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# SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF COMBINED THROMBOXANE RECEPTOR ANTAGONIST/THROMBOXANE SYNTHASE INHIBITORS: PYRIDINE-CONTAINING AMINO-PROSTANOIDS

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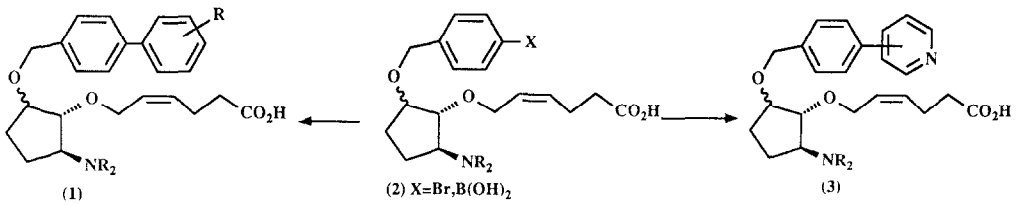
**Abstract:** Incorporation of a pyridine moiety into amino-prostanoids, previously shown to be thromboxane receptor antagonists, has provided a series of compounds which also show potent thromboxane synthase inhibitory activity. **GR 83783**, an $^{n}$propyl-substituted pyridine demonstrates these properties both *in vitro* and *in vivo*.

The powerful platelet aggregatory effects and constrictor properties on vascular smooth muscle of thromboxane $A_2$ ($TxA_2$) have implicated it in an array of thrombotic diseases[1]. There has therefore been considerable interest in generating agents that modulate the actions of $TxA_2$ and this has resulted in the discovery of a variety of thromboxane receptor antagonists and synthase inhibitors[2,3]. The reason why the synthase inhibitors have failed to achieve their expected therapeutic potential is probably because the accumulated prostaglandin endoperoxides can directly activate the thromboxane receptor, an effect which opposes the initial synthase inhibition[4]. Although the clinical efficacy of thromboxane receptor antagonists has yet to be unequivocally demonstrated, it is likely that they will be more effective than synthase inhibitors[1]. However, more recently it has been shown that superior anti-thrombotic effects can be achieved when a thromboxane receptor antagonist is combined with a synthase inhibitor[5,6].

Rather than use two separate drug entities, we have chosen to synthesise agents that have both activities in the same molecule[7]. Our strategy was based on the incorporation of a pyridine group, believed in other inhibitors to complex the haem atom in the enzyme active site, into compounds that already possessed the desired thromboxane receptor blocking activity[7]. This report describes our preliminary findings using this approach.

We have previously described the synthesis of a series of amino-modified prostanoids (**1**), generated *via* the bromide/boronic acid intermediate (**2**), which are potent and long-acting thromboxane receptor antagonists[8]. By utilising an appropriately substituted bromopyridine or pyridine boronic acid, the preparation of analogues (**3**) using a palladium catalysed aryl-aryl bond coupling strategy was easily accomplished[9].

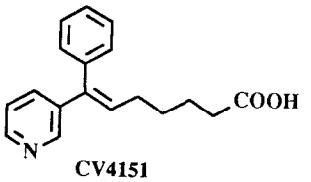



(1) (2) X=Br,$B(OH)_2$ (3)

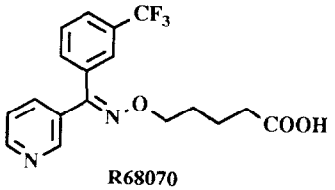
The Table shows that compounds of structure (3) are capable of potently inhibiting U-46619-induced platelet aggregation in human whole blood and also attenuating the U-46619-induced contraction of the rat isolated thoracic aortic strip[10]. These two results demonstrate blocking activity at both the platelet and vascular thromboxane receptor and that prostanoids (3) behave in a similar fashion to their biphenyl counterparts (1). However, in contrast to (1), many of the pyridine containing compounds also inhibit collagen-induced thromboxane $A_2$ production in human citrated whole blood, this reflecting their ability to inhibit thromboxane synthase, an effect independent of their thromboxane receptor blocking activity[6].

Examination of the *in vitro* pharmacological data (Table) reveals that, as in the previously reported series[8], substitution in the terminal aryl ring enhances receptor blocking activity. Although the position and nature of this pyridine substitution is not critical for antagonist potency, both factors affect enzyme inhibitory activity. For example only 3,4-disubstituted pyridines possess the desired enzyme inhibitory properties with the preferred additional substituent being a small alkyl group; polar moieties and larger groups either reduce the inhibitory activity significantly or abolish it completely. It is apparent that the structure-activity relationships for the two biological effects do not parallel each other and that a compromise on the two activities is necessary. Based on this reasoning the propyl substituted compound, **GR 83783**, was chosen for further evaluation.

Several of the compounds reported (see Table) have similar thromboxane synthase inhibitory activity to the pyridines **CV4151**[7a] and **R68070**[7b], both claimed as drugs with accompanying thromboxane receptor antagonist activity. However, the amino-prostanoids described are approximately three orders of magnitude more potent as receptor blocking drugs than either pyridine[6].

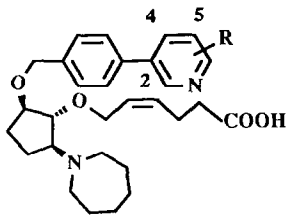



**CV4151**

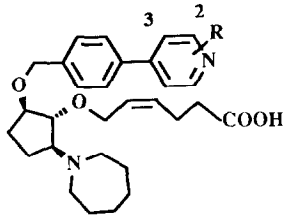



**R68070**

**TABLE. $TXA_2$ RECEPTOR ANTAGONIST/SYNTHASE INHIBITORY ACTIVITIES *IN VITRO***




A *META SERIES*




B *PARA SERIES*

| | | $TxA_2$ ANTAGONIST ACTIVITY | | $TxA_2$ SYNTHASE ACTIVITY |
|---|---|---|---|---|
| A OR B | R[a] | $pA_2$[b] HWB[c] | $pA_2$[b] Rat Aorta[d] | $pIC_{50}$[e] |
| A | H | 7.9 | 7.4 | 6.2 |
| A | 4-Me | 8.6* | 7.5 | 7.0 |
| A | 4-Et | 9.0* | 8.0 | 5.6 |
| A | 4-$^n$Pr **(GR 83783)** | 8.6[f]* | 7.5 | 7.0 |
| A | 4-$^n$Bu | 8.4* | 8.0 | 7.0 |
| A | 4-$^t$Bu | g | g | <5.0[h] |
| A | 4-$CH_2OH$ | 8.5* | 7.9 | 5.8 |
| A | 5-$CH_2OH$ | 8.2 | 7.6 | <5.0[h] |
| A | 2-$CH_2OH$ | g | g | <5.0[h] |
| A | 4-$(CH_2)_2OH$ | g | g | <5.0[h] |
| B | H | 8.0 | 7.1 | 6.2 |
| B | 3-$CH_2OH$ | 8.5 | 7.8 | <5.0[h] |
| B | 3-Me | 8.2 | 7.7 | 5.8 |
| B | 3-Et | 8.2* | 7.8 | 6.4 |
| **CV4151** | | 4.8 | 5.2 | 7.0 |
| **R68070** | | 5.4 | 5.9 | 7.5 |

a. Other substituents studied include $NH_2$, NHAc, OMe, $CH_2CONH_2$, $CH_2CO_2Et$, Ph, $CH_2F$. All were highly potent $TxA_2$ receptor antagonists ($pK_B$'s 7.0-8.5, estimated from a single concentration-ratio value) but were less active as $TxA_2$ synthase inhibitors ($pIC_{50}$'s <6.0). b. $pA_2$ values are a mean of at least two determinations. Schild analysis gave slopes not significantly different from unity, unless indicated by * where the slope was >1.0. 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. e. Inhibition of collagen-induced $TxA_2$ production in human citrated whole blood. f. Each enantiomer[13] of **GR 83783** demonstrated a similar pharmacological profile to racemic material. g. $pA_2$ values can only be estimated and are between 7.5-8.5. h. No significant effect at 10$\mu$M.

The antagonism of U-46619-induced platelet aggregation in human whole blood exhibited by **GR 83783** was characterised by non-parallel displacements of the aggregation curves, resulting in the slope of the Schild regression[11] being greater than unity. This may be due to a very slow rate of dissociation of the drug from the platelet $TxA_2$ receptor[12]. However, despite this observation the antagonism was specific since **GR 83783** (1-10$\mu$M) had no affect upon PAF-induced or the primary phase of ADP-induced aggregation. In human clotting blood **GR 83783** (0.1-10$\mu$M) exhibited a potent inhibitory effect upon serum $TxA_2$ formation, whilst concomitantly elevating $PGE_2$ and $PGD_2$ concentrations, a profile consistent with a

specific action of the drug at the $TxA_2$ synthase enzyme with no effect at the cyclooxygenase enzyme. Interestingly, both the individual enantiomers[13] of **GR 83783** possessed a very similar *in vitro* pharmacological profile and therefore evaluation *in vivo* was carried out on racemic material. When administered orally to the conscious dog, **GR 83783** (3mg/kg) inhibited collagen-induced platelet aggregation *ex vivo* for up to 24h but only inhibited serum $TxA_2$ formation for 2h.

The above preliminary data demonstrates that **GR 83783** is an orally effective, specific and long-acting thromboxane receptor antagonist. Unfortunately, accompanying inhibition of the synthase enzyme is only apparent for 2h and hence future work will concentrate on improving the duration of action of enzyme inhibition.

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9. The conditions utilised for the palladium-catalysed coupling reactions [$(Ph_3P)_4Pd$, DME, 2N $Na_2CO_3$, reflux] between (**2**, X=$B(OH)_2$) and appropriately substituted bromopyridines, afforded the products (**3**) in 50-95% yield. All new compounds possessed satisfactory spectroscopic and/or analytical data.
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13. The individual enantiomers were synthesised in an identical fashion using the chiral intermediates described in ref. 8. 1R-**GR 83783**, 96.8%e.e.; 1S-**GR 83783**, 98.8%e.e.