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Pharmacological evaluation of both enantiomers of (R,S)-BM-591 as thromboxane A₂ receptor antagonists and thromboxane synthase inhibitors

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Abstract

The aim of this work is to evaluate the anti-thromboxane activity of two pure enantiomers of (R,S)-BM-591, a nitrobenzene sulfonylurea chemically related to torasemide, a loop diuretic. The drug affinity for thromboxane A₂ receptor (TP) of human washed platelets has been determined. In these experiments, (R)-BM-591 (IC₅₀ = 2.4 ± 0.1 nM) exhibited a significant higher affinity than (S)-BM-591 (IC₅₀ = 4.2 ± 0.15 nM) for human washed platelets TP receptors. Both enantiomers were stronger ligands than SQ-29548 (IC₅₀ = 21.0 ± 1.0 nM) and sulotroban (IC₅₀ = 930 ± 42 nM), two reference TXA₂ receptor antagonists. Pharmacological characterisations of (S)-BM-591 and (R)-BM-591 were compared in several models. Thus, (R)-BM-591 strongly prevented platelet aggregation induced by arachidonic acid (AA) (600 μM) and U-46619 (1 μM) while (S)-BM-591 showed a lower activity. On isolated tissues pre-contracted by U-46619, a stable TXA₂ agonist, (S)-BM-591 was more potent in relaxing guinea-pig trachea (EC₅₀ = 0.272 ± 0.054 μM) and rat aorta (EC₅₀ = 0.190 ± 0.002 μM) than (R)-BM-591 (EC₅₀ of 9.60 ± 0.63 μM and 0.390 ± 0.052 μM, respectively). Moreover, at 1 μM, (R)-BM-591 totally inhibited TXA₂ synthase activity, expressed as TXB₂ production from human platelets, while at the same concentration, (S)-BM-591 poorly reduced the TXB₂ synthesis (22%). Finally, in rats, both enantiomers lost the diuretic activity of torasemide. In conclusion, (R)-BM-591

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exhibits a higher affinity and antagonism on human platelet TP receptors than (S)-BM-591 as well as a better thromboxane synthase inhibitory potency. In contrast, (S)-BM-591 is more active than the (R)-enantiomer in relaxing smooth muscle contraction of rat aorta and trachea guinea pig. Consequently, (R)-BM-591 represents the best candidate for further development in the field of thrombosis disorders. © 2004 Elsevier Inc. All rights reserved.

Keywords: Thromboxane antagonist; Thromboxane synthase; Pharmacological evaluation; Thrombosis disorders

1. Introduction

Thromboxane A₂ (TXA₂) is a cellular lipidic mediator resulting from metabolism of arachidonic acid by cyclooxygenases and thromboxane synthase [1] (Fig. 1). This prostanoid is mainly produced by platelets, macrophages and lung parenchyma [2]. It is a potent inducer of both platelet aggregation and vascular and bronchial smooth muscles contraction leading to arterial thrombosis, hypertension and bronchoconstriction. Due to these properties, TXA₂ is implicated in several physiopathological states such as myocardial infarction and bronchial asthma [3–5] where high concentrations of this prostanoid have been detected

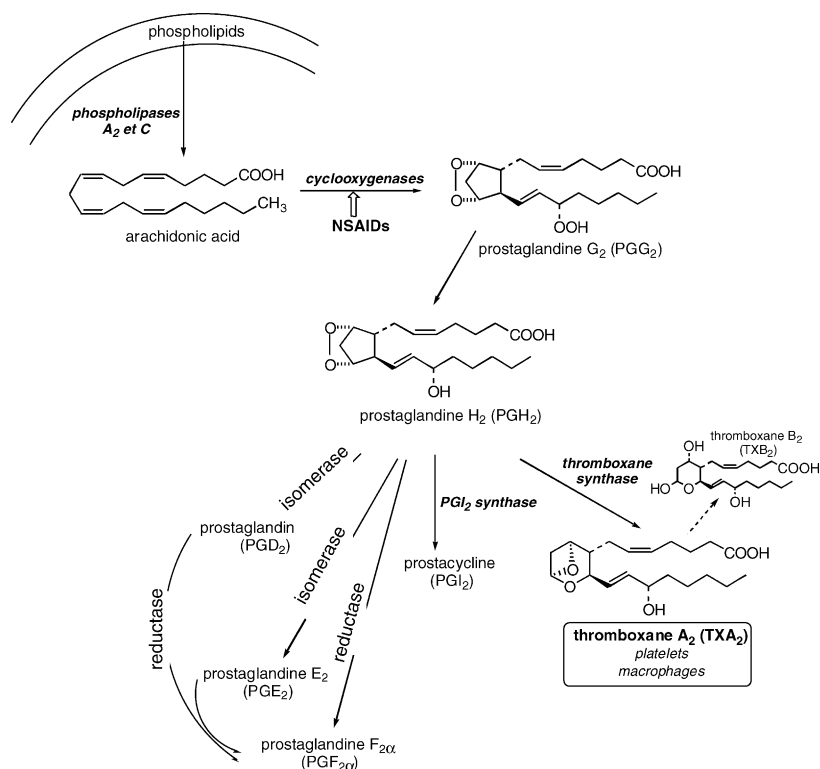


Fig. 1. Thromboxane A₂ production by metabolism of arachidonic acid by cyclooxygenases.

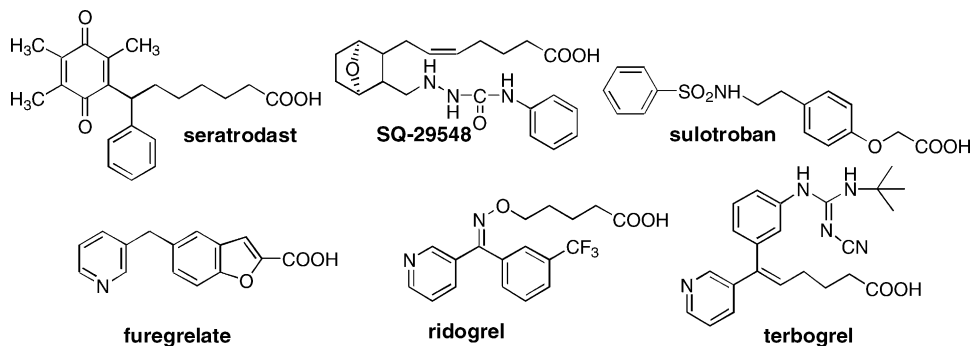


Fig. 2. Chemical structures of reference TXRAs (seratrodist, SQ-29548, sulotroban) and TXSI (furegrelate) or dual TXRA/TXSI compounds (ridogrel, terbogrel).

[6,7]. Thus, molecules combining a dual thromboxane synthase inhibition and TXA₂ receptor antagonist such as ridogrel and terbogrel and picotamide have been developed with the aim of reducing TXA₂ production and preventing the deleterious activity of TXA₂ and PGH₂ at a receptor level (Fig. 2) [8,9]. This type of compounds presents more advantages than the TXA₂ receptor antagonists (TXRA, i.e. SQ-29548, sulotroban) or TXA₂ synthase inhibitors (TXSI, i.e. furegrelate) used alone (Fig. 2). Moreover, it has been demonstrated that drugs acting only on TXA₂ receptors (TP-receptors) reduce the severity of myocardial infarction [10] and bronchial asthma [11]. In 1992, torasemide (Fig. 3), a renal Na⁺ K⁺ 2Cl[−] co-transporter inhibitor was shown to relax the dog coronary artery precontracted by carbocyclic TXA₂ [12,13]. The affinity for TXA₂ receptors and the activity of several compounds chemically related to torasemide were evaluated on smooth muscle TP-receptors and on thromboxane synthase in several in vitro and ex vivo models. These studies

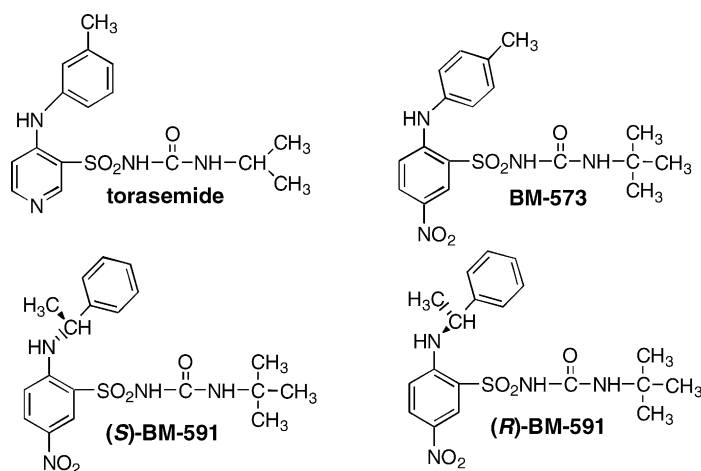


Fig. 3. Chemical structures of torasemide and its original chemically related TXRA, BM-573, (S)-BM-591, (R)-BM-591.

revealed molecules showing a high affinity for human platelet TXA₂ receptor (1 nM < IC₅₀ < 20 nM). Most of these molecules were also able to completely inhibit human TXA₂ synthase at 1 µM and to antagonise smooth muscle TXA₂ receptors [14–17]. The purpose of this work was to evaluate and compare the pharmacological profiles of two enantiomers ((R)-BM-591 and (S)-BM-591)) chemically related to torasemide and BM-573, a potent dual TXRA/TXSI in vitro and in vivo previously studied for the prevention of pig coronary infarction [17–19].

2. Materials and methods

2.1. Drugs and chemicals

Torasemide and its chemically related compounds were synthesized in the Laboratory of Medicinal Chemistry of the University of Liège according to general synthetic pathways previously described [14,15,20]. SQ-29548 was purchased from RBI (Bioblock, Illkirch, France) and sulotroban was synthesized as previously reported [21]. Seratrodast was isolated from BronicaTM, a generous gift of Takeda Chemicals Industry (Osaka, Japan). Concentrated drug solutions were prepared in dimethylsulphoxide (DMSO) and the final concentration of DMSO (0.1%) had no effect on the parameters measured. U-46619 (9α, 11α-methanoepoxy-PGH₂) purchased from Cayman Chemicals Company (Ann Arbor, MI) and supplied in methyl acetate, was diluted with incubation buffer. [5,6-³H]SQ-29548 (0.25 mCi) was obtained from NEN (Boston, MA, USA). Stock solution of sodium arachidonate (5 mM, Sigma, Belgium) was prepared with physiologic serum.

3. Pharmacology

3.1. Radioligand binding assays

The TXA₂ receptor binding studies were carried out on human washed platelet [16,22,23]. Human platelet-rich plasma (PRP) was provided by the Belgian Red Cross. Washed platelet suspension was prepared in a calcium- and magnesium-free Tyrode-Hepes buffer (mM: NaCl 137, KCl 2.7, NaH₂PO₄ 0.4, NaHCO₃ 12, D-glucose 5, Hepes; pH 7.4) to a concentration 2×10^8 cells/mL. Freshly prepared samples of this suspension (500 µL) were incubated with [5,6-³H]SQ-29548 (5 nM final concentration, 100 µL) for 60 min at 25 °C. The displacement was initiated by addition of the studied ligand dissolved in the same buffer (400 µL). After incubation (30 min, 25 °C), ice-cold Tris-HCl buffer (10 mM, pH 7.4; 4 mL) was added, the sample was rapidly filtered through a glass-fibre filter (Whatman GF/C) and the tube rinsed twice with ice-cold buffer (4 mL). The filters were then placed in plastic scintillation vials containing an emulsion-type scintillation mixture (4 mL) and the radioactivity was counted by scintillation spectroscopy. The amount of [5,6-³H]SQ-29548 specifically bound to human platelet TXA₂ receptor (Bs, %) was calculated from the following equation: $Bs = 100 \times (B - NSB) / B_t$, where B_t (total binding) and NSB (non-specific binding) are the radioactivity of [5,6-³H]SQ-29548

(5 nM) bound to the platelets incubated in the absence of any competing ligand and in the presence of unlabelled SQ-29548 (50 μ M), respectively. B is the radioactivity of the filtered platelets incubated with [3 H]SQ-29548 (5 nM) and the studied compound at a fixed concentration of 10^{-6} M or at concentrations ranging from 10^{-5} to 10^{-10} M. In each experiment, NSB varied between 5 and 7% of Bt. For each drug, three concentration–response curves were performed in triplicate. The drug concentration which reduced the amount of specifically bound [3 H]SQ-29548 by 50% (IC₅₀) was determined by non-linear regression analysis (GraphPad Prism software). The results are expressed as mean \pm standard error.

3.2. Human platelet aggregation

The anti-aggregatory potency has been determined according to a turbidimetric method [24]. Platelet-rich plasma and platelet-poor plasma (PPP) were prepared as previously described [14–16,19,23]. Platelet concentration of PRP was adjusted to 3×10^8 cells mL $^{-1}$ by dilution with PPP. Platelet aggregation of PRP was studied [24] using a double-channel aggregometer (Chronolog Corporation) connected to a linear recorder [25]. PRP (225 μ L) was added in a silanized cuvette and stirred (1100 rpm $^{-1}$). Each drug was prepared in DMSO (20 μ L) and preincubated in PRP for 3 min at 37 °C before the addition of the aggregating agent. Platelet aggregation was initiated by addition of 5 μ L arachidonic acid (600 μ M final) or by addition of 10 μ L U-46619 (1 μ M final). To evaluate platelet aggregation, the maximum increase in light transmission was determined from the aggregation curve 6 min after addition of the inducer. The minimal drug concentrations (EC₁₀₀) preventing any platelet aggregation were determined when arachidonic acid was used as inducer. The drug concentrations (EC₅₀) required to induce 50% of platelet aggregation when U-46619 was used as inducer, were calculated by non-linear regression analysis (GraphPad Prism software) from at least four dose-response curves. The results are expressed as mean \pm standard deviation.

3.3. Rat aorta relaxation

Endothelium-denuded thoracic aortic rings obtained from rats (Wistar, 250–300 g) anaesthetized with sodium pentobarbital (40 mg kg $^{-1}$, i.p.) were suspended under a tension of 1 g in Krebs's solution (mM: NaCl 118, KCl 5.4, CaCl₂ 2.5, MgCl₂·6H₂O 1.5, NaHCO₃ 25, NaH₂PO₄ 1.2 and glucose 10; pH 7.4) which was bubbled with O₂–CO₂ 95–5 at 37 °C in a 20 mL tissue bath (EMKA Technologies). The muscle tension of the aortic rings was isometrically recorded with a force-displacement transducer IT1 (EMKA Technologies). The buffer was renewed at 15-min intervals during the equilibrium period (40 min) before exposing the rings to U-46619 (20 nM). When a stable tension was obtained (15 min), the potential antagonist was added at a cumulative increasing concentration to the bath up to the tension returned to the base-line value. The EC₅₀ value of each drug was assessed for at least four concentration–response curves obtained from separate preparations. EC₅₀ is expressed as the concentration which reduces of 50% of the tension induced by U-46619 (20 nM). The EC₅₀ values were calculated by non-linear regression analysis (GraphPad Prism software). The results are expressed as mean \pm standard error.

3.4. Guinea-pig trachea relaxation

Trachea was removed from guinea pigs (Hartley, 250–300 g) anaesthetized with sodium pentobarbital (40 mg kg⁻¹, i.p.), and carefully cleaned of connective tissue. Trachea strips were suspended in the muscle bath (20 mL) and the experiment progressed in the same conditions as those described above for the rat aorta except for the concentration of U-46619 (10 nM), the contraction inducer. The results are expressed as mean \pm standard error.

3.5. Thromboxane synthase activity

PRP and PPP were prepared as previously described [14–16,19,23]. Each drug was dissolved in DMSO and diluted with a Tyrode-Hepes buffer (mM: NaCl 137, KCl 2.7, NaH₂PO₄·H₂O 0.4, D-glucose 5, NaHCO₃ 12, Hepes; pH 7.4). To 900 μ L of PRP, 50 μ L NaCl 0.9% and 10 μ L of drug solution were added. After 6 min incubation at 37 °C under stirring (600 rpm), TXA₂ synthesis was induced by sodium arachidonate as substrate (40 μ L, 600 μ M final concentration). After 4 min, the reaction was stopped by indomethacine (50 μ L at 0.02 M in ethanol). The sample was immediately centrifuged (17,500 \times g, 10 s), the supernatant removed and frozen (–80 °C) until assayed for TXB₂ dosage. Basal and maximal productions of TXB₂ were estimated in the absence and in the presence of arachidonic acid, respectively. Thromboxane synthase activity was expressed as the TXB₂ production which was measured by using a competitive enzyme immunoassay [26] (Thromboxane B₂ Enzyme Immunoassay Kit, Cayman Chemical Company, Ann Arbor, MI, USA).

3.6. Rat diuresis

The methodology was previously described [19,20]. Briefly, Wistar rats (200–250 g) received a single oral saline charge (40 mL kg⁻¹ of NaCl 0.9%) with or without drug (30 mg kg⁻¹) or intraperitoneal injection of the drugs (10 mg kg⁻¹). Rats were allowed free access to food and drink until the beginning of the experiment and were housed in groups of three in metabolism cages. Urine was collected during 4 h after drug administration and diuresis (mL kg⁻¹) was expressed as mean of urinary volume (mL) collected from the cage.

4. Results

In binding experiments, the affinity of (S)-BM-591 and (R)-BM-591 for human platelet TP receptors was evaluated. This affinity was determined on human washed platelets by measuring the ability of these drugs to displace [5,6-³H]SQ-29548, a potent competitive ligand of TP receptors, from its binding site. Concentrations-response curves were realised and led to calculate the required drug concentration (IC₅₀) displacing 50% of the radioligand bound to the human platelets TP receptor (Table 1). The pure enantiomers, (S)-BM-591 (IC₅₀ = 4.2 \pm 0.15 nM) and (R)-BM-591 (IC₅₀ = 2.4 \pm 0.10 nM) showed a higher affinity than that of SQ-29548 (IC₅₀ = 21 \pm 1.0 nM) and sulotroban (IC₅₀ = 930 \pm 42 nM), the two

Table 1
Affinity for the human platelet TP receptor

Drug	TXA2 receptor affinity IC ₅₀ (nM) ^a
SQ-29548	21 ± 1.0
Sulotroban	930 ± 42
Toraseamide	2690 ± 35
BM-573	1.3 ± 0.05 ^b
(R,S)-BM-591	3.5 ± 0.05
(S)-BM-591	4.2 ± 0.15
(R)-BM-591	2.4 ± 0.10

^a Drug concentration required to displace 50% of [³H]SQ-29548 from TP-receptor of human platelets. Values are mean ± S.E.M., *n* = 4.

^b From [17].

TXRAs selected as reference. The IC₅₀ of the racemate BM-591 was intermediate between the affinity of its enantiomers (IC₅₀ = 3.5 nM ± 0.05).

Then, the ability of both enantiomers to prevent human platelet aggregation induced by arachidonic acid (600 μM) or by U-46619 (1 μM), a TXA₂ agonist, has been investigated. Platelet aggregation was dose-dependently prevented by (S)-BM-591 and (R)-BM-591 as observed for SQ-29548 and sulotroban whatever the inducer used. When arachidonic acid was used, the anti-aggregating efficacy of (R)-BM-591 (EC₁₀₀ = 0.12 ± 0.01 μM) was 20 times more potent than that of the other enantiomer, (S)-BM-591 (EC₁₀₀ = 2.5 ± 0.2 μM) but lower than that of SQ-29548 (EC₁₀₀ = 0.034 ± 0.001 μM). In the same conditions, BM-573 was as potent as the R-enantiomer previously studied [17]. Toraseamide (EC₁₀₀ = >100 μM) had no activity while sulotroban (EC₁₀₀ = 10.2 ± 1.05 μM) exhibited a weak TXRA. When U-46619 triggered platelet aggregation, EC₅₀ of (R)-BM-591 and (S)-BM-591 were 0.5 ± 0.1 μM and of 1.3 ± 0.3 μM, respectively.

The antagonism of these original drugs has also been investigated on isolated smooth muscle of rat aorta and guinea-pig trachea, two tissues rich in TP receptors. The required concentration (EC₅₀) reducing of 50% of the smooth muscle tension was calculated from concentration–response curves (Fig. 4). Studies on isolated rat thoracic aorta pre-contracted by U-46619 (20 nM), a TXA₂ receptor agonist, showed that (S)-BM-591 (EC₅₀ = 188 ± 2 nM) and (R)-BM-591 (EC₅₀ = 390 ± 52 nM) had a better activity than sulotroban (EC₅₀ = 1620 ± 182 nM) but were less potent than SQ-29548 (EC₅₀ = 2.3 ± 0.07 nM) and seratrodoast (EC₅₀ = 49 ± 4.5 nM) which are the most active compounds (Table 2).

On isolated guinea-pig trachea pre-contracted by U-46619 (10 nM), (R)-BM-591 (EC₅₀ = 9600 ± 630 nM) could be considered as a weak TXRA two-fold less active than toraseamide (EC₅₀ = 4020 ± 380 nM) while (S)-BM-591 (EC₅₀ = 272 ± 54 nM) was more active than sulotroban (EC₅₀ = 465 ± 39 nM), but less than BM-573 (EC₅₀ = 17.7 ± 3.9 nM), SQ-29548 (EC₅₀ = 3.8 ± 0.5 nM) and seratrodoast (EC₅₀ = 5.7 ± 0.9 nM).

Since there is a great therapeutic interest for dual-acting compounds (TXRA/TXSI), we investigated the ability of both novel enantiomers to inhibit human platelet TXA₂ synthase. The activity of this enzyme has been evaluated as the platelet TXB₂ production induced by arachidonic acid (600 μM). Furegrelate, a well described TXSI, was chosen as reference drug [27,28]. If (R)-BM-591 at a concentration of 1 μM completely abolished the TXB₂

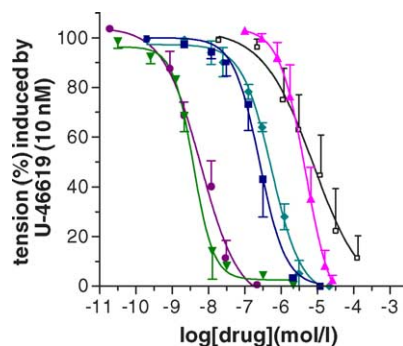


Fig. 4. Relaxation by both enantiomers ((S)-BM-591 (■), (R)-BM-591 (□), by torasemide (▲) by reference compounds (seratrodist (●), SQ-29548 (▼), sulotroban (◆)) of the isolated guinea pig trachea precontracted by U-46619 (10 nM). Each point represent the mean \pm S.E.M. of the response in at least three different experiments.

production, the TXB2 synthesis was only reduced by 22% in presence of (S)-BM-591 (1 μ M) and by 58% in presence of furegrelate (10 μ M) (Fig. 5).

By using the method described by de Leval, both enantiomers (100 μ M) did not reduced the PGE2 production induced by purified ovine COX-1 and COX-2 isoforms (data not shown) [29].

Due to a chemical structure close to that of torasemide, a high ceiling diuretic, we measured the urinary volume excreted by rats for 4 h following a single oral (30 mg kg⁻¹) and intraperitoneal (10 mg kg⁻¹) administration of (S)-BM-591 or (R)-BM-591. The urinary volume excreted by rats treated with (S)-BM-591 (31.1 mL kg⁻¹ h⁻¹, 4.9 ± 0.8 mL kg⁻¹ h⁻¹, respectively) and with (R)-BM-591 (23.7 mL kg⁻¹ h⁻¹, $5.2 \pm$

Table 2

Pharmacological activity of original enantiomer TXA₂ receptor antagonists compared to reference compounds

Drug	Aggregometry		Relaxation of precontracted	
	U-46619 EC ₅₀ (μ M) ^a	Arachidonic acid EC ₁₀₀ (μ M) ^b	Rat aorta EC ₅₀ (nM) ^c	Guinea-pig trachea EC ₅₀ (nM) ^c
Seratrodist	nd ^d	nd ^d	49 \pm 4.5	5.7 \pm 0.9
SQ-29548	nd ^d	0.034 \pm 0.001	2.3 \pm 0.07	3.8 \pm 0.5
Sulotroban	11.3 \pm 2.0	10.2 \pm 1.05	1620 \pm 182	465 \pm 39
Torasemide	>100	>100	3610 \pm 260	4020 \pm 380
BM-573	0.24 \pm 0.09	0.12 \pm 0.01 ^e	28.4 \pm 4.5 ^e	17.7 \pm 3.9 ^e
(S)-BM-591	1.3 \pm 0.1	2.5 \pm 0.2	188 \pm 2.0	272 \pm 54
(R)-BM-591	0.5 \pm 0.05	0.12 \pm 0.01	390 \pm 52	9600 \pm 6300

^a EC₅₀ is the minimal drug concentration preventing 50% of human platelet aggregation induced by 1 μ M of U-46619.

^b EC₁₀₀ is the minimal drug concentration preventing 100% of human platelet aggregation induced by 600 μ M of arachidonic acid.

^c EC₅₀ is the minimal drug concentration reducing by 50% the rat aorta and the guinea-pig trachea tension induced by 20 nM and 10 nM, respectively.

^d Not determined. Values are mean \pm S.E.M., $n \geq 4$.

^e From [17].

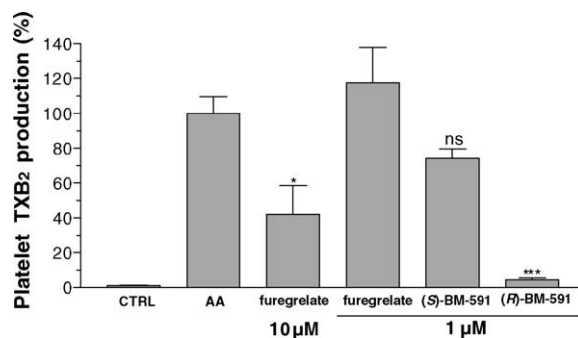


Fig. 5. Effect of (S)-BM-591, (R)-BM-591 on TXB₂ production induced by arachidonic acid (600 μM). Results are expressed as mean ± S.E.M. **p* < 0.05, ****p* < 0.001, ns: non-significant as compared with TXB₂ production to arachidonic acid.

1.2 mL kg⁻¹ h⁻¹, respectively) was not significantly different to that of the control rats (21.1 mL kg⁻¹ h⁻¹, 3.8 ± 0.8 mL kg⁻¹ h⁻¹, respectively). Both enantiomers lost all the diuretic properties of torasemide (30 mg kg⁻¹ p.o., 10 mg kg⁻¹ i.p.), their chemical parent (88.3 mL kg⁻¹ h⁻¹, 56.38 ± 7.2 mL kg⁻¹ h⁻¹, respectively).

5. Discussion

Consequently to the surprising discovery of Uchida et al. [13] who showed that torasemide, a high-ceiling diuretic [12], was a weak TXRA, several original molecules structurally related to this drug were synthesised with the aim of improving this TP-receptor antagonism. First, the affinity of more than hundred torasemide derivatives was studied on TP-receptor of human washed platelets. From this screening, some sulfonylureas and sulfonylcyanoguanidines emerged as high potent ligands [14–17,22,23]. Among them, the racemate (R,S)-BM-591 (IC₅₀ = 3.5 nM) exhibited a strong ability to displace [5,6-³H]SQ-29548 from TP receptors of human platelets. The affinity of this derivative was higher than that of sulotroban and SQ-29548, one of the most potent TXRA described to date.

Each pure enantiomer of (R,S)-BM-591 was then separately synthesized and examined for their affinity for TP-receptors of human washed platelets. From these experiments, the (R)-enantiomer (EC₅₀ = 2.4 nM) exhibited a significantly (*P* < 0.05) higher affinity than that of the (S)-enantiomer (EC₅₀ = 4.2 nM). (R)-BM-591 was characterized by an affinity two-fold lower than that of BM-573 (IC₅₀ = 1.3 nM), a potent and well-described TXRA [17].

Both enantiomers prevented platelet aggregation induced by arachidonic acid or by U-46619. The (R)-enantiomer (EC₁₀₀ = 0.12 μM) was 20-fold more potent than (S)-BM-591 (EC₁₀₀ = 2.5 μM) as human platelet anti-aggregating agent with arachidonic acid. When U-46619 was used to induce platelet aggregation, (R)-BM-591 (EC₅₀ = 0.5 μM) was only 2-fold more potent than the (S)-BM-591 (EC₅₀ = 1.3 μM). If these results are consistent with the trend observed for their affinity of TP-receptor, the discrepancy observed between

their affinity for the platelet TP-receptor and their antiaggregant activity could be attributed to a strong binding to plasma proteins present in the PRP used for aggregation experiments (Tables 1 and 2). This has been already mentioned for some hypoglycaemic sulfonylureas [32].

The TXA₂ antagonism of both enantiomers was confirmed on smooth muscle preparations contracted by U-46619. On the contrary to the results obtained on human platelets, the (S)-enantiomer was respectively 2- and 35-fold more active than (R)-BM-591 on isolated rat aorta and guinea-pig trachea, respectively. As the 3D-structure of both enantiomers is quite different, this discrepancy could be relevant of a better fitting of the S-enantiomer with the smooth muscle cell TP receptor which is probably different from that expressed on platelets. Indeed, these results are consistent with the hypothesis claiming expression of different TP receptor isoforms depending of the type of tissue [33]. On smooth muscle preparations, the (R)-enantiomer was 25-fold more potent on pre-contracted rat aorta ($EC_{50} = 0.39 \mu\text{M}$) than on isolated guinea-pig trachea ($EC_{50} = 9.6 \mu\text{M}$), whereas the (S)-enantiomer showed a similar potency on both preparations. These observations could only be explained by the hypothesis supporting the existence of interspecies differences in TP receptors [34–36].

At $1 \mu\text{M}$, the (R)-enantiomer completely blocked the platelet TXB₂ production. As TXB₂ is the non-enzymatic metabolite of TXA₂, this result suggests that (R)-BM-591 completely inhibited TXA₂ synthase. Indeed, we demonstrated that both enantiomers had no inhibitory potency on both ovine cyclooxygenase isoforms (data not shown) according to a previously described method [29]. Moreover, (R)-BM-591 was more potent than furegrelate chosen as reference TXSI [27,28]. On the contrary, the TXB₂ production induced by human platelets is non-significantly and poorly reduced (–22% at $1 \mu\text{M}$) by (S)-BM-591 (Fig. 5). Thus (R)-BM-591 could be considered as a potent drug acting as a dual TXRA/TSI mainly on the cardiovascular system (platelets and aorta). This pharmacological profile, combined with a poor TP-receptor antagonism on guinea-pig trachea, led to propose (R)-BM-591 as the best candidate for further developments in the field of cardiovascular diseases such as myocardial infarct and thrombosis disorders involving overproduction of TXA₂.

In vivo experiments in rats demonstrated that both enantiomers lost all diuretics properties of their chemical parent, torasemide, after oral and intraperitoneal administration of drug.

At a chemical point of view, the acidic sulfonylurea moiety of these molecules is negatively charged at the physiological pH [29], mimicked the carboxylate function present in all the previously described thromboxane modulators and probably bound the Arg295 residue through an ionic interaction [30]. Indeed, these compounds are the first TXRA deprived of a carboxylic group (Fig. 3) which is a common feature of all the previously described TXRA (Fig. 2). According to the ligand-TP receptor interaction model proposed by Yamamoto [31], the p-toluyll moiety of BM-573 and the methylbenzyl residue of the enantiomers (S)-BM-591 and (R)-BM-591 probably occupied a hydrophobic pocket of the TXA₂ receptor. The presence of a tert-butyl on the sulfonylurea side-chain also enhanced the affinity by occupying a second hydrophobic pocket [23,29]. Examination of the activity of these enantiomers on different smooth muscles taken from the same species will contribute to clarify the activity of these enantiomers. Moreover, complementary binding investigations of both enantiomers on TP isoforms separately expressed will probably contribute to the comprehension of the differences observed.

Further in vivo experiments are warranted to confirm the therapeutical interest of (R)-BM-591 in the prevention or the treatment of cardiovascular diseases involving TXA₂.

References

- [1] Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci USA* 1975;72:2994.
- [2] Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A₂, and prostacyclin. *Pharmacol Rev* 1978;30:293.
- [3] Wenzel SE. Arachidonic acid metabolites: mediators of inflammation in asthma. *Pharmacotherapy* 1997;17:35.
- [4] Patrono C. Prevention of myocardial infarction and stroke by aspirin: different mechanisms? Different dosage? *Thromb Res* 1998;92:S7.
- [5] Dogné JM, de Leval X, Benoit P, Rolin S, Pirotte B, Masereel B. Therapeutic potential of thromboxane inhibitors in asthma. *Expert Opin Invest Drugs* 2002;11:275.
- [6] Sladek K, Dworski R, Fitzgerald GA, et al. Allergen-stimulated release of thromboxane A₂ and leukotriene E₄ in humans. Effect of indomethacin. *Am Rev Respir Dis* 1990;141:1441.
- [7] Gurbel PA, Murugesan SR, Lowry DR, Serebruany VL. Plasma thromboxane and prostacyclin are linear related and increased in patients presenting with acute myocardial infarction. *Prostaglandins Leukot Essent Fatty Acids* 1999;61:7.
- [8] Wilson TW, Quest DW. Ridogrel: an antiplatelet agent with antihypertensive properties. *Cardiovasc Drug Rev* 2000;18:222.
- [9] Galie N, Manes A, Branzi A. The new clinical trials on pharmacological treatment in pulmonary arterial hypertension. *Eur Respir J* 2002;20:1037.
- [10] Savage MP, Goldberg S, Bove AA, et al. Effect of thromboxane A₂ blockade on clinical outcome and restenosis after successful coronary angioplasty. Multi-hospital eastern Atlantic restenosis trial (M-HEART II). *Circulation* 1995;92:3194.
- [11] Hoshino M, Sil J, Shimizu K, Nakayama H, Koya A. Effect of AA-2414, a thromboxane A₂ receptor antagonist, on airway inflammation in subjects with asthma. *J Allergy Clin Immunol* 1999;103:1054.
- [12] Friedel HA, Buckley MM. Torasemide. A review of its pharmacological properties and therapeutic potential. *Drugs* 1991;41:81.
- [13] Uchida T, Kido H, Yamanaga K, Okita M, Watanabe M. A novel loop diuretic, torasemide, inhibits thromboxane A₂-induced contraction in the isolated canine coronary artery. *Prostaglandins Leukot Essent Fatty Acids* 1992;45:121.
- [14] Dogné JM, Rolin S, de Leval X, et al. Pharmacology of the thromboxane receptor antagonist and thromboxane synthase inhibitor BM-531. *Cardiovasc Drug Rev* 2001;19:87.
- [15] Dogné JM, Wouters J, Rolin S, et al. Design, synthesis and biological evaluation of a sulfonylcyanoguanidine as thromboxane A₂ receptor antagonist and thromboxane synthase inhibitor. *J Pharm Pharmacol* 2001;53:669.
- [16] Dogné JM, de Leval X, Kolh P, et al. Pharmacological evaluation of the novel thromboxane modulator BM-567 (I/II). Effects of BM-567 on platelet function. *Prostaglandins Leukot Essent Fatty Acids* 2003;68:49.
- [17] Rolin S, Dogné JM, Michaux C, Delarge J, Masereel B. Activity of a novel dual thromboxane A₂(2) receptor antagonist and thromboxane synthase inhibitor (BM-573) on platelet function and isolated smooth muscles. *Prostaglandins Leukot Essent Fatty Acids* 2001;65:67.
- [18] Rolin S, Petein M, Tchana-Sato V, et al. BM-573, a dual thromboxane synthase inhibitor and thromboxane receptor antagonist, prevents pig myocardial infarction induced by coronary thrombosis. *J Pharmacol Exp Ther* 2003;306:59.
- [19] Dogné JM, Hanson J, De Leval X, et al. Pharmacological characterization of BM-573, a novel thromboxane A₂ receptor antagonist and thromboxane synthase inhibitor in a rat model of arterial thrombosis and its effects on bleeding time. *J Pharmacol Exp Ther* 2004;309:498–505.
- [20] Masereel B, Renard P, Schynts M, Pirotte B, de Tullio P, Delarge J. Synthesis and pharmacology of pyrid-3-yl sulfonylureas and sulfonylthioureas as inhibitors of the astrocytic Na⁺2Cl⁻K⁺ co-transporter. *Eur J Med Chem* 1994;29:527.

- [21] Nuhrich A, Varache-Lembège M, Lacan F, Devaux G. Use of infrared spectroscopy in monitoring of a new method for the preparation of sulotroban, an antithrombotic drug. *J Chem Educ* 1996;319:689.
- [22] Masereel B, Damas J, Fontaine J, et al. Thromboxane A2 receptor antagonism in man and rat by a sulphonylcyano guanidine (BM-144) and a sulphonylurea (BM-500). *J Pharm Pharmacol* 1999;51:695.
- [23] Dogné JM, de Leval X, Neven P, et al. Effects of a novel non carboxylic thromboxane A2 receptor antagonist (BM-531) derived from torasemide on platelet function. *Prostaglandins Leukot Essent Fatty Acids* 2000;52:311.
- [24] Born GV, Cross MJ. The aggregation of blood platelets. *J Physiol* 1963;168:178–95.
- [25] Harris DN, Asaad MM, Phillips MB, Goldenberg HJ, Antonaccio MJ. Inhibition of adenylate cyclase in human blood platelets by 9-substituted adenine derivatives. *J Cyclic Nucleotide Res* 1979;5:125.
- [26] Pradelles P, Grassi J, Maclouf J. Enzyme immunoassays of eicosanoids using acetylcholine esterase as label: an alternative to radioimmunoassay. *Anal Chem* 1985;57:1170.
- [27] Johnson RA, Nidy EG, Aiken JW, Crittenden NJ, Gorman RR. Thromboxane A2 synthase inhibitors. 5-(3-Pyridylmethyl)benzofuran-2-carboxylic acids. *J Med Chem* 1986;29:1461.
- [28] Mohrland JS, Vander Lugt JT, Gorman RR, Lakings DB. Thromboxane synthase activity and platelet function after furegrelate administration in man. *J Clin Pharmacol* 1989;29:53.
- [29] de Leval X, Delarge J, Devel P, et al. Evaluation of classical NSAIDs and COX-2 selective inhibitors on purified ovine enzymes and human whole blood. *Prostaglandins Leukot Essent Fatty Acids* 2001;64:211.
- [30] Michaux C, Rolin S, Dogne JM, et al. Structure determination and comparison of BM567, a sulfonylurea, with terbogrel, two compounds with dual action, thromboxane receptor antagonism and thromboxane synthase inhibition. *Bioorg Med Chem Lett* 2001;11:1019.
- [31] Yamamoto Y, Kamiya K, Terao S. Modeling of human thromboxane A2 receptor and analysis of the receptor-ligand interaction. *J Med Chem* 1993;36:820.
- [32] Balant L. Clinical pharmacokinetics of sulfonylurea hypoglycemic drugs. *Clin Pharmacokinet* 1981;6:215–41.
- [33] Moore F, Asboth G, Bernal AL. Thromboxane receptor signalling in human myometrial cells. *Prostaglandins Other Lipid Mediat* 2002;67:31.
- [34] Ogletree ML, Allen GT. Interspecies differences in thromboxane receptors: studies with thromboxane receptor antagonists in rat and guinea pig smooth muscles. *J Pharmacol Exp Ther* 1992;260:789.
- [35] Zhang R, Ogletree ML, Moreland S. Characterization of thromboxane A2/prostaglandin endoperoxide receptors in aorta. *Eur J Pharmacol* 1996;317:91.
- [36] Kemp BK, McPherson GA. Interspecies differences in thromboxane A2 receptors are distinguished by glibenclamide. *Eur J Pharmacol* 1998;354:173.