New Antianginal Nitro Esters with Reduced Hypotensive Activity. Synthesis and Pharmacological Evaluation of 3-[(Nitrooxy)alkyl]-2*H*-1,3-benzoxazin-4(3*H*)-ones

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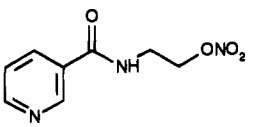
New nitro ester 3-[(nitrooxy)alkyl]-2*H*-1,3-benzoxazin-4(3*H*)-ones show marked inhibitory activity against ischemia-induced electrocardiographic changes, with only limited systemic hemodynamic effects, and are reported in the present study. These new nitro vasodilators are potent inhibitors of the electrocardiographic T-wave and S-T segment elevation induced by intravenous or intracoronary administration of Arg-vasopressin or methacholine in the anesthetized rat. The most active compounds are up to 300- and 600-fold more potent than glyceryl trinitrate or Nicorandil, respectively. These nitro esters relax in a concentration-dependent manner the isolated rabbit aorta, at higher concentrations (2–40-fold) than glyceryl trinitrate, and reduce the mean arterial blood pressure at doses 7–300-fold higher than those required by glyceryl trinitrate to exert a similar hypotensive effect. Remarkably, these compounds retain their anti-ischemic and hemodynamic profile after oral (po) administration. These new nitro ester derivatives, endowed with a marked antianginal activity, which is not associated with concurrent and pronounced falls in systemic blood pressure, represent the leads of a new class of selective nitrovasodilators having a preferential action on large coronary vessels, which could be clinically relevant in the treatment of coronary artery diseases.

Introduction

Organic nitrates and glyceryl trinitrate (GTN) have been mainstays of cardiovascular therapy[1] for many years and of particular benefit in the treatment of an angina pectoris attack,[2,3] unstable angina,[4,5] and early stages of acute myocardial infarction.[6] They have shown a real efficacy in the presence of coronary atherosclerosis, hypercholesterolemia, and other associated vessel wall disorders involving endothelial dysfunction[7,8] and disordered vasodilatory capacity of the coronary arteries.[8,9] This group of drugs termed nitrovasodilators have a unique spectrum of efficacy, in that they dilate at low doses the large coronary conductance vessels and, as the dose is increased, dilate resistance vessels as well.[9] However, the preferential action of nitrovasodilators on large epicardial arteries is often limited by the concurrent effect on the arteriolar vessels,[10,11] which leads to a fall in coronary vascular resistance, a fall in mean arterial blood pressure (MABP), and a reflex increase in heart rate (HR). Despite the structural diversity of the molecular entities[12] belonging to this class, very few coronary dilators have shown a potent antianginal activity not associated with the effect on small coronary and systemic vessels and thus with limited hypotensive effect.

Nicorandil (1, Chart 1) is a hybrid molecule endowed with both a potassium channel-opening activity associated with the nicotinamide moiety and a guanylate cyclase activation property, attributed to the nitro ester side chain.[13] In order to obtain new organic nitrates devoid of any potassium channel-opening activity, we have designed new aryl amides of (nitrooxy)alkylamines, in which the pyridine ring was substituted with a simple aromatic group. Some of these compounds were chemically unstable because of the presence of a primary amide group. So this group was blocked as an intramolecular aminal using 2-hydroxybenzoic acid as an aromatic ring, giving 2*H*-1,3-benzoxazin-4(3*H*)-one molecules.

Chart 1



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This paper reports a systematic investigation on the synthesis and evaluation of a new series of compounds derived from 3-[(nitrooxy)alkyl]-2*H*-1,3-benzoxazin-4(3*H*)-ones (**6a–q**). The biological activities were compared with those of GTN and Nicorandil as reference compounds. ITF 296 (**6a**),[14] the lead compound of this series, was previously reported as a potent anti-ischemic agent[15,16] whose action being, at low doses, devoid of major systemic hemodynamic changes,[17,18] suggesting a marked preferential action on large coronary vessels.

Chemistry

Compounds **6a–o** were synthesized starting from the corresponding methyl salicylic esters (**2a–o**) which were converted to 2-hydroxy-*N*-(hydroxyalkyl)benzamides (**3a–o**) according to the procedure described by Black and Wade[19] (Scheme 1). The conversion of benzamides **3a–o** to the corresponding 3-substituted-2,3-dihydro-4*H*-1,3-benzoxazin-4(3*H*)-ones (**4a–o**) was carried out in a two-step procedure involving a cyclization with paraformaldehyde under acidic conditions[20] and the hydrolysis of the intermediate. The nature of the intermediate depends on the acid used in the cyclization. If *p*-toluenesulfonic acid was used, beside the ring-closing reaction, there was the formation of acetals on

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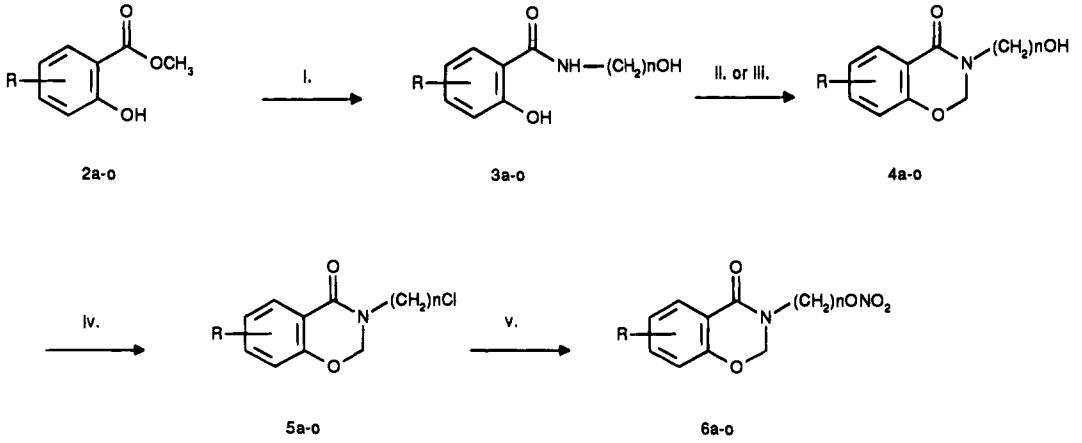
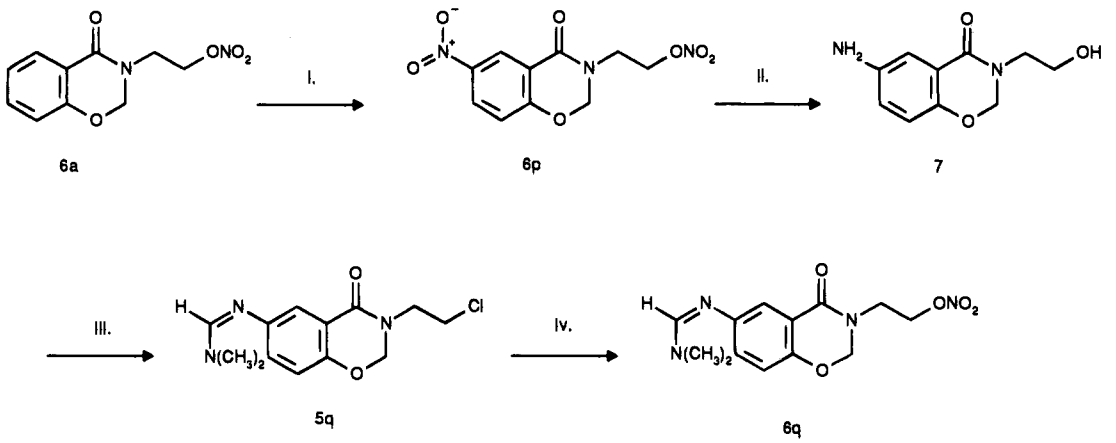
Scheme 1[a]



[a] (i) $NH_2(CH_2)_nOH$, 170 °C; (ii) $(CH_2O)_n$, AcOH, HCl, $CHCl_3$, then Na_2CO_3, CH_3OH; (iii) $(CH_2O)_n$, *p*-toluensulfonic acid, toluene, reflux, then HCl, THF; (iv) $SOCl_2$, $CHCl_3$, reflux; (v) $AgNO_3$, acetonitrile, reflux.

Scheme 2[a]



[a] (i) HNO_3, −10 °C; (ii) H, Pd/C, MeOH; (iii) $SOCl_2$, DMF, 0 °C; (iv) $AgNO_3$, acetonitrile.

the terminal hydroxy group, and in this case the hydrolysis was obtained with aqueous acid. If hydrochloric acid and acetic acid were used, beside the cyclization, the acetylation of the terminal hydroxy group occurs. It was then hydrolyzed using aqueous inorganic bases. Treatment of alcohols **4a–o** with thionyl chloride gave chloro derivatives **5a–o** which were converted into nitrate esters **6a–o** by reaction with silver nitrate in refluxing acetonitrile.[21]

6-Nitro compound **6p** was obtained directly from nitration of **6a** with nitric acid (Scheme 2). Catalytic hydrogenation of **6p** yielded the amino alcohol **7** which was transformed into compound **5q** by reaction with thionyl chloride in the presence of dimethylformamide. Compound **5q** was converted into **6q** using standard conditions.

All new organic nitrates **6a–q** were isolated by flash chromatography, recrystallized, and characterized by ^{1}H-NMR and elemental analysis. The analytical data corresponding to each compound are reported in Table 1.

Results

In Vitro and In Vivo Pharmacology. The compounds **6a–q** were tested for their vasorelaxing activity in the isolated endothelium-denuded rabbit aorta and compared with GTN or Nicorandil. All the compounds studied induced a concentration-dependent relaxation of the rabbit aorta precontracted by norepinephrine, being less active (IC_{50}: from 2.1×10^{-8} to 9.8×10^{-8} M) than GTN (EC_{50}: 1.6×10^{-8} M) (Table 2). Some of them (**6e,i,n,q**) presented a very poor vasorelaxing activity ranging from 20- to 90-fold lower than that of GTN. Nicorandil (IC_{50}: 1.1×10^{-6} M) was found to be a very weak vasorelaxing compound, being markedly less active than GTN. As compared with compounds **6a–q**, Nicorandil was about 2-fold less active than the weakest vasorelaxing compound of this series (**6q**).

In vivo, even though no correlation was found between the vasorelaxing activity and the hypotensive effect, the same overall pattern was observed; none of the compounds **6** tested was more active than GTN in reducing MABP. The most active compound in reducing MABP was 7-fold less active than GTN (**6l**, ED_{20}: 37 vs 5.4 μg/kg). Nicorandil induced a decrease in MABP, with a potency 30-fold lower than that of GTN (ED_{20}: 160 μg/kg).

Antianginal Effect. 2*H*-1,3-Benzoxazin-4(3*H*)-ones **6a–q**, given intravenously, inhibited the ECG changes (induced by the intravenous administration of vasopressin). All the compounds tested have shown a significant and dose-dependent inhibition of the T-wave elevation, being 5-fold (compound **6m**, ED_{50}: 107 μg/kg) to more than 200-fold (compound **6i**, ED_{50}: 1.5 μg/kg; **6a**, ED_{50}: 2.1 μg/kg) more active than GTN (ED_{50}:

Table 1. Physical Data for Compounds **6a–q**

compd	R	n	mp (°C)	^{1}H-NMR (200 MHz, DMSO)	anal.[a]
6a	H	2	49–51 (*n*-hexane)	7.81 (1H,dd), 7.52 (1H,dt), 7.14 (1H,t), 7.05 (1H,d), 5.36 (2H,s), 4.71 (2H,t), 3.85 (2H,t)	$C_{10}H_{10}N_2O_5$
6b	H	3	41–42 (*n*-hexane)	7.82 (1H,dd), 7.53 (1H,dt), 7.16 (1H,t), 7.07 (1H,d), 5.35 (2H,s), 4.57 (2H,t), 3.88 (2H,t), 1.99 (2H,m)	$C_{11}H_{12}N_2O_5$
6c	H	5	39–40 (*n*-hexane)	7.78 (1H,dd), 7.51 (1H,dt), 7.14 (1H,t), 7.04 (1H,d), 5.30 (2H,s), 4.50 (2H,t), 3.45 (2H,t), 1.76–1.26 (6H,m)	$C_{13}H_{16}N_2O_5$
6d	5-CH_3	2	71–73 (*n*-hexane)	7.38 (1H,t), 6.96 (1H,d), 6.91 (1H,d), 5.28 (2H,s), 4.71 (2H,t), 3.86 (2H,t), 2.60 (3H,s)	$C_{11}H_{12}N_2O_5$
6e	8-CH_3	2	75–76 (*n*-hexane)	7.65 (1H,d), 7.41 (1H,d), 7.06 (1H,t), 5.38 (2H,s), 4.73 (2H,t), 3.86 (2H,t), 2.21 (3H,s)	$C_{11}H_{12}N_2O_5$
6f	7-CH_3	2	89–91 (Et_2O–*n*-hexane)	7.96 (1H,d), 7.67 (1H,d), 6.85 (1H,s), 5.32 (2H,s), 4.69 (2H,t), 3.82 (2H,t), 2.32 (3H,s)	$C_{11}H_{12}N_2O_5$
6g	7-Cl	2	86–88 (*n*-hexane)	7.81 (1H,d), 7.24 (1H,s), 7.22 (1H,d), 5.42 (2H,s), 4.72 (2H,t), 3.86 (2H,t)	$C_{10}H_9ClN_2O_5$
6h	7-OCH_3	2	97–98 (*n*-hexane)	7.72 (1H,d), 6.73 (1H,dd), 6.62 (1H,d), 5.35 (2H,s), 4.72 (2H,t), 3.83 (2H,t), 3.82 (3H,s)	$C_{11}H_{12}N_2O_6$
6i	7-CF_3	2	50–51 (*n*-hexane)	8.02 (1H,d), 7.52 (1H,m), 7.50 (1H,m), 5.49 (2H,s), 4.74 (2H,t), 3.90 (2H,t)	$C_{11}H_9F_3N_2O_5$
6l	6-CH_3	2	76–78 (Et_2O–*n*-hexane)	7.61 (1H,d), 7.35 (1H,dd), 6.97 (1H,d), 5.33 (2H,s), 4.72 (2H,t), 3.86 (2H,t), 2.30 (3H,s)	$C_{11}H_{12}N_2O_5$
6m	6-Cl	2	98–99 (*n*-hexane)	7.72 (1H,d), 7.57 (1H,dd), 7.12 (1H,d), 5.38 (2H,s), 4.70 (2H,t), 3.84 (2H,t)	$C_{10}H_9ClN_2O_5$
6n	6-OCH_3	2	62–63 (*n*-hexane)	7.28 (1H,d), 7.14 (1H,dd), 7.03 (1H,d), 5.33 (2H,s), 4.70 (2H,t), 3.87 (2H,t), 3.78 (3H,s)	$C_{11}H_{12}N_2O_6$
6o	6-CN	2	132–134 (AcOEt)	8.21 (1H,d), 8.01 (1H,dd), 7.30 (1H,d), 5.50 (2H,s), 4.73 (2H,t), 3.88 (2H,t)	$C_{11}H_9N_3O_5$
6p	6-NO_2	2	97–99 (Et_2O)	8.54 (1H,d), 8.40 (1H,dd), 7.36 (1H,d), 5.56 (2H,s), 4.74 (2H,t), 3.90 (2H,t)	$C_{10}H_9N_3O_7$
6q	6-N=CHN(CH_3)$_2$	2	140–141 (Et_2O)	12.27 (1H,s), 8.79 (1H,s), 7.97 (1H,d), 7.84 (1H,dd), 7.19 (1H,d), 5.41 (2H,s), 4.74 (2H,t), 3.89 (2H,t), 3.37 (6H,s)	$C_{13}H_{17}ClN_4O_5$

[a] Combustion analyses gave results within 0.4% of theoretical values.

Table 2. Antianginal and Hypotensive Effects of Compounds **6a–q**

compd	R	n	vasorelaxation IC_{50}[a]	MABP ED_{20}[b]	ECG changes ED_{50}[c]	selectivity index[d]
6a	H	2	0.094	190	2.1	90
6b	H	3	0.077	1800	4.1	44
6c	H	5	0.098	730	58	13
6d	5-CH_3	2	0.125	300	4.6	65
6e	8-CH_3	2	0.360	880	58.2	15
6f	7-CH_3	2	0.110	220	12	17.5
6g	7-Cl	2	0.070	790	11	72
6h	7-OCH_3	2	0.047	290	2.0	147
6i	7-CF_3	2	0.220	>300	1.5	200
6l	6-CH_3	2	0.035	37	6.0	8.6
6m	6-Cl	2	0.037	88	107	0.87
6n	6-OCH_3	2	0.230	>1000	5.1	421
6o	6-CN	2	0.071	>300	11.5	26
6p	6-NO_2	2	0.021	76	64	1.2
6q	6-N=CHN(CH_3)$_2$	2	0.640	1270	9.7	131
Nicorandil			1.1	160	940	0.17
GTN			0.016	5.4	545	0.01

[a] Vasorelaxation of the endothelium-denuded rabbit aorta, expressed as IC_{50} (μM). [b] Reduction in mean arterial blood pressure, expressed as ED_{20} (μg/kg), after intravenous administration. [c] Inhibition of vasopressine-induced ECG changes, expressed as ED_{50} (μg/kg). [d] Selectivity index as the ratio of doses required to induce a reduction in MABP and that required to prevent ECG changes.

545 μg/kg) (Table 2). Remarkably, under such experimental conditions, Nicorandil (ED_{50}: 940 μg/kg) presented a weak preventive action against electrocardiographic changes induced by ischemia, being about 2-fold less active than GTN and much less potent than compounds **6a,b,l,n,o,q.** The inhibitory effect of compound **6a** on T-wave elevation is illustrated in Figure 1.

In order to assess the anti-ischemic potential of 2*H*-1,3-benzoxazin-4(3*H*)-ones **6**, the main active compounds (ED_{50} < 10 μg/kg in the vasopressin assay) were tested for their capacity to inhibit the S-T segment elevation induced by the intracoronary administration of methacholine, which was shown to be a very potent coronary vasoconstrictor in the rat, most likely mimicking a coronary vasospasm situation.[22] The antianginal actions of compounds **6a,b,l,n,q** were fully retained on this model as well (ED_{50}: 1.3, 1.0, 0.41, 13.0, and 17.1 μg/kg, respectively) (Table 3). ITF 296 (compound **6a,** ED_{50}: 1.3 μg/kg) induced a significant prevention of the ECG changes, being at least 30-fold more active than GTN (ED_{50}: 36 μg/kg) and Nicorandil (ED_{50}: 47 μg/kg) (Table 3). The most active compound in preventing ischemia-induced electrocardiographic changes, namely compound **61,** was about 100-fold more potent than GTN or Nicorandil.

Remarkably, the potent anti-ischemic activity of compounds **6a,l,n** was retained even after oral (po) administration (ED_{50}: 118, 142, and 100 μg/kg) (Table 3). Under similar experimental conditions, Nicorandil has been reported[23] to reduce by 50% the methacholine-induced S-T segment elevation at 3 mg/kg, while GTN was almost inactive in various models of angina after oral administration.[24,25]

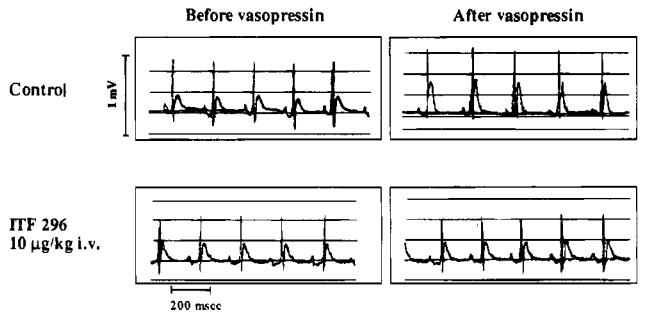


Figure 1. Representative tracings of ECG changes induced by iv administration of vasopressin (1 IU/kg) in control and ITF 296-treated rats. Vasopressin was administered 10 min after vehicle or drug. The T-wave elevation reached the maximum 30 s after the administration of vasopressin.

Table 3. Effect of Compounds **6** on Methacholine-Induced ECG Changes in the Anesthetized Rat

compd	ECG changes Meth-ED_{50} iv[a]	ECG changes Meth-ED_{50} po[a]
6a	1.3	118
6b	1.0	–
6d	>10	–
6h	>10	>300
6i	82	>300
6l	0.41	142
6n	13	100
6q	17.1	>300
Nicorandil	47	3000[b]
GTN	36	*c*

[a] Inhibition of methacholine-induced ECG changes in the anesthetized rat, expressed as ED_{50} (μg/kg), after intravenous (iv) or oral (po) administration. [b] Reference 23. [c] Reference 24.

Hemodynamic Parameters. 2*H*-1,3-Benzoxazin-4(3*H*)-ones **6** at doses which, after intravenous administration prevent significantly the T-wave or S-T segment elevation, were devoid of any significant hypotensive effect (Table 2). ITF 296 (compound **6a**) was found to induce a marked inhibition of the S-T segment elevation (ED_{50}: 2.1 μg/kg), while a 90-fold higher dose was required to induce 20% reduction of mean arterial blood pressure (ED_{20}: 190 μg/kg). The selectivity index taken as the ratio of anti-ischemic activity vs hypotension was higher than that of either GTN or Nicorandil. Compounds **6b,d,h,i,o,q** have also shown a quite good range of selectivity (Table 2). The selectivity was maintained after oral administration as well. Compound **6a,** after oral administration, prevented the methacholine-induced ECG changes with an ED_{50} of 118 μg/kg, while a dose higher than 3 mg/kg, per os, was required to lower the MABP by about 20%. Remarkably, at doses corresponding to the ED_{50} required to trigger a potent anti-ischemic effect, no consistent effect on heart rate was noted, assessing the primary observation suggested that these compounds are poor hypotensive agents.

Conversely, GTN exerted its antianginal action at doses (ED_{50}: 545 μg/kg) at least 100-fold higher than that required to induce a significant hypotensive response (ED_{20}: 5.4 μg/kg) (Table 2). A slight increase in heart rate accompanied the 20% reduction in the mean arterial blood pressure. Moreover, Nicorandil, at the dose corresponding to the ED_{50} (940 μg/kg) of the anti-ischemic action, induced a marked fall in MABP (−33%) and a significant increase in heart rate (25%).

Chart 2

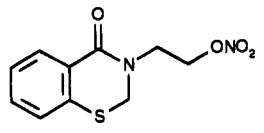


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Table 4. QSAR Parameters

compd	R	selectivity index[a]	log selectivity index	log *P*[b]	σ **6**[c]	σ **7**[c]
6a	H	90	1.954	1.85	0.00	0.00
6f	7-CH_3	17.5	1.243	2.41	0.00	0.07
6g	7-Cl	72	1.857	2.56	0.00	0.37
6h	7-OCH_3	147	2.167	1.83	0.00	0.12
6i	7-CF_3	200	2.301	2.73	0.00	0.43
6l	6-CH_3	8.6	0.934	2.41	−0.17	0.00
6m	6-Cl	0.87	−0.060	2.56	0.23	0.00
6n	6-OCH_3	421	2.624	1.83	−0.27	0.00
6o	6-CN	26	1.415	1.28	0.66	0.00
6p	6-NO_2	1.2	0.079	1.57	0.78	0.00

[a] See Table 3. [b] log *P* value for **6a** was experimentally determined, and the others were calculated using tabulated additive values starting from the log *P* of the unsubstituted compound **6a**. [c] Electrostatic effect of the substituents toward the oxygen in position 1 of the benzoxazin-4-one.

Discussion

The structure−activity study was mainly based on the definition of the role of the (nitrooxy)alkylamine chain and the influence of the presence of substituents at the 5−8 positions of the benzoxazin-4-one ring in the derivative containing a (nitrooxy)ethyl side chain. The elongation of the methylene chain of the alkanolamine from two to five (compounds **6b,c** vs **6a**) did not influence the vasorelaxing activity but appeared to be responsible for decreasing the antianginal activity.

The substitution of positions 5−7 with a methyl group led to compounds with both a slightly reduced vasorelaxing and anti-ischemic activity (compounds **6d,f,**l vs **6a**). However a methyl group in position 8 caused a strong decrease in both of these activities (compound **6e** vs **6a**). Due to the steric hindrance of the methyl group, this data suggests the critical role of oxygen in position 1 of the 2*H*-1,3-benzoxazin-4(3*H*)-one molecule. This indication was confirmed by the strong reduction of the activities when we substituted the oxygen with heteroatoms such as in compound **8** (Chart 2).[14] The substitution of position 7 with electron-donating or electron-withdrawing groups led to compounds with a slightly reduced anti-ischemic activity as compared with the nonsubstituted compound (**6f-i** vs **6a**), indicating that this position is not sensitive to the electronic influence of the substituent. The substitution of the 6 position with an electron-donating group, such as a methyl or methoxy group, led to compounds with good antianginal activity (**6l,n** vs **6a**). Conversely, the substitution with an electron-withdrawing group in the 6 position, such as nitro or chlorine, reduced considerably the anti-ischemic activity (**6m,p** vs **6a**).

Quantitative structure−activity relationships were investigated for compounds **6a,f−q** (Table 4), using the selective index (Table 2) as the biological variable, electronic parameters (σ) of the substituents, and the log *P* of the compounds. The linear regression analysis, performed using 2R stepwise regression of BMDP

statistical package,[26] yielded to eq 1

$$\log(\text{selective index}) = 5.33 - 1.90 \log P - 2.59\sigma_6 + 4.47\sigma_7 \quad (1)$$

$$n = 10 \qquad r^2 = 0.9173 \qquad (p < 0.05)$$

From this equation it seems that in order to have a high selectivity index it is necessary to have the presence of an electron-donating group in position 6 and an electron-withdrawing group in position 7 of the 2*H*-1,3-benzoxazin-4(3*H*)-one system. However the introduction of substituent must not increase the hydrophobicity of the final molecule too much. Further detailed study of structure–activity is now necessary to identify other substituents and their respective physicochemical parameters allowing to increase the antianginal potency and/or the coronary selectivity.

Benzoxazinone nitro ester derivatives have shown a substantially different hemodynamic profile than reference compounds tested in this study. Conversely to GTN or Nicorandil, their antianginal activity occurred at doses which did not affect systemic pressure and heart rate. The design and synthesis of new 2*H*-1,3-benzoxazin-4(3*H*)-ones, using this structure–activity strategy, are currently under investigation in our laboratory, for assessing the molecular and biochemical rationales of their potent antianginal activity, not associated with significant changes in systemic hemodynamic parameters.

Among the main active compounds (**6a,b,d,h,i,l,n,o,q**), only compound **6l** has shown a relatively small range of selectivity in that its antianginal effect took place at doses 10-fold lower than those required for inducing a concurrent hypotension. The other active compounds from this series are much more selective than either Nicorandil or GTN. Furthermore, the antianginal activity and the small reduced effect on systemic hemodynamic parameters were retained after oral administration as well, differentiating ITF 296 (**6a**) and its analogues from GTN, whose antianginal efficacy is strongly limited by its extensive first-pass effect.[24]

This structure–activity suggests that compounds **6a–q** may induce a preferential dilatatory effect on coronary vessels and a very limited vasodilatation of the arteriolar vessels which would support their potential coronary selectivity and thus their potential beneficial therapeutic action in angina pectoris. Supporting this assertion, the main active compound, ITF 296, has been reported to selectively increase the diameter of epicardial coronary arteries at doses which do not affect coronary resistances or systemic hemodynamics.[16] The relaxation of vascular smooth muscle induced by ITF 296 is endothelium-independent and inhibited "in vitro" by methylene blue or hemoglobin, suggesting that the relaxation is mediated by a NO-dependent activation of guanylate cyclase.[15] Moreover, the vasorelaxating effect of ITF 296 on the isolated rabbit femoral artery was not affected by glibenclamide (data not shown), thus excluding an action on ATP-dependent K^+-channels and differentiating this compound from Nicorandil.

The selective action of nitrovasodilators on large coronary arteries may be mediated by a different catalytic process involved in vessels of different size, modulated by the presence or absence of sulfhydryl groups, required for the conversion of and the NO release from this compound and the final activation of soluble guanylate cyclase.[27] According to this hypothesis, the coronary selectivity seen with compounds **6** could be the result of a conversion involving a different enzymatic pathway or alternatively a difference in the kinetics of NO release at different levels of the vascular tree.

Since hypotension and reflex tachycardia are well-known major side effects in the therapeutic daily use of nitro derivatives such as GTN, compounds **6** could therefore offer an attractive alternative to the classical nitrate therapy of the coronary heart diseases on account of their improved antianginal efficacy and limited systemic effects. In addition, the long lasting anti-ischemic effect of ITF 296 in the rat[17] as well as the maintenance of its vasodilating action on coronary artery during long term administration in the conscious dog[28] could witness a reduced tolerance phenomenon and thus add to the beneficial action of such agents on myocardial protection.

Experimental Section

General. Melting points were determined on a Buchi apparatus in glass capillary tubes and are uncorrected. Thin-layer chromatography was performed on silica gel glass-backed plates (5719) purchased from E. Merck & Co., and flash chromatography was performed on silica gel 60 (230–400 mesh ASTM) (E. Merck & Co). ^{1}H-NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer, and the chemical shifts are given in ppm (δ) referenced to DMSO-d_6 (2.50 ppm). Elemental analyses were carried out on a Perkin Elmer 240 instrument. The analytical data of compounds **6a–q** are illustrated in Table I.

***N*-(2-Hydroxyethyl)-2-hydroxybenzamide (3a).** The compound was synthesized using the procedure described by Black and Wade.[19]

Compounds 3b–o were synthesized following the same procedure, and the analytical data (^{1}H-NMR and elemental analysis) were in agreement with the proposed structure.

3-(2-Hydroxyethyl)-2*H*-1,3-benzoxazin-4(3*H*)-one (4a). Method A. Paraformaldehyde (5.5 g, 182 mmol) and acetic acid (11 mL) were added to a solution of 2-hydroxybenzamide **3a** (18.5 g, 102 mmol) in $CHCl_3$ (500 mL). The reaction mixture was cooled to 0 °C, and then the solution was saturated with HCl gas. After the reaction mixture was stirred for 24 h at room temperature, the chloroformic phase was separated from an oily residue by decantation, washed with water (200 mL), and dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The crude product was dissolved in methanol (400 mL), and Na_2CO_3 (5.4 g, 51 mmol) was added. The reaction mixture was stirred at room temperature for 12 h; then the salts were filtered off, and the solvent was evaporated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (400 mL) and water (200 mL). The two phases were separated, the organic phase was dried with Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2–acetone, 9:1), yielding 9.5 g of pure **4a** (yield 45%): mp 59–60 °C (*n*-hexane); ^{1}H-NMR (200 MHz, DMSO) δ 7.82 (1H, dd), 7.54 (1H, td), 7.18 (1H, t), 7.07 (1H, d), 5.34 (2H, s), 3.57 (4H, s). Anal. ($C_{10}H_{11}NO_3$) C,H,N.

Method B. Paraformaldehyde (28 g, 927 mmol) and *p*-toluenesulfonic acid (5.8 g, 31 mmol) were added to a solution of salicylamide **3a** (56 g, 310 mmol) in toluene (840 mL). The reaction mixture was refluxed, and water was removed by azeotropic distillation (for 2 h). The mixture was cooled at room temperature, and the solvents were evaporated under reduced pressure. The crude mixture was dissolved in THF (600 mL), and a 3 N aqueous solution of HCl (600 mL) was added. The solution was refluxed, and after 24 h the reaction mixture was cooled to room temperature, THF was removed

under reduced pressure, and the aqueous solution was extracted with ethyl acetate (2 × 400 mL). The organic phase was dried with anhydrous Na_2SO_4, and the solvents were evaporated under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane–ethyl acetate, 1:1), yielding 45 g of pure **4a** (yield 75%).

Compounds 4b–o were synthesized starting from compounds **4b–r** using either method A or method B, and the analytical data (^{1}H-NMR and elemental analysis) were in agreement with the proposed structure.

3-(2-Chloroethyl)-2*H*-1,3-benzoxazin-4(3*H*)-one (5a). Thionyl chloride (3.54 g, 48 mmol) was slowly added at 0 °C to a solution of alcohol **4a** (9 g, 46 mmol) in $CHCl_3$ (70 mL), and the solution was refluxed for 3 h. The solvent was removed under reduced pressure, and the crude product was dissolved in fresh $CHCl_3$ (50 mL) and evaporated to dryness three times. Purification of the crude product by flash chromatography (*n*-hexane–ethyl acetate, 8:2) yielded 9.3 g of pure **5a** (yield 95%): mp 48–49 °C (*n*-hexane); ^{1}H-NMR (200 MHz, DMSO) δ 7.83 (1H, dd), 7.53 (1H, dt), 7.16 (1H, t), 7.06 (1H, d), 5.39 (2H, s), 3.83 (4H, s). Anal. ($C_{10}H_{10}ClNO_2$) C,H,Cl,N.

Compounds 5b–o were synthesized using the same procedure, and the analytical data (^{1}H-NMR and elemental analysis) were in agreement with the proposed structure.

3-[2-(Nitrooxy)ethyl]-2*H*-1,3-benzoxazin-4(3*H*)-one (6a). A solution of **5a** (5 g, 23 mmol) and silver nitrate (6 g, 35 mmol) in acetonitrile (85 mL) was refluxed, and after 2 h the reaction mixture was cooled to room temperature, the salts were filtered off, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (*n*-hexane–ethyl acetate, 8:2). The product was then treated with hot *n*-hexane, filtered, and dried, yielding 4.8 g of pure **6a** (yield 88%). Analytical data are illustrated in Table 1.

Compounds 6b–o were synthesized using the same procedure starting from compounds **5b–o**, and the analytical data of each compound are illustrated in Table 1.

3-[2-(Nitrooxy)ethyl]-6-nitro-2*H*-1,3-benzoxazin-4(3*H*)-one (6p). Fuming nitric acid (50 mL, 1.2 mol) was added dropwise to solid **6a** (50 g, 210 mmol), and the solution was stirred at −10 °C for 15 min and then poured into ice and extracted with $CHCl_3$ (3 × 1000 mL). The combined organic phases were washed with water, then with 0.1 N solution of NaOH, and finally with water again. The solution was then dried with anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was treated with hot ethyl ether, filtered, and dried, yielding 43 g of **6p** (yield 70%). Analytical data are illustrated in Table 1.

3-(2-Hydroxyethyl)-6-amino-2*H*-1,3-benzoxazin-4(3*H*)-one (7). Pd/C 10% (4.2 g) was added to a solution of **6p** (42 g, 148 mmol) in methanol (6300 mL), and the mixture was hydrogenated at room temperature at 15 psi. When the hydrogen consumption was over, the catalyst was filtered off and the solvent was removed under reduced pressure. The crude product was treated with hot ethyl ether, filtered, and dried, yielding 30 g of **7** (yield 88%): mp 94–96 °C (ethyl ether); ^{1}H-NMR (200 MHz, DMSO) δ 7.02 (1H, s), 6.78 (2H, m), 5.18 (2H, s), 5.05 (2H, s), 4.09 (1H, t), 3.53 (4H, m). Anal. ($C_{10}H_{12}N_2O_3$) C,H,N.

3-(2-Chloroethyl)-6-(*N',N'*-dimethyl-*N*-formimido)-2*H*-1,3-benzoxazin-4(3*H*)-one (5q). Amino alcohol **7** (10 g, 48 mmol) was slowly added to a solution of thionyl chloride (200 mL) and dimethylformamide (5 mL, 64 mmol) at 0 °C. The reaction mixture was then poured into ice, and the aqueous solution was neutralized with solid NaOH at 0 °C and then extracted with $CHCl_3$ (3 × 100 mL). The combined organic phases were dried with anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was treated with ethyl ether, filtered, and dried, yielding 9.2 g of pure **5q** (yield 67%): mp 94–95 °C (ethyl ether); ^{1}H-NMR (200 MHz, DMSO) δ 7.75 (1H, s), 7.30 (1H, d), 7.10 (1H, dd), 6.93 (1H, d), 5.32 (2H, s), 3.82 (4H, s), 3.01 (3H, s), 2.93 (3H, s). Anal. ($C_{13}H_{16}ClN_3O_2$) C,H,Cl,N.

3-[2-(Nitrooxy)ethyl]-6-(*N',N'*-dimethyl-*N*-formimido)-2*H*-1,3-benzoxazin-4(3*H*)-one Hydrochloride (6q). A solution of **5q** (9, g, 32 mmol) and silver nitrate (7.7 g, 45 mmol) in acetonitrile (100 mL) was refluxed for 2 h. The reaction was cooled to room temperature, the salts were filtered off, and the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl ether and treated with a solution of HCl in ethyl ether. The solid was filtered and dried, yielding 4.5 g of pure **6q** (yield 41%). Analytical data are illustrated in Table 1.

Evaluation of Vasorelaxing Activity. The vasorelaxation induced by each compound was evaluated in the endothelium-denuded rabbit aorta strip previously contracted by norepinephrine.[29] Male New Zealand rabbits weighing 2–3 kg were sacrificed by a blow on the neck. The thoracic aorta was exposed, rapidly dissected out from surrounding tissues, and cut into rings (0.3 cm length) whose endothelium was removed by gently rubbing the luminal surface with a cotton bud. Rings were suspended in organ chambers containing 10 mL of warm (37 °C), oxygenated (95% O_2, 5% CO_2) Krebs–Henseleit (pH: 7.4) solution of the following composition (mM): NaCl, 113; KCl, 4.7; $CaCl_2$ $2H_2O$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; K_2HPO_4, 1.2; and glucose, 11.5; and allowed to equilibrate for 2 h under a resting tension of 2.0 g.

At the end of the equilibration period, tissues were contracted by adding norepinephrine (final concentration 10^{-7} M) directly to the organ bath and the response was recorded with isometric transducers. When the contraction had reached a stable plateau, dose–response curves were constructed by adding increasing concentrations for each compound, in a cumulative manner, in the organ chamber. IC_{50}s were calculated from the dose–response curves.

Evaluation of Anti-ischemic and Hemodynamic Activities. The in vivo antianginal action was monitored in the urethane-anesthetized rat as the inhibitory effect of the T-wave elevation induced by intravenous bolus of vasopressine[30] and the S-T segment elevation induced by intracoronary administration of methacholine.[22] Both antianginal activities were reported as ED_{50} in μg/kg. The action on mean arterial blood pressure was expressed as the ED_{20}, in μg/kg, corresponding to the dose necessary to induce a 20% reduction of MABP. The heart rate was monitored throughout the experiment.

Male Sprague–Dawley rats, weighing 350–400 g, were anesthetized with urethane (1.25 g/kg) given ip and the trachea cannulated with poly(ethylene)tubing (PE 240) to facilitate breathing. Blood pressure was monitored by a poly(ethylene) catheter (PE 50) inserted into the carotid artery and connected with a Statham pressure transducer (P23ID). Mean arterial blood pressure was continuously recorded on a Battaglia-Rangoni polygraph. Heart rate was derived from the pressure wave. The femoral vein was cannulated (PE 50) to allow the compounds administration which were given in a final volume of 1 mL/kg over 20 s. Results are expressed as percent of variation over basal values. ED_{20}s (corresponding to the dose necessary to induce a 20% reduction of the MABP) were calculated from the dose–response curve.

The standard limb lead II of the electrocardiogram was recorded on a Battaglia-Rangoni electrocardiograph. Arg^8-vasopressin (1 IU/kg) was administered into the femoral vein in a final volume of 1 mL/kg over 20 s. The Arg^8-vasopressin-induced rise in T-wave amplitude was measured in millivolts. Each compound, or the corresponding vehicle, was administered intravenously 10 min before the injection of vasopressin. GTN was given 1 min before. Groups of 8 rats were used for each tested dose. For each group, the percent of the inhibition of vasopressin-induced T-wave elevation was calculated from the ratio of the mean T-wave elevation over the mean value of the control group. ED_{50}s were calculated from the dose–effect regression line.

In a separate group of animals, a poly(ethylene) (PE 50) tube was inserted into the coronary ostia through the right carotid artery to a point near the aortic valve to allow the administration of methacholine into the coronary arteries. The S-T-wave elevation induced by methacholine, given at 8.0 μg in 0.02 mL over 1 s, was measured and expressed in millivolts. The methacholine injection was repeated at different times after drug administration. Drugs were administered intravenously or orally to separate groups of rats, each animal receiving a

single administration. Groups of five rats were used for each tested dose. The inhibition of methacholine-induced S-T segment elevation was calculated in percent over basal value. ED_{50}s were calculated from the dose–effect regression line.

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