7.28 (2H, imidazole H^4 and H^5), 7.47 (1H, br s, phenyl H^4), 7.89 (1H, br s, imidazole H^2), 12.33 (1H, br s, OH). Anal $C_{15}H_{14}N_2O_2$ (C, H, N).

Method E
Ethyl 5,6-dihydro-7-(1H-imidazol-1-yl)-2-naphthalene carboxylate 10
Absolute ethanol (14.4 ml) was added slowly to $SOCl_2$ (2.2 ml, 0.029 mol) at 0°C, the mixture was heated to room temperature and **7** (7 g, 0.029 mol) was added portionwise. The reaction mixture was refluxed for 1 d and then stirred overnight at room temperature. The solvent and the excess of $SOCl_2$ were evaporated under vacuum and the residue was chromatographed on silica gel, eluant $CHCl_3$-CH_3OH (50:5), to give 5.60 g (72%) of **10**; mp = 113–116°C; NMR ($CDCl_3$) δ 1.62 (3H, t, CH_3-CH_2), 2.80–3.40 (4H, m, CH_2-CH_2), 4.39 (2H, q, CH_3-CH_2), 6.92 (1H, br s, *CH*=C-N), 7.28–8.00 (6H, m, aromatics). Anal $C_{18}H_{18}N_2O_2$ (C, H, N).

Method F

5,6-Dihydro-7-(1H-imidazol-1-yl)-2-naphthalenemethanol ***15***

A solution of **10** (2.7 g, 0.01 mol) in dry THF (50 ml) was added dropwise at 0°C to a stirred suspension of $LiAlH_4$ (0.208 g, 0.0055 mol) in dry tetrahydrofuran under nitrogen atmosphere. The reaction was refluxed for 1 d and then stirred overnight at room temperature. H_2O (40 ml) was added and the precipitate formed filtered off. The filtrate was concentrated, extracted with $CHCl_3$, dried (Na_2SO_4) and evaporated under vacuum to give 2 g (88.5%) of **15**; mp = 88–91°C; NMR ($CDCl_3$) δ 2.80 (4H, m, CH_2-CH_2), 4.60 (2H, s, *CH*$_2$ OH), 5.26 (1H, s, OH), 6.40 (1H, s, *CH*=C-N), 7.08–7.63 (6H, m, aromatics). Anal $C_{14}H_{14}N_2O$ (C, H, N).

Method G

5,6-Dihydro-7-(1H-imidazol-1-yl)-2-naphthalenecarboxamide ***11***

A suspension of **7** (0.5 g, 0.0021 mol) in DMF (10 ml) was treated with $SOCl_2$ (2 ml, 0.027 mol) and then cooled in an ice bath; gaseous NH_3 was passed through the reaction mixture, with stirring, for 5 h. The reaction mixture was stirred for a further 12 h at room temperature. The ammonium salt was filtered off and ether was added giving a precipitate which, chromatographed on silica gel, eluant $CHCl_3$-CH_3OH-AcOH (45:5:2.5), furnished 0.37 g (75%) of **11**; mp = 205–210°C; NMR (DMSO–d_6) δ 2.94 (4H, m, CH_2CH_2), 6.85 (1H, s, *CH*=C-N), 7.06 (1H, s, imidazole H^4), 7.24 (1H, d, phenyl H^4), 7.28 (1H, s, imidazole H^5), 7.65 (1H, dd, phenyl H^3), 7.67 (2H, m, imidazole H^2 + one amidic NH), 7.90 (1H, br s, one amidic NH), 8.14 (1H, br s, phenyl H^1); $IR_{(C=O)}$ (KBr) 1670 cm^{-1}. Anal $C_{14}H_{13}N_3O$ (C, H, N).

Method H

7,8-Dihydro-6-(1H)-imidazol-1-yl)-2-naphthalenecarboxilic acid ***8*** *and 7,8-dihydro-6-(1H-imidazol-1-yl)-2-naphthalene-carboxamide* ***12***

A mixture of 1-(6-bromo-3,4-dihydronaphthalen-2-yl)-1H-imidazole (4 g, 0.0146 mol, mp = 107–110°C, prepared in a four-step synthesis from the known 6-bromo-1-tetralone), CuCN (1.62 g, 0.018 mol) and DMF was refluxed for 6 h. The reaction mixture was added to a solution of $FeCl_3$ (6 g, 0.037 mol) in H_2O (10 ml) and concentrated HCl (2 ml) and the mixture was maintained at 60°C for 30 min. A dark precipitate was filtered off, water (30 ml) was added and the pH of the solution was adjusted to neutrality with 10% NaOH. The light brown precipitate was filtered, washed with water and dried under vacuum, giving 2.91 g of crude 7,8-dihydro-6-(1H-imidazol-1-yl)-2-naphthalenecarbonitrile. The latter was refluxed in 23% HCl (30 ml) for 8 h. The solution precipitate was filtered, washed with water and dried. Flash chromatography on silica gel, eluant $CHCl_3$-CH_3OH (180:20), furnished two products: at R_f = 0.30, 1.36 g (39%) of **8**; mp = 290–293°C; NMR (DMSO–d_6) δ 3.04 (4H, m, CH_2-CH_2), 7.17 (1H, s, *CH*=C-N), 7.72–9.38 (6H, m, aromatics). Anal $C_{14}H_{12}N_2O_2$ (C, H, N); at R_f = 0.26, 1.15 g (33%) of **12**; mp = 217–219°C; NMR (DMSO–d_6) δ 2.97 (4H, m, CH_2-CH_2), 6.91 (1H, s, *CH*=C-N), 7.18–7.90 (6H, m, aromatics). Anal $C_{14}H_{13}N_3O$ (C, H, N).

Method I

5,6-Dihydro-7-(1H-imidazol-1-yl)-2-naphthaleneacetic acid ***16***

A suspension of **15** (10 g, 0.044 mol) in pyridine (3.88 ml, 0.044 mol) and CH_2Cl_2 (70 ml) was cooled to 0°C and $SOCl_2$ (4.78 ml, 0.064 mol) was added dropwise. The reaction mixture was refluxed 3 h. Water (100 ml) was added and the pH was adjusted to neutrality with 10% NaOH. The organic layer was separated and the aqueous solution extracted with CH_2Cl_2 (3 x 70 ml). The combined organic phases were washed with water, dried over $CaCl_2$ and evaporated under vacuum, giving 9.35 g of crude 1-(7-chloromethyl-3,4-dihydro-2-naphthalenyl)-1H-imidazole, mp = 180–188°C. The latter was added to a solution of NaCN (3.04 g, 0.064 mol) in H_2O (10 ml) and ethanol (20 ml). The reaction mixtures were heated, under stirring, at 100°C for 3 h. The organic solvent was evaporated under vacuum and the residue taken up with $CHCl_3$ (100 ml) and H_2O (100 ml). The aqueous solution was extracted with $CHCl_3$ and the combined organic layers dried over $CaCl_2$. Evaporation under vacuum of the solvent gave 7.13 g of crude 5,6-dihydro-7-(1H-imidazol-1-yl)-2-naphthaleneacetonitrile, which, without further purification, was hydrolized with 23% HCl, in the same manner described for **8**. The crude reaction product was chromatographed on silica gel, eluant $CHCl_3$-CH_3OH (7:1), giving 5.6 g (50% from **15**) of **16**; mp = 155–158°C; NMR (DMSO–d_6) δ 2.88 (2H, t, CH_2-CH_2-C-N), 3.30 (2H, t, CH_2-CH_2-C-N), 3.50 (2H, s, *CH*$_2$COOH), 6.80 (1H, s, *CH*=C-N), 7.04–8.12 (6H, m, aromatics). Anal $C_{15}H_{14}N_2O_2$ (C, H, N).

Method J

5,6-Dihydro-7-(1H-imidazol-1-yl)-2-naphthalenyl-oxyacetic acid ***17***

A mixture of **2** (6 g, 0.028 mol), potassium *tert*-butoxide (3.8 g, 0.34 mol), ethyl α-bromoacetate (3.14 ml, 0.028 mol) and *tert*-butanol (60 ml) was refluxed for 3 h. The solvent was evaporated under vacuum and the residue taken up with CH_2Cl_2 and H_2O. The organic layer was separated and the aqueous solution extracted with CH_2Cl_2. The combined organic layers were washed with 3% HCl and brine and then dried over $CaCl_2$. The solvent was evaporated and the crude product chromatographed on silica gel, eluant CH-Cl_3-CH_3-OH (180:20), giving 7.04 g of ethyl (5,6-dihydro-7-(1H-imidazol-1-yl)-2-naphthalenyl) oxyacetate, oil. The latter was hydrolized by refluxing with N/2 methanolic KOH solution (150 ml) for 4 h. The solvent was evaporated and the product taken up with H_2O (200 ml). The solution was acidified with AcOH and the precipitate was filtered, washed with H_2O and dried under vacuum, giving 5.3 g (70%) from **2** of **17**; mp = 206–208°C; NMR (DMSO–d_6) δ 2.85 (4H, m, CH_2CH_2), 4.65 (2H, s, CH_2COOH), 6.80 (1H, br s, *CH*=C-N), 6.68–81.5 (6H, m, aromatics); $IR_{(C=O)}$ (KBr) 1735 cm^{-1}. Anal $C_{15}H_{14}N_2O_3$ (C, H, N).

Method K

7-(1H-Imidazol-1-yl)-2-naphthalenecarboxylic acid ***23***

A mixture of **10** (5 g, 0.019 mol) and finely powdered elemental sulfur (20 g) was heated, under vigorous stirring, at 130°C for 8 h. The mixture was cooled and taken up with AcOEt (100 ml) and the sulfur was filtered off and washed with AcOEt. The filtrates were extracted with 8% HCl and the acidic solution was neutralized with 10% NaOH and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The residue was chromatographed on silica gel, eluant AcOEt + 0.25% of concentrated NH_4OH, giving 2.67 g of ethyl 7-(1H-imidazol-1-yl)-2-naphthalencarboxylate which was converted to the free acid by hydrolysis in N/2 methanolic KOH (100 ml). Usual work-up (see *Method J*) and crystallization from 95% EtOH furnished 2.17 g (48% from **10**) of **23**; mp = 292–295°C; NMR (DMSO–d_6) δ 7.17 (1H, br s, imidazole H^4), 7.92 (1H, br s, imidazole H^5), 8.00 (2H, m, H^3 and H^6), 8.08 and 8.18 (2H, two doublets, H^4 and H^5), 8.42 (2H, m, H^1 and H^8), 8.63 (1H, s, imidazole H^2). Anal $C_{14}H_{10}N_2O_2$ (C, H, N).

Method L

Trans-*3-(2-(1H-Imidazol-1-yl)-vinyl)benzoic acid* ***31***

Thionyl chloride (50 ml, 0.69 mol) was cooled to 0°C and 3-(1-hydroxy-2-(1H-imidazol-1-yl)ethyl)benzoic acid (mp = 170–173°C, prepared from known 3-(2-bromo-1-oxo)ethylbenzoic acid) (2 g, 0.0086 mol) was added portionwise with vigorous stirring. The mixture was refluxed for 1 h and the excess of $SOCl_2$ was evaporated under vacuum. The gummy residue was dissolved in N/2 methanolic KOH (150 ml) and the solution stirred at room temperature for 16 h. The precipitate was filtered off and the alcoholic solution evaporated to dryness. The residue was taken up with H_2O (25 ml) and the pH was adjusted to 6.5 with 23% HCl. The precipitate was filtered, washed with water and dried under vacuum. Crystallization from EtOH furnished 1.2 g (65%) of **31**; mp = 231–233°C; NMR (CD_3COCD_3) δ 7.06 (1H, dd, imidazole H^4), 7.08 (1H, d, J = 14.7 Hz, *CH*=C-N), 7.50 (1H, dd, H^5), 7.60 (1H, dd, imidazole H^5), 7.75 (1H, ddd, H^4), 7.94 (1H, ddd, H^5), 7.95 (1H, d, J = 14.7 Hz, CH=*CH*-N), 8.28 (2H, m, H^2 and imidazole H^2); $IR_{(C=O)}$ (KBr) 1680 cm^{-1}. Anal $C_{12}H_{10}N_2O_2$ (C, H, N).

Method M

1-(7-Carboxy-3,4-dihydro-2-naphthalenyl)-3-methyl-1H-imidazolium hydroxide inner salt ***32***

A mixture of **7** (1.6 g, 0.0066 mol), methyl iodide (0.82 ml, 0.013 mol), KOH (1.12 g, 0.02 mol), EtOH (75 ml) and H_2O (7.5 ml) was refluxed for 6 h. The solvent was evaporated under vacuum and the residue was chromatographed on silica gel, eluant $CHCl_3$-CH_3OH-CH_3COOH (90:10:5), giving 0.86 g (51%) of **32**; mp = 267–269°C, hygroscopic; NMR (D_2O) δ 2.88–3.10 (4H, m, CH_2CH_2), 4.09 (3H, s, CH_3), 6.94 (1H, br s, *CH*=C-N), 7.34–8.99 (6H, m, aromatics). Anal $C_{15}H_{14}N_2O_2$ (C, H, N).

Molecular modelling

The molecular mechanics energy calculations were determined by the standard parameters of the SYBYL software package. The structures were built using normal values of bond lengths and angles and the energy was then minimized with a combination of SIMPLEX and Newton-Raphson algorithm. The conformational analyses of **7**, **22** and dazoxiben were performed allowing the rotation of all the free-to-move tortional angles, excluding the carboxyl group which was taken as planar with the phenyl group. Compounds **7** and **22** were analysed with an angle increment of 5°, excluding all the conformational energies above 15 kcal/mol and measuring the distances between the nitrogen of the dummy atom and the carboxyl group. For the dazoxiben, comprising up to 4 rotatable bonds, we used an angle increment of 15° (which gives rise to 331 776 theoretical conformations) and energy cut of 15 kcal/mol and the distance constraints previously obtained for the pharmacophoric groups of **7**. The conformations obtained for the two couples of compounds were then fitted together using as reference points the N3, dummy atom and carboxylic group. The best matching molecules in terms of root mean square (RMS), are those reported in figure 3a (RMS = 0.10) and 3b (RMS = 0.16).

Pharmacology

In vitro *experiments*

Male New Zealand White rabbits (Charles River, Italy) weighing about 3 kg and fasted for 22 h were used. The blood (20 ml) was withdrawn from the intermedial auricular caudal artery of the animal in steryl disposable syringe containing 3.15% sodium citrate (ratio 1:9) and was divided into portions of 3 ml. Products or vehicle were added to each portion at the required concentration in a volume of 30 µl. Samples were incubated for 10 min at 37°C. Collagen was then added at the final concentration of 1 µg/ml for stimulating membrane phospholipase and activating arachidonic acid metabolism. The samples were incubated for 10 additional min at 37°C and then the plasma was separated by centrifugation at 3000 rpm for 15 min, collected and stored at –20°C until tested. Each value is the mean of 6 experiments.

The selectivity of compounds for TxA_2 synthase was assayed *in vitro* by evaluating the production of TxB_2 and PGE_2 in clotting blood from male rats of Milan normotensive strain (MNS). Experiments were performed as follows: blood samples (10 ml) collected from the abdominal aorta of the animals under light ether anaesthesia were immediately transferred (in portions of 0.5 ml) into glass tubes containing 5 µl of the solution of the test compound or saline. The samples were allowed to clot for 1 h at 37°C. Time related TxB_2 production was determined in preliminary experiments. Serum was separated by centrifuging blood at 3000 rpm for 10 min, collected and stored at –20°C until assay.

Ex vivo *experiments*

Male IVA-SDIV rats (Ivanovas, GmbH, Germany) weighing about 260 g and fasted for 16 h were used. Products were administered by gavage as a suspension in a vol of 5 ml/kg body weight. The stock suspensions were prepared by homogenizing the substances with a Braun potter in 0.5% methocel in distilled water. The working suspensions were prepared by diluting the respective stock suspension with methocel. Each compound was tested on 10 rats. Two hours after product administration, blood samples (10 ml) were collected from the abdominal aorta of the rats under CO_2 anaesthesia using sterile disposable syringes. Samples were allowed to clot for 1 h at 37°C for TxB_2 determination. The serum was separated by centrifugation at 3000 rpm for 15 min, collected and stored at –20°C until tested.

Determination of TxB_2 and PGE_2 levels

Thromboxane A_2 (TxA_2) synthase inhibition was assayed by determining the TxB_2 (stable TxA_2 metabolite) levels in rabbit or rat whole blood *in vitro* and in rat serum after oral treatment (*ex vivo*). The TxB_2 levels were determined by radioimmunological techniques using New England Nuclear (NEN; Dreieich, Germany) RIA kits according to the supplier's instructions. The effects of the compounds on TxA_2 synthesis was evaluated as the percentage of change *vs* the control. Variables were submitted to analysis of variance [57] and Dunnett's *t*-test was performed to compare control groups with each treated group [58].

Only in experiments for determining selectivity for TxA_2 synthase, were TxB_2 and PGE_2 levels determined by RIA using more selective antibodies according to a described method [59].

An aliquot of 100 µl of the appropriately diluted samples was added to 400 µl of phosphate buffer 20 mM pH 7.4 + BSA 0.1% (Sigma Chemical Co, St Louis, MO, USA) and to 1 ml of buffer containing ^3H-TxB_2 (5500 dpm) and TxB_2 rabbit antiserum (1:125 000) or ^3H-PGE_2 (5000 dpm) and PGE_2 rabbit antiserum (1: 30 000). The incubation was performed at 4°C for 16 h. At the end of the incubation 0.1 ml of phosphate buffer + 10% BSA and 0.1 ml of 100 mg/ml charcoal (Carlo Erba, Milano, Italy) were added. The incubation mixture was centrifuged at 3800 x 10' and the supernatant collected; 10 ml of Instagel II was added and the samples were finally counted in a β-Counter Tricarb 1900 (Packard Instrument Co, Downers Grove, IL, USA). The intrassay variability was 7%.

Experimental thrombosis in the rabbit

The test was performed according to the method of Silver [60] and Randall [61]. Male New Zealand White rabbits (Charles River, Italy), weighing about 3 kg and fasted for 22 h, were treated by gavage with the product suspended in 0.5% methocel in distilled water or vehicle. The thrombosis was induced 2 h after the treatment by injection of arachidonic acid solution at the final concentration of 1.4 mg/kg in 1 min. The animals were observed for 48 h after arachidonic acid injection and the mortality was recorded. Statistical analysis was performed on the mortality percentage and ED_{50} values were calculated by Finney's probit analysis technique [62].

LD_{50} determination

Male ICEM:CER (SPF Caw) mice weighing about 20 g, were used. The tested products were suspended in 0.5% methocel 400 cps in distilled water; all doses were administered at a constant volume of 1 ml/20 g of animal weight. The animals were kept under observation for 7 days after dosing. At the end of the observation period, the LD_{50} value and confidence limits for $P = 0.95$ for each of the tested products were calculated by the method of Litchfield and Wilcoxon (63).

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References

1 Swenson J, Hamberg M, Samuelsson B (1975) *Acta Physiol Scand* 94, 222
2 Hamberg M, Swenson J, Samuelsson B (1975) *Proc Natl Acad Sci USA* 72, 2994
3 Gryglewski RJ, Bunting S, Moncada S, Flower RJ, Vane JR (1976) *Prostaglandins* 12, 685
4 Bunting S, Gryglewski RJ, Moncada S, Vane JR (1976) *Prostaglandins* 12, 897
5 Moncada S, Gryglewski RJ, Bunting S, Vane JR (1976) *Nature (Lond)* 263, 663
6 Vesterqvist O, Edhag O, Green K, Henriksson P (1985) *Thromb Res* 37, 459
7 Fitzgerald DJ, Roy L, Catella S, Fitzgerald GA (1986) *New Engl J Med* 315, 983
8 Udvardy M, Torok I, Rak K (1987) *Thromb Res* 47, 479
9 Ball HA, Cook JA, Wise WC, Halushka PV (1986) *Intensive Care Med* 12, 116
10 Dunn M (1987) *Drugs* 33 (suppl 1) 56
11 Patrono C, Ciabattoni G, Remuzzi G, Gatti E, Bombardieri S, Di Munno O, Tartarelli G, Cinotti GA, Simonetti BM, Pierucci A (1985) *J Clin Invest* 76, 1011
12 Lefort J, Rotilio D, Vergafttig BB (1984) *Br J Pharmacol* 82 (3), 565
13 Defreyn G, Deckmyn H, Vermylen J (1982) *Thromb Res* 26, 389
14 Vane JR (1982) *J Endocrinol* 95, 3 p
15 Vermylen J, Deckmyn H (1983) *Br J Clin Pharmacol* 15, 175
16 Vermylen J, Defreyn G, Carreras LO, Machin SJ, Van Schaeren J, Verstraete M (1981) *Lancet* i, 1073
17 Gryglewski RJ, Zmuda A, Korbut R, Krecioh E, Bieron K (1977) *Nature* 267, 627
18 Moncada S, Hermann AG, Higgs EA, Vane JR (1977) *Thromb Res* 11, 323
19 Tai HH, Yuan B (1978) *Biochem Biophys Res Commun* 80, 236
20 Yoshimoto T, Yamamoto S, Hayaishi O (1978) *Prostaglandins* 16, 529
21 Iizuka K, Akahane K, Momose D, Nakazawa M, Tanouchi T, Kawamura M, Ohyama I, Kajiwara I, Iguchi Y, Okada T, Taniguchi K, Miyamoto T, Hayaishi M (1981) *J Med Chem* 24, 1139
22 Tanouchi T, Kawamura M, Ohyama I, Kajiwara I, Iguchi Y, Okada T, Miyamoto T, Taniguchi K, Hayaishi ML, Iizuka K, Nakazawa M (1981) *J Med Chem* 24, 1149
23 Cross PE, Dickinson RP, Parry MJ, Randall MJ (1981) *Agents and Actions* 11, 275
24 Greenberg R, Antoniaccio MJ, Steinbacher T (1982) *Eur J Pharmacol* 80, 19
25 Anderegg K, Anzeveno P, Cook JA, Halushka PV, McCarthy J, Wagner E, Wise WC (1983) *Br J Pharmacol* 78, 725
26 Wynalds MA, Liggett WF, Fitzpatrick FA (1983) *Prostaglandins* 26, 311
27 Ford MF, Browne LJ, Campbell T, Genenden C, Goldstein R, Gude C, Wasley JWF (1983) *J Med Chem* 28, 164
28 Kato K, Ohkawa S, Terao S, Terashita Z, Nishikawa K (1985) *J Med Chem* 28, 287
29 Cross PE, Dickinson RP, Parry MJ, Randall MJ (1986) *J Med Chem* 28, 1427
30 Cross PE, Dickinson RP, Parry MJ, Randall MJ (1986) *J Med Chem* 29, 342.
31 Wright WB, Press JB, Chan PS, Marsico JW, Hang MF, Lucas J, Tanher J, Tomcufcik AS (1986) *J Med Chem* 29, 523
32 Johnson RA, Nidy EG, Aiken JW, Crittenden MJ, Gorman RR (1986) *J Med Chem* 29, 1461.
33 Sincholle D, Coquelet C, Bonne C (1986) *Arzneim-Forsch* 36 (I), 117.
34 Cross PE, Dickinson RP, Parry MJ, Randall MJ (1986) *J Med Chem* 29, 1643.
35 Press JB, Wright WB, Chan PS, Hang MF, Marsico JW, Tomcufcik AS (1987) *J Med Chem* 30, 1036.
36 Manley PW, Allanson NM, Booth RFG, Buckle PE, Kuzniar EJ, Lad N, Lai SMF, Lunt DO, Tuffin DP (1987) *J Med Chem* 30, 1588.
37 Martinez GR, Walker KAM, Hirschfeld DR, Maloney PJ, Yang DS, Rosenkranz RP (1989) *J Med Chem* 32, 890.
38 Amemiya Y, Terada A, Wachi K, Miyazawa H, Hatakeyama N, Matsuda K, Oshima T (1989) *J Med Chem* 32, 1265.
39 Kanao M, Watanabe Y, Kimura Y, Saegusa J, Yamamoto K, Kanuo H, Kanaya M, Kubo H, Ashida S, Ishikawa F (1989) *J Med Chem* 32, 1326.
40 Hornby EJ, Skidmore IF (1982) *Biochem Pharmacol* 31, 1158.
41 Fitzgerald GA, Reilly IAG, Pedersen AK (1985) *Circulation* 72, 1194
42 Lefer AM (1986) *Drugs of today* 21 (6), 283
43 Smith EF III, Lefer AM, Smith JB, Nicolaou KC (1981) *Archiv Pharmacol* 318, 130
44 Gresele P, Van Houtte E, Arnout J, Deckmyn H, Vermylen J (1984) *Thromb Haemost* (Stuttg) 52 (3), 364
45 Imura Y, Terashita Z, Shibouta Y, Nishikawa K (1988) *Eur J Pharmacol* 147, 359
46 De Clerck F, Beetens J, de Chaffoy de Courcelles D, Freyne E, Janssen PAJ (1989) *Thromb Haemost* 61, 35

47 Cozzi P, Carganico G, Pillan A, Branzoli U (1983) UK Pat 2122997A; *Chem Abstr* 100, 174829a
48 Cozzi P, Mongelli N, Pillan A (1984) *J Het Chem* 21, 311
49 Cozzi P, Branzoli U, Lovisolo PP, Orsini G, Carganico G, Pillan A, Chiari A (1986) *J Med Chem* 29, 404
50 Rane DF, Fishman AG, Pike RE (1984) *Synthesis* 8, 694
51 Bencze WL, Barsky LJ (1962) *J Med Pharm Chem* 5, 1298
52 Ulrich V, Haurand M (1983) *Adv Pros Thrombox Leukotr Res* 11, 105
53 Van Opdenbosch N, Cramer R, Giarrusso FF (1985) *J Mol Graphics* 3, 110
54 SYBYL Manual (1985) Tripos Associates, St Louis (Mo)
55 Salvati P, Pugliese F, Ferti C, Pierucci L, Ferrario R, Patrono C (1988), 21st Annual Meeting Am Soc Nephrology, San Antonio, Kidney Int (1989), 35, 149
56 Alessandrini P, Salvati P, Pugliese F, Ciabattoni G, Patrono C (1990), 7th Int Conference on Prostaglandins and selected compounds, Florence, Abstract Book, p 307
57 Huitson A (1966) *In: The analysis of variance*, Kendall MG ed, C Griffin Lts, London, pp 37
58 Dunnett CW (1955) *J Am Stat Assoc* 50, 1096
59 Patrono C, Ciabattoni G, Pinca E, Pugliese F, Castrucci G, De Salvo A, Satta MA, Peskar BA (1980) *Thromb Res* 17, 317
60 Silver MJ (1974) *Science* 183, 1085
61 Randall MJ, Parry MJ, Hankeswood E, Cross PE, Dickinson RP (1981) *Thromb Res* 23, 145
62 Finney DJ (1964) *In: Probit Analysis*, Carmbridge University Press, pp 249
63 Litchfield JT, Wilcoxon F (1949) *J Pharmacol Exptl Ther* 96, 99