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Synthesis and preliminary pharmacological investigation of some 2-[4-(dialkylaminoalkoxy)phenyl]benzotriazoles and their N-oxides

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Abstract

A set of 2-[4-(dialkylaminoalkoxy)phenyl]benzotriazoles and corresponding N-oxides was prepared. In a preliminary pharmacological investigation concerning some of these compounds, several in vitro and in vivo activities were shown. At concentrations in the range of 3–10 μM all tested compounds strongly inhibited (50–100%) the guinea pig ileum contractions induced either electrically or by means of several agonists; of particular interest was the antagonism to leukotriene D_4. Compound **5b** inhibited platelet aggregation induced by thromboxane A_2, PAF and ADF (but not by arachidonic acid) and increased the bleeding time in mice. Compounds **5b** and **6b** protected mice from potassium cyanide hypoxia and exerted anti-hypercholesterolemic action; the first compound produced a ratio between HPL and total serum cholesterol concentrations below 0.92, thus indicating a potential anti-atherogenic activity. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Benzotriazole derivatives; Benzotriazole N-oxides; Leukotriene D_4 antagonists; Thromboxane A_2 antagonists; Hypocholesterolemic agents

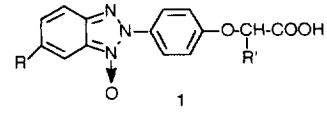
1. Introduction

During the systematic investigation of pharmacophoric potentialities of benzotriazole nucleus, undertaken for many years by Sparatore and co-workers [1–6], a set of 4-(benzotriazol-1-oxide-2-yl) phenoxy alkanoic acids (**1**) was prepared [4].

Most of these compounds at oral doses ≤25 mg/kg exhibited strong anti-inflammatory, myorelaxant and diuretic activities. The activity on CNS, clearly shown in the rotarod test, was indicated also by some effects on spontaneous motility (inhibition, sometimes preceded by stimulation), on pentamethylene tetrazole induced convulsions, on pentobarbital narcosis and on sensitivity to tail pinch in mice. The results in these tests were not impressive, but it must be noted that the effects of doses higher than 25 mg/kg were not investigated.

Strong depressive effects on CNS were observed by Buu-Hoi et al. [7] with a derivative of 2-(4-hydroxyphenyl)benzimidazole which is isosteric with the heteroaromatic moiety present in our compounds, namely 2-[4-(2-diethylaminoethoxy)phenyl]benzimidazole (**2**). These effects (abolition or substantial reduction of spontaneous motility and loss of equilibrium reflexes on 'rotarod') were observed with doses (666–66 mg/kg) much higher than ours; at a dose of 30 mg/kg, comparable with ours, only a modest reduction of spontaneous motility was observed.

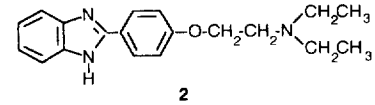
The benzimidazole derivative **2** was studied also from a completely different point of view, that is as inhibitor of cholesterol biosynthesis [8]. Actually, when administered orally at the dose of 15 mg/kg for 1 week in rats, it produced a strong reduction of plasma and tissue cholesterol with simultaneous accumulation of 7-dehydro cholesterol whose reductive conversion to cholesterol is blocked.



1

R = H, Cl, CH_3, OCH_3

R' = H, CH_3



2

In such a situation we deemed it of interest to investigate the effect of the exchange of the alkanoic chain of compounds **1** with the diethylaminoethyl chain of compound **2** and also

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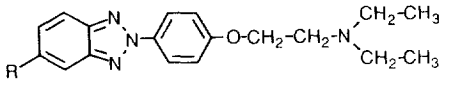
with the dimethylaminopropyl chain often present in neuropsychoactive drugs. Moreover the corresponding benzotriazoles, which are structurally closer to the benzimidazole derivative **2**, were examined.

Thus the prepared compounds are represented by the general formulas **3–6**.



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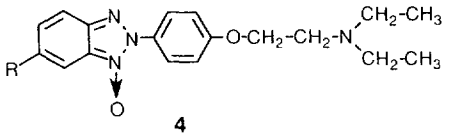

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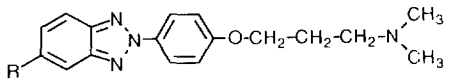
R = H (a); Cl (b)



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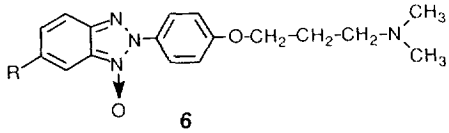


6

R = H (a); Cl (b); CF_3 (c); OCH_3 (d)

2. Chemistry

Compounds of formulas **3–6** were obtained by reacting the different 2-(4-hydroxyphenyl)-6-*R*-benzotriazoles (and corresponding N-oxides) with the required tert-aminoalkylchloride in ethanolic sodium hydroxide solution.

The suitable 2-(4-hydroxyphenyl)-6-*R*-benzotriazoles and the corresponding N-oxides were prepared through the reductive cyclization of 2-nitro-4-*R*-4′-hydroxyazobenzenes, in turn obtained by coupling the diazotized 2-nitro-4-*R*-anilines with phenol (Scheme 1).

Although some of the intermediates **7**, **8** and **9** were already described in the literature, their preparations were not always satisfactory, so that the setting up of particular operating conditions was required in each step of Scheme 1.

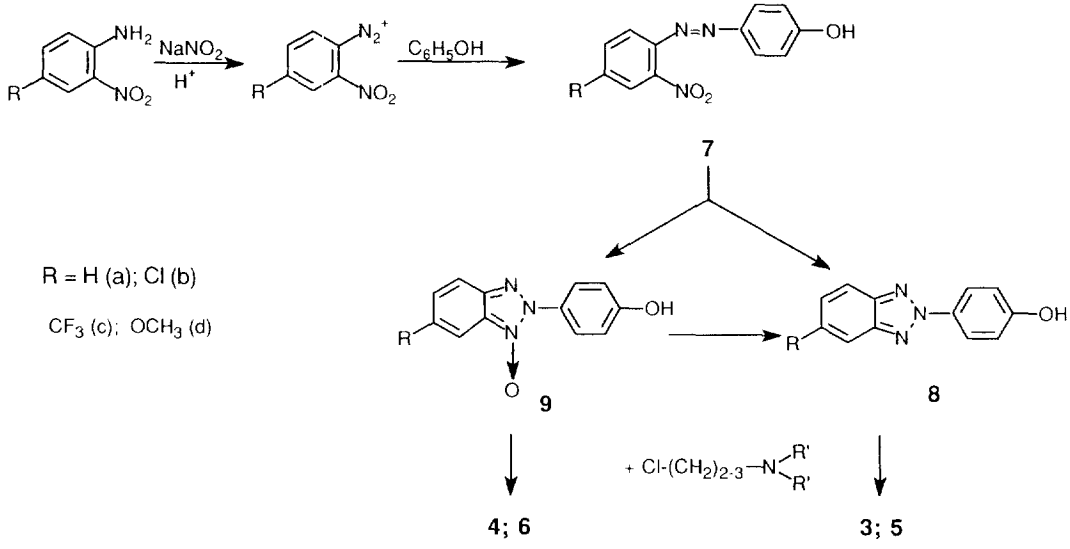
Many reducing agents have been employed in the past for the conversion of 2-nitroazobenzene to benzotriazoles or to benzotriazole N-oxides, such as sodium dithionite [4,9,10], zinc dust in aqueous or ethanolic alkali [11,12], zinc dust in pyridine [13,14], glucose and ethanolic alkali [12], aminoiminomethanesulfinic acid (thiourea-dioxide) [15,16], hydroxylamine in ethanolic sodium hydroxide solution [17], hydrazine hydrate in the presence of alkali hydroxide [18], triethylphosphite [19], carbon oxide in the presence of triethylamine [20], baker's yeast [21] and some others.

Most of the above methods when applied to compounds **7** gave erratic results, with poor yields or formation of mixtures of benzotriazoles **8** and benzotriazole N-oxides **9** and also of amino compounds, the separation of which was troublesome.

In our cases satisfactory yields of benzotriazoles **8** and of benzotriazole N-oxides **9** were obtained by reducing 2-nitro-4-*R*-4′-hydroxyazobenzene (**7**), respectively, with zinc dust in 2 N NaOH solution/ethanol (1:2 vol./vol.) [12] and with hydroxylamine in aqueous ethanolic sodium hydroxide solution [17]. The ratio among the reactants was of paramount importance for good yields and selectivity of the reduction reactions.

The structures of the final compounds **3–6** were supported by the elemental analytical results and UV spectra, which conform with those of similar compounds [4,19,22,23].

The ^1H NMR ($CDCl_3$) spectra did not exhibit any unusual features; thus only the spectrum of compound **5a** is described as an example: δ 8.3–8.2 and 7.1–7.0 (2 pseudo doublets, 4H, *p*-phenylene); 8.0–7.85 and 7.5–7.35 (2 dd, 4H, ben-



Scheme 1.

zotriazole); 4.03–4.17 (t, 2H, $-O-CH_2$); 2.55–2.40 (t, 2H, CH_2-N); 2.27 (s, 6H, $2CH_3$); 2.1–1.9 (m, 2H, $-C-CH_2-C$).

3. Experimental

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

The elemental analyses were performed at the Microanalytical Laboratory of the 'Dipartimento di Scienze Farmaceutiche' of Genoa University and the analytical results for the indicated elements were within ±0.3% of the calculated values.

UV spectra were recorded with a Perkin-Elmer Lambda 16 spectrophotometer; ^1H NMR spectra were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ as solvent with TMS as internal standard.

3.1. 4'-Hydroxy-2-nitro-4-R-azobenzenes (7a–d)

Compounds **7a** and **7b** were prepared according to the described method [4], while for **7c** (unknown) and for **7d** the method was modified as follows.

7c: 2-nitro-4-trifluoromethylaniline (2.06 g, 10 mmol) was triturated with 6.3 ml of conc. hydrochloric acid. To the stirred ice cooled mixture, a solution of 1.1 g (16 mmol) of sodium nitrite in 10 ml of water was added slowly; after 15 min 90 ml of iced water were added and a solution of 1.07 g (11.4 mmol) of phenol and 280 mg (5 mmol) of potassium hydroxide in 7.5 ml of water was dropped slowly. The mixture was stirred further for 45 min at 0°C and then for 15 min at room temperature. The solid was filtered, washed with warm water and the joined solutions were set apart, while the solid was treated with 0.5 M sodium hydroxide solution. To the alkaline solution acetic acid was added till pH = 5–6; the precipitate was collected, washed with water and, after drying, with a mixture (1:1) of dichloromethane–petrol ether. To the solution that was set apart, sodium acetate was added till pH = 5–6 and the azo compound was collected and washed as above.

7d: to a solution of 4-methoxy-2-nitroaniline (1.68 g = 10 mmol) in 5 ml of concentrated hydrochloric acid, 17 ml of water were added so that a fine precipitate of salt was formed. To this ice cooled suspension a solution of sodium nitrite (750 mg = 10.8 mmol) in 4 ml of water was dropped slowly and the solution was stirred for additional 30 min at 0–2°C. The diazonium salt solution was then poured slowly in a solution of phenol (940 mg = 10 mmol), sodium carbonate (5.63 g = 53 mmol) and sodium acetate (1.69 g = 20 mmol) in 15 ml of water. The mixture was stirred for 45 min at 0–1°C and then for 15 min at room temperature. The precipitate was filtered, washed with warm water, dried and finally washed with a 1:1 mixture of dichloromethane–petrol ether.

3.2. 2-(4-Hydroxyphenyl)-6-R-benzotriazoles (8a–d)

The 4'-hydroxy-2-nitro-4-*R*-azobenzenes (10 mmol) were dissolved in a mixture of 56 ml of ethanol and 27.8 ml of 2 N NaOH (55.6 mmol) and to the solution 2.78 g (42.5 mmol) of zinc dust were added. To improve the reduction rate of compound **7c**, 33 ml of 2 N NaOH solution and 3.3 g of zinc dust were used. The mixture was boiled under reflux and under a stream of nitrogen for a time variable from 30 to 90 min. The formation of the benzotriazoles was followed through TLC on silica using dichloromethane/methanol (20:1) as eluent.

After cooling, ethanol was removed under vacuum from the reaction mixture, water was added and the unreacted zinc was filtered. The solution was acidified with 1 M HCl and extracted with ether. The ether extract was dried over sodium sulfate and evaporated to dryness. The solid residue was washed with a mixture of dry ether–petrol ether and crystallized from the solvent indicated in Table 1.

Table 1
4'-Hydroxy-2-nitro-4-*R*-azobenzenes (**7a–d**), 2-(4-hydroxyphenyl)-6-*R*-benzotriazoles (**8a–d**) and corresponding 1-oxides (**9a–d**)

Compound	Formula [a]	M.p. (°C)	Solvent [b]	Yield (%)	M.p. (°C) from literature
7a	$C_{12}H_9N_3O_3$	157–160	A	87	157 [11]
7b	$C_{12}H_8ClN_3O_3$	184–186	A	42	187–188 [4]
7c	$C_{13}H_8F_3N_3O_3$	130–132	B	69	
7d	$C_{13}H_{11}N_3O_4$	172–174	C [c]	72	167–168 [4]
8a	$C_{12}H_9N_3O$	212–214	E [c]	90	219 [9]
8b	$C_{12}H_8ClN_3O$	188–190	D	77	203–205 [16]
8c	$C_{13}H_8F_3N_3O$	149–151	D	74	
8d	$C_{13}H_{11}N_3O_2$	169–170	D	92	
9a	$C_{12}H_9N_3O_2$	233–234	D [c]	80	232–233 [9]
9b	$C_{12}H_8ClN_3O_2$	229–230	F	76	213–214 [4]
9c	$C_{13}H_8F_3N_3O_2$	193–194	D	62	
9d	$C_{13}H_{11}N_3O_3$	225–227	D	90	223–224 [4]

[a] Analytical results for C, H, N were within ±0.3% of the calculated values.
[b] A = dichloromethane; B = petrol ether; C = dichloromethane/petrol ether (1:1); D = dry ether; E = dry ether/petrol ether (1:1); F = ethanol.
[c] Only washing.

4.1. Material and methods

All tested compounds were used as hydrochlorides.

For in vivo tests the substances were generally administered by oral route using a gastric tube; they were prepared as aqueous solutions or finely homogenized suspensions in 'tween 80' (2%). In a few cases the substances were introduced intraperitoneally, dissolved in water. Groups of 3 or 5 animals (mice or rats) were used. For the in vitro assays, sometimes, it was necessary to increase the solubility by means of dimethyl sulfoxide in a concentration not interfering with tests (0.1% for platelet aggregation and 0.5% for all others).

Doses (mg/kg) or concentration (μg/ml or μM) indicated in the methods were the highest utilized routinely, depending on toxicity; when significant activity was detected, lower doses or concentrations were tested in order to define the minimal effective ones, and secondary tests were performed to have some insight for the possible mechanism of action.

The procedure for most assays has already been described [24–29]; the pertinent reference is indicated near each assay name in the results Tables, which are relative to the more significant ones only.

Procedures not previously described are given in the following section.

4.2. Thromboxane A_2 receptor antagonism [30]

A single rat aortic ring, bathed in physiological salt solution at 37°C (containing atropine, phentolamine, mepyramine and cyproheptadine at 0.1 μg/ml, and also propranolol and indomethacin at 1 μg/ml) plus test substance (100 μM conc) are contracted submaximally by the thromboxane mimetic agent U 46619 (0.03 μM conc.). Antagonism to this response by more than 50% indicates TxA_2 receptor antagonist activity.

4.3. Naloxone antagonism (opiate agonism) [31]

The effect of test substance (3 μM conc) on guinea pig ileum contractions induced by electrical transmural stimulation in physiological salt solution at 37°C is determined [25].

If reduction of contractile responses elicited by the minimum inhibitory concentration of the test substance is reversed more than 50% by naloxone (27 μM conc) an opioid mechanism of action is indicated (+).

4.4. Vas deferens contractility [32,33]

The prostatic portion of rat vas deferens is maintained in physiological salt solution at 37°C and contracted by transmural field stimulation (70% of maximum, 0.1 Hz). Reduction of resultant neurogenic twitch responses by more than 50% within five minutes after addition of the test substance (100 μM conc) indicates significant activity.

5. Results and discussion

The most significant results are collected in Tables 3–6, while some others are described in the following discussion.

On the whole compounds **3a**, **5b** and **6b** were well tolerated up to the dose of 300 mg/kg p.os and up to 100 mg/kg i.p. in mice observed for acute toxic symptoms or autonomic effects during the subsequent 72 h.

Mice treated with compound **3a** exhibited a modest irritability and increased explorative activity, while those treated with compoud **6b** exhibited a slight muscle relaxation. No effects at all were observed with compound **5b**.

Results of in vivo tests directed to show any specific activity on CNS (as analgesia (phenylquinone writhings; tail flick), anti-convulsive activity (against metrazole and electroshock induced convulsions), 5-methoxy-*N*,*N*-dimethyltryptamine potentiation, apomorphine and oxotremorine antagonism, tetrabenazine hypothermia antagonism) were completely negative, apart from a slight reduction of writhings observed for compound **6b**.

The increased explorative activity observed with compound **3a** contrasts with the strong inhibition exhibited by compound **2** [7], of which it is isosteric.

Concerning the *cardiovascular apparatus*, the tested compounds exhibited only a modest anti-hypertensive activity in spontaneously hypertensive rats [24,25] (2–4% blood pressure reduction during the 4 h of observation, at the dose of 100 mg/kg), associated with a moderate reduction (up to 8% for compound **5b**) of heart rate. These results are only partially accounted for by the results of related in vitro tests (Tables 3 and 4).

Actually, the three tested substances exerted negative chronotropic activity on isolated guinea pig right atria, antagonism on type L calcium channel in guinea pig ileum and rat portal vein, but not on guinea pig left atria. Compounds **3a** and **5b** exhibited significant α_1-adrenergic antagonism in the rat vas deferens test, but they reduced by only 12% and 25% the norepinephrine induced mydriasis in mice [27], when administered i.p. at 30 mg/kg dose. On the other hand, compounds **3a** and **6b** showed apparent β_1-adrenergic antagonism, since they reduced the isoproterenol-induced positive inotropic activity in guinea pig atria; however the direct moderate negative inotropic activity exerted on guinea pig atria suggests possible physiologic rather than pharmacologic receptor antagonism.

In spite of the negative chronotropic activity observed in spontaneously beating guinea pig right atria, no protective activity was seen against chloroform induced arrhythmia in mice (at 100 mg/kg i.p.).

It is noteworthy that the tested compounds were able to *prevent the death* of mice *due to the hypoxia* [27] produced by a dose i.v. of 2.4 mg/kg (DL_{95}) of potassium cyanide. Compounds **5b** and **6b** were almost equiactive (60–80% protection respectively at 100 mg/kg p.os and 40% protection for both at 30 mg/kg), while **3a** gave a protection of only 20% at the oral dose of 100 mg/kg. This activity is rarely

Table 3
In vitro tests related to cardiocirculatory function and tracheal relaxation

Test [a]	Conc. (μM)	Effect	Response of compound			Reference drug	Conc. (μM)	Response
			3a	**5b**	**6b**			
Cardiac inotropy [25] (guinea pig left atria)	100	[b]	−31	+4	−30			
Cardiac chronotropy [25] (guinea pig right atria)	100	[b]	−37	−16	−28	clonidine	3	−18
	30		−17	−5	−14			
	10		−5	nt	−9			
Adrenergic β_1 antag. [27] (guinea pig left atria)	100	[c]	62	3	65	propranolol	0.3	82
	30		14	nt	14			
Ca^{2+} antagonism [25] (guinea pig left atria)	100	[d]	0	10	10			
Rat portal vein [24,25] spontan. activated	100	[e]	53	15	28	cromakalim	0.3	81
	30		19	nt	nt			
Rat portal vein [25] potassium depolarized	100	[f]	55	15	23	nifedipine	0.1	76
	30		18	nt	nt			
Rat vas deferens contractility	100	[g]	69	11	78	2-chloroadenosine	1	73
	30		9	nt	33			
Adrenergic α_1 antag. [29] (rat vas deferens)	100	[h]	83	57	26	prazosin	0.08	70
	30		43	32	nt			
Tracheal contrac. [25] (guinea pig)	100	[i]	88	72	80	epinephrine	0.3	79
	30		25	18	0			

[a] References for the methods employed (when no reference is cited, the method is described in the present paper).
[b] % Variation in contractile force or rate.
[c] % Reduction of isoproterenol induced positive inotropic effect.
[d] % Reduction of Ca^{2+} induced increase in contractile force.
[e] % Inhibition of spontaneous movements.
[f] % Inhibition of K^+ induced contractions.
[g] % Reduction of neurogenic twitch response.
[h] % Reduction of phenylephrine induced contractile response.
[i] % Inhibition of tracheal tone relative to relaxation induced by 0.3 μg/ml epinephrine.
nt: not tested.

observed and further investigations are warranted. Among the compounds able to protect mice from cyanide hypoxia are flunarizine and cinnarizine, that at the dose of 30 mg/kg protected 60% of animals. Both the cited drugs are calcium antagonists, as our compounds are (Tables 3 and 4); however the role of calcium channel blockade in the protection from KCN hypoxia is not well defined. Actually compound **3a** that was the most active as a calcium antagonist was less active as an anti-hypoxia agent. On the other hand the anti-hypoxia effect might be related also to the strong *antagonism for leukotriene* D_4 displayed by the three substances, that at 3 μM concentration inhibited by 45–73% guinea pig ileum contractions (Table 4). Actually leukotrienes are formed by brain tissue and increased synthesis occurs under various pathological conditions such as ischaemic insults [34]. Ibudilast [2-methyl-1-(2-isopropylpyrazolo[1.5-*a*]pyridin-3-yl)-1-propanone], a potent leukotriene D_4 antagonist, was launched recently in Japan for the treatment of asthma and for improving cerebral blood flow, although its cerebral vasodilating action might be also related to the potentiation of dilator prostanoids [35,36].

The same antagonism for leukotriene D_4 could account for the observed *relaxation of guinea pig trachea*. Actually this activity did not appear to be related to either β-adrenoceptors agonism, not being antagonized by propranolol, or to cyclooxygenase inhibition since platelet aggregation induced by sodium arachidonate was not inhibited. Moreover the active concentration (100 μM) failed to produce positive inotropic activity in guinea pig atria, but, on the contrary, strong negative activity was seen in guinea pig atria, thus excluding also a theophylline-like mechanism of action.

As mentioned, all three tested compounds were completely inactive against the platelet aggregation induced by arachidonic acid, but exhibited a quite different activity against *platelet aggregation* induced by thromboxane A_2 and PAF (Table 5).

While compounds **3a** and **6b** were still completely inactive against the first aggregating agent and only slightly active against the latter, compound **5b** inhibited strongly the aggregation induced by both agents and also by ADP. Since compound **6b** is the N-oxide of **5b**, it should be inferred that the reductive conversions of the former to the latter is not pos-

Table 4
In vitro tests for activity on guinea pig ileum contractions induced by several agents

Test [a]	Conc. (μM)	Effect	Response of compound			Reference drug	Conc. (μM)	Response
			3a	**5b**	**6b**			
Ca^{++} antagonism [25] (channel type L)	100	[b]	100	100	100	nifedipine	0.01	78
	30		100	85	88			
	10		88	60	53			
	3		27	19	9			
Leukotriene LTD_4 antagonism [26]	100	[b]	97	100	100	LY-171883 [c]	1	74
	30		80	100	100			
	10		79	100	80			
	3		45	73	64			
	1		nt	43	12			
Bradykinin B_2 antagonism [25]	100	[b]	100	100	97	nifedipine	0.03	60
	30		89	81	75			
	10		56	31	53			
	3		24	nt	11			
Neurokinin NK_1 antagonism [25]	100	[b]	100	100	100	spantide	1	65
	30		95	100	69			
	10		55	61	36			
	3		0	8	nt			
Cholecystokinin CCK_A antag. [25]	100	[b]	100	79	100	devazepide	0.03	70
	30		100	61	81			
	10		67	36	58			
	3		32	nt	24			
Methacholine antagonism [25]	100	[b]	100	100	100	atropine	0.03	70
	30		99	100	97			
	10		83	100	88			
	3		38	79	40			
	1		nt	22	nt			
Angiotensin I inhibition [25]	100	[b]	100	65	100	captopril	10	67
	30		91	34	89			
	10		53	nt	21			
	3		16	nt	nt			
Angiotensin II antagonism [25]	100	[b]	100	86	92	[Sar^1, Thr^8]-angiotensin II	0.1	94
	30		96	17	26			
	10		58	nt	nt			
	3		21	nt	nt			
Inhibition electrically stimulated contractions [25]	100	[c]	+	+	+	atropine	0.1	+
	30		+	+	+			
	10	[d]	+	+	+			
	3		–	–	+			
	1		nt	nt	–			
Naloxone antag. (opiate agonism)	10	[d]	–	–	nt			
	3		nt	nt	–			

[a] References for the methods employed (when no reference is cited, the method is described in the present paper).
[b] % Reduction of contractile response to the various agents.
[c] % Inhibition of contractions by more than 50% is recorded as significant (+). Inhibition less than 50% is recorded as (–).
[d] Reversal by naloxone of the reduction of contractile response elicited in the foregoing assay by the minimum inhibitory concentration, may indicate an opioid mechanism (+).
[e] 5-[4-(4-acetyl-3-hydroxy-2-propylphenoxy)butyl]tetrazole.
nt: not tested.

sible, at least in in vitro operating conditions, or that it is very slow.

Concerning the activity against thromboxane A_2 induced platelet aggregation, compound **5b** was just a little less active than the established reference drug sulotroban; however **5b** was clearly less active than sulotroban in relaxing a single rat aortic ring contracted by the thromboxane mimetic agent U 46619. Nevertheless we consider the benzotriazole derivative **5b** as an interesting lead for further research, since it differs largely from the known thromboxane A_2 receptor antagonists for both the aromatic moiety and the basic side chain. Actually, as far as we know, all thromboxane antagonists, like thromboxane itself, contain an acidic function [37,38]. Since compound **3a** was devoid of activity, the nature of the basic chain seems to be a determinative factor for thromboxane antagonism.

Table 5
Inhibition of rabbit platelet aggregation induced by several agents and some related in vitro and in vivo assays

Test [a]	Conc. (μM) dose (mg/kg)	% Inhibition by compound 3a	5b	6b	Reference drug	Conc. (μM) dose (mg/kg)	% Inhibition
	in vitro						
Aggregation induced by:							
sodium arachidonate [25]	100	0	0	0			
thromboxane A_2 [26]	100	0	100	0	sulotroban	10	60
	30	nt	68	nt			
	10	nt	20	nt			
PAF [25]	100	23	100	15	apafant	0.3	68
	30	nt	62	nt	(WEB 2086)		
	10	nt	21	nt			
ADP [25] [b]	100	nt	100	nt	2-chloroadenosine	3	74
	30	nt	52	nt			
	10	nt	20	nt			
Tromboxane A_2 antag. (rat aorta) [c]	100	nt	40	nt	sulotroban	3	50
	in vivo						
Bleeding time [27] (mice) [d]	p.o.; 100	nt	59	nt	aspirin	p.o.; 30	55
	30	nt	16	nt			
PAF antag. [25] (mice) [e]	p.o.; 100	nt	20	nt	L-652731 [f]	p.o.; 100	60

[a] References for the methods employed (when no reference is cited, the method is described in the present Note).
[b] Tested only compounds exhibiting more than 50% inhibition in one of the foregoing assays.
[c] % Inhibition of contraction of a rat aortic ring induced by the thromboxane mimetic agent U 46619 (tested only compound active against thromboxane induced aggregation).
[d] % Prolongation of bleeding time relative to a contro group of animals (tested only compound with significant antiaggregatory activity).
[e] % Protection from mortality induced by PAF i.v. administration (tested only compounds active against PAF induced aggregation).
[f] *Trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran.
nt: not tested.

Because of its anti-aggregating properties, compound **5b** prolonged significantly the bleeding time in mice after an oral dose of 100 mg/kg.

The benzotriazole N-oxide derivative **6b** exhibited a significant *saluretic* (+170%) and moderate *kaluretic* (+70%) activity, associated with increased (+80%) urine volume output in hydrated rats [24,25] after an oral dose of 30 mg/kg. The behavior of the corresponding benzotriazole derivative **5b** was somewhat intriguing, showing at the same dose a clearly minor saluretic (+50%), but an identical (+80%) diuretic (urine volume output) activity. However when the dose was lowered to 10 mg/kg, compound **5b** was more active (+50%) as diuretic than **6b** (−30%). The possible in vivo reductive conversion of **6b** to **5b** could play a part in these apparent discrepancies. Compound **3a** was practically inactive in this field.

Most interesting was the strong *hypocholesterolemic activity* shown by compounds **5b** and **6b**, while compound **3a** was, once again, inactive. Since the main structural difference between active and inactive compound was the nature of the basic chain, the remaining benzotriazole derivatives **5a**, **5c** and **5d**, containing the dimethylaminopropoxy chain, were investigated in order to define the eventual contribution to the activity from the substituents on the aromatic nucleus. In addition to the total serum cholesterol concentration, either the heparin precipitating beta lipoprotein (HPL; corresponding to LDL and VLDL) concentrations or the high density betalipoprotein (HDL) concentrations were determined.

Reduction in the HPL/cholesterol ratio below 0.92, or reduction of the cholesterol/HDL ratio equal to or higher than 40% respect the ratio found for untreated hypercholesterolemic animals, indicate an anti-atherogenic activity.

The results in Table 6 indicate that compounds **5b** and **5d** met the above requirements and were comparable to bezafibrate; compound **6b** (N-oxide of **5b**) exhibited good cholesterol lowering activity, but its HPC/cholesterol ratio was not satisfactory. Some activity was seen also for compound **5a**.

In spite of the small number of compounds so far examined for hypocholesterolemic activity, the following structure–activity relationships may be tentatively proposed.

(i) The dimethylamino propoxy chain is more suitable for the activity than the diethylaminoethoxy one (compare **5a** and **3a**), although the latter is present in the potent compound **2** (isosteric of **3a**) and in many other hypocholesterolemic agents [39].

(ii) The substituents introduced on the benzene ring can strongly influence the activity, with chloro and methoxy substitution giving, in the order, the best results; a potent electron-withdrawing group such as trifluoromethyl seems to be quite detrimental.

(iii) The oxidation of benzotriazole to benzotriazole N-oxide does not abolish activity, but some reduction was

Table 6
Hypocholesterolemic activity in diet-induced hypercholesterolemic mice and related assays [29]

Test	Dose, p.os	Response produced by test compound [a]							
	(mg/kg)	**3a**	**5b**	**6b**	Bezafibrate	**5a**	**5c**	**5d**	Bezafibrate
Total cholesterol [b]	300	−6	−33	−32	nt	−16	−5	−30	−39
	100	nt	−15	−11	−25	nt	nt	+9	nt
	30	nt	0	nt	nt	nt	nt	nt	nt
Heparin precipitating lipoproteins HPL [c]	300	−2	−49	−35	nt				
	100	nt	−23	−8	−30				
	30	nt	0	nt	nt				
Ratio HPL/total cholesterol [d]	300	1.04	0.76	0.96	nt				
	100	nt	0.91	1.03	0.93				
	30	nt	0.96	nt	nt				
High density lipoproteins HDL [e]	300					+6	−1	+17	+15
	100					nt	nt	−8	nt
Ratio total cholesterol/HDL	300					3.25	3.96	2.46	2.19
	100					nt	nt	4.11	nt
% Variation ratio cholesterol/HDL [f]	300					−21	−4	−40	−47
	100					nt	nt	−18	nt

[a] Experiments with compounds **3a**, **5b**, **6b** and compounds **5a**, **5c**, **5d** were made in two stages and the reference drug bezafibrate was tested in each of them.
[b] Average percent reduction in serum cholesterol concentration ≥ 15% relative to hypercholesterolemic control mice indicates significant activity (established control animals have a serum total cholesterol content of 350 ± 28 mg/dl).
[c] Reduction of serum HPL concentration ≥ 20% indicates significant activity.
[d] Reduction of ratio below 0.92 indicates potential anti-atherogenic activity.
[e] Established hypercholesterolemic mice have a serum HDL content of 85.0 ± 6.7 mg/dl and a ratio total cholesterol/HDL equal to 4.12.
[f] Reduction of ratio equal to or higher than 40% respect the ratio found for untreated hypercholesterolemic animals indicates potential anti-atherogenic activity.
nt: not tested.

observed (compare **5b** and **6b**); however at the moment it is impossible to say if the activity observed for **6b** is due to **6b** itself or to compound **5b**, formed through in vivo reduction of the N-oxide function.

The simultaneous presence in the same molecule (**5b**) of hypocholesterolemic and platelet anti-aggregating activities could represent an outstanding property shared by only few other anti-hypercholesterolemic agents (as fibrates).

On the basis of these results, further investigations are warranted in order to eventually improve the hypocholesterolemic activity and, on the other hand, to define if the mechanism of action is similar to that of 2-[4-(2-diethylaminoethoxy)phenyl]benzimidazole (**2**) [8,40] ($\Delta^{5,7}$-sterol-7-reductase inhibition) or quite different [41–43]. The inhibition of later steps of cholesterol biosynthesis could give rise to build up of cholesterol precursors to toxic levels, but, in any case, the possibility of interruption of sterol biosynthesis in non-mammalian systems offers opportunities for original approaches in the treatment of parasitic diseases [41,44].

As already observed passim in the foregoing discussion, compounds **3a**, **5b** and **6b**, at concentrations in the range 3–10 μM, were able to *inhibit* by 50–100% *the guinea pig ileum contractions* induced either electrically or by means of several agonists (Table 4) such as angiotensin I and II, bradykinin B_2, calcium ion, cholecystokinin CCK_A, leukotriene D_4, methacholine and neurokinin NK_1 (= substance P). Opioid receptors seems to be not involved, since activity was not abolished by naloxone.

To these in vitro activities did not correspond in vivo some expectable actions such as the anti-hypertensive one and the inhibition of symptons induced by oxotremorine administration [26]. Such a pharmacological profile is rarely observed and may, therefore, merit further investigations for potential use of the tested substances (particulary **6b**) in localized intestinal disorders such as the so-called irritable bowel syndrome.

Finally, compound **5b** was able to inhibit the growth of *Staphylococcus aureus* (methycillin resistant strain ATCC 33591) with MIC = 10 μg/ml, but was inactive against gram-negative bacteria and fungi.

6. Conclusions

This preliminary investigation has shown that 2-[4-(dialkylaminoalkoxy)phenyl]benzotriazoles and the corresponding N-oxides were able to display several activities in vitro and in vivo, as a general characteristic of this type of compound or as a peculiarity of a single compound. Among the in vitro activities, the antagonism against leukotriene D_4 (common to all tested compounds) and thromboxane A_2 (**5b**) appeared remarkable, while among the in vivo activities the protection of mice from cyanide hypoxia (**5b**, **6b**) and,

even more so, the ability of compound **5h** to reduce serum total cholesterol and heparin precipitating betalipoproteins with potential anti-atherogenic properties were worthy of note.

Therefore investigations on this type of benzotriazole derivative will be pursued.

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