Bioorganic & Medicinal Chemistry Letters, Vol.1, No.12, pp. 689-694, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL AMINO-PROSTANOIDS: POTENT AND ORALLY EFFECTIVE THROMBOXANE A_2 RECEPTOR ANTAGONISTS

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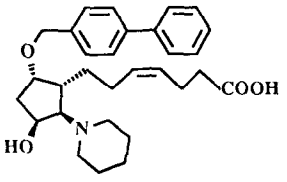
(*Received* 23 *July* 1991)

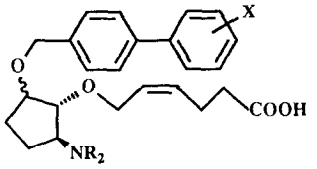
Abstract: Modification of the thromboxane receptor antagonist, **GR 32191**, has led to a series of readily synthesised amino-prostanoids. The most effective compound is **GR 70067**, an orally active agent that may be useful in the treatment of thrombotic disease.

Thromboxane A_2 (TxA_2) is an unstable metabolite derived from arachidonic acid and is a potent inducer of platelet aggregation and constrictor of vascular smooth muscle and as such it is implicated in a variety of thrombotic diseases. We have previously shown that the amino-modified prostanoid **GR 32191** is a potent, specific and long-acting thromboxane receptor antagonist[1,2]. **GR 32191** is currently undergoing clinical evaluation[3].

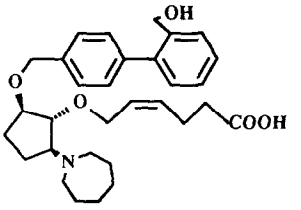
The fact that **GR 32191** has to be synthesised *via* a lengthy procedure and that its aqueous solubility at physiological pH is extremely low persuaded us to search for a synthetically more accessible and water soluble compound with a similar pharmacological profile. This report describes the synthesis and pharmacological evaluation of a novel series of related amino-prostanoids (**1**) one of which, **GR 70067**, fulfils the above criteria.



GR32191 (+)



(1) 5α and 5β X= polar group

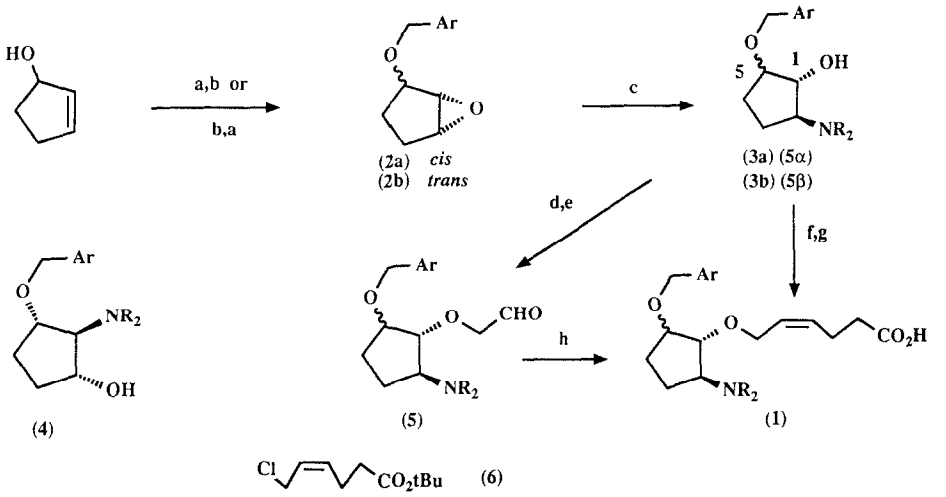


GR70067 (±)

The synthesis of the amino-compounds (**1**) was achieved by the routes illustrated in Schemes 1 and 2 and utilised the *cis* and *trans* epoxy ethers (**2a**) and (**2b**) as key intermediates (Scheme 1). The *cis* isomer (**2a**) was prepared by selective syn-epoxidation of cyclopenten-1-ol followed by alkylation with aralkyl bromides. Reversal of this sequence yielded a mixture of epoxides (**2a**) and (**2b**) (ratio 1:2 respectively) which were separated by chromatography. The amino-alcohols (**3a**) and (**3b**) were obtained by cleavage of (**2a**) and (**2b**) with a secondary amine. Although the reaction with (**2b**) was regioselective, affording only (**3b**), epoxide (**2a**) gave a mixture of (**3a**) and the regioisomer (**4**) (ratio 4:1 respectively). The carboxylic

acid containing chain was attached in two ways. The first method used a three-step sequence which involved alkylation of the amino-alcohols with bromoacetaldehyde dimethylacetal followed by acidic hydrolysis to liberate the aldehyde (**5**). A final Wittig condensation then provided the acids (**1**). Compounds generated in this fashion were a mixture of alkene isomers (Z:E 70:30). However, geometrically pure Z products could be prepared by alkylation of the amino alcohols (**3**) with the allylic chloride (**6**) followed by ester hydrolysis.

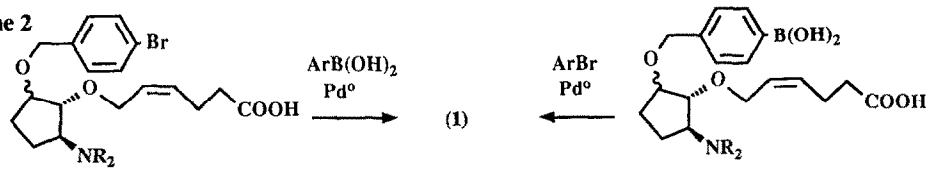
Scheme 1



(a) MCPBA, CH_2Cl_2; (b) NaH, $ArCH_2Br$, DMF; (c) R_2NH, ^nBuOH, heat; (d) NaH, $BrCH_2CH(OMe)_2$, DMF; (e) HCl, aq.acetone; (f) NaH, (**6**), DMF; (g) 2N NaOH, EtOH; (h) $Ph_3P^+(CH_2)_3CO_2H\ Br^-$, KO^tBu, THF.

Although the above methods allowed the synthesis of compounds containing a variety of amino substituents an alternative route was required for derivatives which embodied substituted biphenylmethylether groups (Scheme 2). Bromobenzyl ethers (**7**), generated by the procedures used in Scheme 1, underwent palladium catalysed aryl coupling with a variety of substituted aryl boronic acids to provide acids (**1**)[4]. Alternatively, the prostanoid boronic acids (**8**) could be coupled with aryl halides to also afford acids (**1**). The aryl boronic acids utilised in Scheme 2 were all prepared by a butyl lithium-mediated metal-halogen exchange reaction of the appropriate aromatic bromide/iodide in the presence of an excess of triisopropyl borate[5]. In some circumstances hydroxy and carboxylic acid functions present in the aryl moiety had to be utilised in a protected form and liberated after the desired palladium catalysed coupling process had been achieved. Optical resolution of the amino alcohols (**3**) allowed the synthesis of enantiomerically pure derivatives[6,7].

Scheme 2

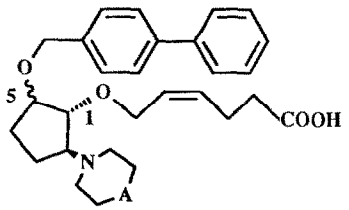


(7) (8)

For most compounds a measure (pA_2 values) of TxA_2 antagonist activity at the platelet and vascular receptor was determined against U-46619-induced aggregation of platelets in human whole blood and contraction of the rat isolated thoracic aortic strip respectively[1]. Tables 1 and 2 which summarise the *in vitro* results on a selection of compounds reveal that these novel amino-prostanoids are potent TxA_2 receptor blocking drugs.

Initial studies with compounds with an unsubstituted biphenylmethylether group concluded that the preferred amino-substituent was either a piperidino or hexahydroazepino moiety; compounds containing morpholino, thiamorpholino, pyrrolidino or acyclic amino groups were usually less potent. Although there was a tendency for the 5α-compounds to be slightly more active than their 5β-counterparts this difference was only marginal and demonstrates that stereochemical integrity of the biaryl moiety is not critical for activity. In the 5α-series the 1R-enantiomer was significantly more potent than the 1S-enantiomer in both tests. However, in contrast, both enantiomers of the 5β-series had similar activity at the platelet receptor but the 1S-enantiomer was much more potent than the 1R-enantiomer at the vascular site (Table 1).

TABLE 1: *IN VITRO* THROMBOXANE RECEPTOR ANTAGONIST ACTIVITY



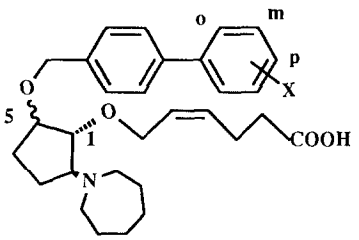
	A[a]	pA_2[b] HWB[c]		pA_2[b] Rat Aorta[d]	
		5α	5β	5α	5β
	CH_2	8.1*	7.8	7.3	7.2
1R	CH_2	8.0*	7.2	7.5	6.1*
1S	CH_2	7.0	7.4*	6.0	7.8
	$(CH_2)_2$	8.3	7.9	7.4	7.6

a. Compounds where A=O or S or when the substituent is pyrrolidine were generally less potent. b. pA_2 values are a mean of at least two determinations. (Schild analysis gave slopes not significantly different from unity except where indicated * when >1). c. Inhibition of U-46619-induced platelet aggregation in human whole blood. d. Inhibition of U-46619-induced contraction of rat isolated thoracic aortic strip.

Because of the encouraging *in vitro* antagonist activity demonstrated by the compounds in Table 1, the racemic 5α and 5β piperidino derivatives were evaluated *in vivo* in the anaesthetised guinea-pig (1mg/kg i.v.). These derivatives produced small decreases in diastolic blood pressure, and were without effect upon tracheal inflation pressure (TIP), a measure of bronchoconstriction, indicating the absence of TxA_2-like agonist effects on both vascular and respiratory smooth muscle. However, these compounds antagonised the vasopressor and bronchoconstrictor effects (increases in TIP) produced by U-46619. The compounds also inhibited collagen-induced platelet aggregation *ex vivo* after oral dosing to the conscious dog (1mg/kg). Disappointingly, they produced a consistently smaller maximal effect than our standard drug **GR 32191** and in addition were shorter acting (i.e. 4-6h vs. >12h).

In an attempt to improve both potency and duration of action, a variety of substituted compounds were synthesised and all shown to have similar *in vitro* activity (Table 2). Most functional groups were tolerated with the exception of $-CO_2H$ and $-CH_2CO_2H$ which both decreased potency substantially (estimated pK_B values <6).

TABLE 2: *IN VITRO* THROMBOXANE RECEPTOR ANTAGONIST ACTIVITY



		pA_2 HWB			pA_2 Rat Aorta		
	X	o	m	p	o	m	p
5α	CH_2OH	8.8*+	8.3	8.4	7.9	7.1	7.3
5α	$NHSO_2Me$	8.2*			7.2		
5β	CH_2OH	8.5*#	8.1*	8.5*	8.2#	7.9	7.7
5β	$(CH_2)_2OH$	8.7+			7.5		
5β	OH	7.8*		8.2*	7.7		8.2
5β	$NHSO_2Me$	8.0	7.9*		8.4	7.3	
5β	$NHCONH_2$	8.1*			7.2		
5β	CH_2CONH_2	8.1		8.4*	6.8		7.7
5β	$CONH_2$	7.8		8.5*	7.8		7.7
5β	CH_2NHAc	7.6		8.6*	6.9		7.7
5β	CH_2SO_2NHMe	8.1		8.5	7.9		7.8

* Schild analysis produces slopes significantly greater than unity. # **GR 70067**. 1R and 1S enantiomers[8] have very similar *in vitro* activity. + Value represents pK_B estimated from a single concentration-ratio value.

In general, the substituted analogues behaved in a similar fashion *in vivo* to the piperidino compounds described above with the exception of several o-substituted compounds in the 5β-series (e.g. X=CH_2OH, $NHSO_2Me$, $NHCONH_2$) which showed an extended duration of action in the conscious dog. In particular, the o-hydroxymethyl compound, **GR 70067**, which also showed the desired enhanced water solubility (**GR 70067**, 0.4mg/ml; **GR 32191**, 0.05mg/ml, at pH7.4), was the most interesting compound to emerge from this series.

GR 70067 possessed potent thromboxane receptor blocking activity *in vitro* (Table 2). However, whilst antagonism on rat isolated aorta was specific and surmountable (pA_2=8.2; slope=1.0), quantification of the potency on human platelets using U-46619 was complicated by an apparent non-surmountable profile of antagonism (pK_b 8.5). Despite this profile **GR 70067** was specific, PAF-induced and the primary phase of ADP-induced aggregation being unaffected by concentrations up to 10μM. Furthermore, the drug did not induce platelet shape change (up to 30μM) nor inhibit platelet thromboxane synthase (up to 10μM). In the anaesthetised guinea-pig, **GR 70067** (1mg/kg, i.v.) produced substantial antagonism of U-46619-induced vasoconstriction and bronchoconstriction. An oral dose of 1mg/kg administered to both the conscious guinea-pig and dog inhibited collagen-induced platelet aggregation *ex vivo* for up to 24 hours.

In summary, **GR 70067**, an amino-modified prostanoid related to **GR 32191** is a potent, specific and long-acting thromboxane receptor antagonist. The compound is synthesised in only 6 steps from readily available starting materials and is 10 times more water soluble than **GR 32191**. The pharmacology presented suggests that **GR 70067** could be of value in the treatment of thrombotic disease.

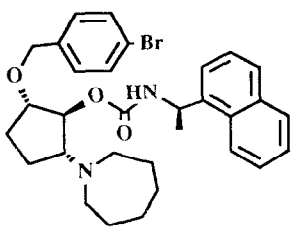
Acknowledgements: The authors wish to express their thanks to Mr. M. Boys, Miss M. Menhenitt, Dr. J. Morris, Miss K. Seager and Mr. K. Wharton for their expert technical assistance.

References and Notes:

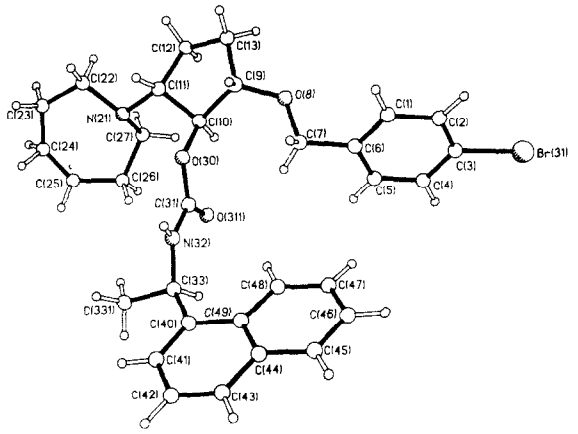
1. Lumley, P.; White, B.P.; Humphrey, P.P.A. *Br. J. Pharmacol.*, **1989**, *97*, 783.
2. Humphrey, P.P.A.; Hallett, P.; Hornby, E.J.; Wallis, C.J.; Collington, E.W.; Lumley, P. *Circulation*, **1990**, *81*, Suppl. 1, 42.
3. Fiddler, G.I.; Lumley, P. *Circulation*, **1990**, *81*, Suppl. 1, 69.
4. The conditions employed [$(Ph_3P)_4Pd$, DME, 2N Na_2CO_3, reflux] in the coupling of (7) and (8) afforded the products (1) in 50-95% yield. All new compounds gave satisfactory spectroscopic and/or analytical data.
5. Thompson, W.J.; Gaudino, J.G. *J. Org. Chem.*, **1984**, *49*, 5237.
6. The enantiomers of (**3b**) were generated by resolution with D- and L-dibenzoyl tartaric acids. (1S-**3b**), $[\alpha]_D$ -1.64° (EtOH); (1R-**3b**), $[\alpha]_D$ +1.72° (EtOH). The enantiomeric purity of these intermediates was >98%e.e. (chiral h.p.l.c.).
7. The absolute configuration of (1S-**3b**) was established from an X-ray structure of the carbamate (1S-**9**).

Crystal data for (9)

$C_{31}H_{37}N_2O_3Br$, M=565.55, monoclinic, space group $P2_1$ (No. 4) a=14.227(6), b=5.162(1), c=19.899(6) A, β=105.29(3)°, U=1410(2) A^3, λ(Cu-Kα)=1.54184 A, Z=2, D=1.33gcm^{-3}, F(000)=592. Colourless plates. Crystal dimensions 0.27 x 0.13 x 0.02mm, μ(Cu-Kα) = 2.25mm^{-1}. Siemens R3m/V diffractometer, 1840 unique data recorded (2θ <105) of which 1298 with I > 2.5σ(I) were used in the subsequent analysis and least-squares refinement (SHELXTL PLUS). Anisotropic temperature factors for all non-hydrogen atoms; hydrogens (except for that on N32) in calculated positions with common isotropic temperature factors for phenyl-type [U=0.08(1)], secondary CH_2 type [U=0.13(1)], tertiary CH type [U=0.03(1)] and methyl [U=0.11(2)] atoms. The absolute configuration was determined using Rogers' eta refinement and an R-factor test. Weighting scheme $w^{-1}=\sigma^2(F) + 0.00012(F)^2$. Final R=0.040, R_W=0.038. Maximum residual electron density was 0.29eA^{-3} and mean and maximum shift/error in the final refinement were 0.01 and 0.13 respectively. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K. Any request should be accompanied by the full literature citation for this paper.



(9)



X-ray structure of (9)

8. (1R-**GR 70067**), $[\alpha]_D$ +27.9° ($CHCl_3$), 98.7%e.e.; (1S-**GR 70067**), $[\alpha]_D$ -27.8° ($CHCl_3$), 98.6%e.e. The enantiomeric purity was measured by chiral h.p.l.c.