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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF COMBINED THROMBOXANE RECEPTOR ANTAGONIST/SYNTHASE INHIBITORS: PYRIDINE-CONTAINING SULPHONAMIDO ACIDS

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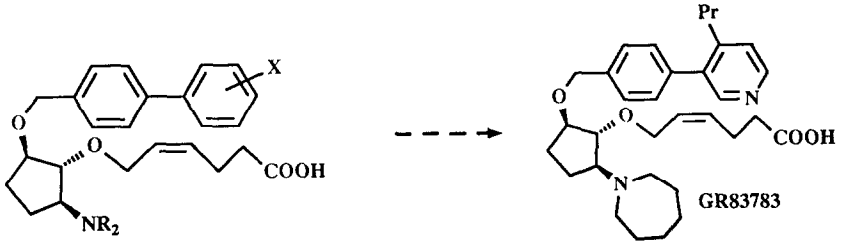
Abstract: A series of compounds which possess both thromboxane receptor antagonist and synthase inhibitory activity has been generated by incorporating an arylsulphonamide group into the synthase inhibitors **CV4151** and **R68070**.

We[1] and others[2] have demonstrated a superior anti-thrombotic effect of combinations of thromboxane receptor blocking drugs and thromboxane synthase inhibitors over either drug alone. The synergistic effect observed is believed to be due to the accumulated prostaglandin endoperoxides being metabolically re-directed to the potent anti-aggregatory substances PGD_2 and PGI_2 but unable to elicit their TxA_2-agonist like effects because of receptor blockade. To avoid the complications associated with administration of two separate drug entities we have embarked upon a programme to synthesise agents that possess both the above biological activities in the same molecule[3]. A previous publication[4] described the synthesis and pharmacological evaluation of novel derivatives derived from the incorporation of a pyridine moiety into known thromboxane receptor antagonists. This approach led to **GR 83783** which demonstrated potent antagonist and enzyme inhibitory properties *in vitro*.

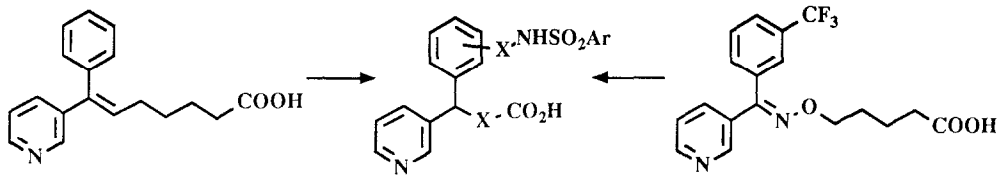
However, although **GR 83783** was long-acting as a thromboxane receptor blocking drug (>24h) following oral administration to the conscious dog (3mg/kg), concomitant enzyme inhibition was present for only 2h. We therefore initiated a search for a different compound class where an extended duration of action could be demonstrated for both biological effects. This letter describes our initial findings.



THROMBOXANE RECEPTOR ANTAGONISTS

THROMBOXANE RECEPTOR ANTAGONISTS/SYNTHASE INHIBITOR

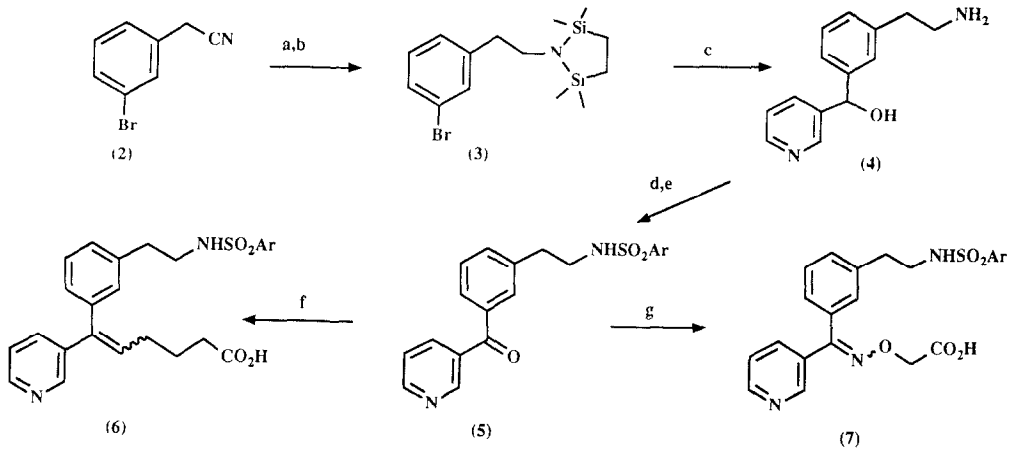
We decided to utilise the potent thromboxane synthase inhibitors **CV4151**[3a] and **R68070**[3b] as templates and attempt to enhance the weak thromboxane receptor antagonist properties already present in these molecules. Since arylsulphonamide groups are present in a variety of thromboxane receptor antagonist structures[5] we reasoned that attachment of this moiety to the pendant phenyl groups of the enzyme inhibitors may confer the required receptor blocking activity. This modification produces hybrid molecules of general structure (**1**).



CV4151 (**1**) X = a linking chain **R68070**

The synthetic route adopted is illustrated with the preparation of analogues (**6**) and (**7**) (Scheme). The nitrile (**2**) was reduced to the corresponding phenylethylamine and then protected as a Stabase derivative[6]. The resultant bromide (**3**) underwent a metal-halogen exchange on reaction with ^{n}BuLi and the lithio intermediate was then added to pyridine-3-carboxaldehyde. This sequence produced the carbinol (4), the amino group being liberated during the acidic work-up procedure. Sulphonylation with an aryl sulphonyl chloride followed by oxidation with manganese dioxide yielded the ketone (**5**). Wittig condensation then provided the olefinic acid (**6**) whereas reaction with the appropriate hydroxylamine derivative afforded the oxime (**7**). Similar methodology enabled the synthesis of all the compounds described in this report.

Scheme

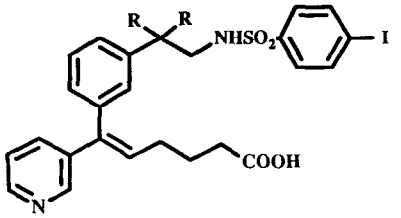


(a) BH_3, THF; (b) [-$CH_2Si(Me)_2Cl]_2$, Et_3N; (c) ^{n}BuLi, THF, -78°; then pyridine-3-carboxaldehyde; 2N HCl; (d) $ArSO_2Cl$, Et_3N, CH_2Cl_2; (e) MnO_2, CH_2Cl_2; (f) $Ph_3P^{+}(CH_2)_3CO_2H\ Br^{-}$, KO^{t}Bu, THF; (g) $H_2NOCH_2CO_2H$, pyridine, EtOH.

Table 1 summarises the *in vitro* activity for a selection of compounds derived from **CV4151** and indicates that they are inhibitors of U-46619-induced platelet aggregation in human whole blood and of U-46619-induced contractions of the rat isolated thoracic aortic strip[7]. These results demonstrate that these hybrid molecules behave as thromboxane receptor blocking drugs at both the platelet and the vascular receptor and in these respects are more active than **CV4151**. The compounds appear to bind avidly to plasma proteins since when inhibition of U-46619-induced platelet aggregation is carried out in a resuspended platelet preparation (i.e. no plasma proteins present) a highly significant (5-10 fold) enhancement of potency is observed (Table 1). Importantly, these hybrid molecules retain their ability to inhibit collagen-induced thromboxane A_2 production in human citrated whole blood[1] and are similar in activity to **CV4151**.

Although Table 1 only lists analogues with a 1,3-substitution pattern on the pendant aromatic ring, the related 1,2- and 1,4-compounds, subsequently described in the patent literature[3e], were prepared but were at least ten fold less active as thromboxane receptor blocking drugs and were therefore not investigated further. The results presented indicate the importance of the spacer groups that separate the sulphonamide and carboxylic acid moieties from the remainder of the framework. For the sulphonamide group a linking chain of two methylene units was found to be optimum. Interestingly a hexenoic acid system is preferred with these hybrid molecules which is in contrast to the heptenoic unit present in **CV4151**. Although substitution in the sulphonamide phenyl ring does not seem to modify markedly either enzyme inhibitory activity or antagonist activity at the platelet receptor, there is a trend for lipophilic substituents to enhance potency at the vascular receptor, with this being optimised with a para-iodo group. Overall it was observed that TxA_2 synthase inhibitory activity was much less sensitive to structural modifications than TxA_2 receptor blocking activity.

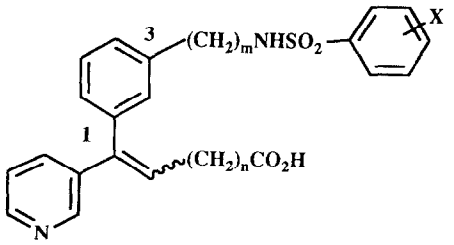
The compound of choice from this limited series was **GR 85305** and this was modified further by incorporation of a gem-dimethyl group at the benzylic position[8]. This resulted in **GR 108774** which was slightly more potent as a receptor blocking drug and similarly active as an enzyme inhibitor.



R=H **GR 85305**

R=Me **GR 108774**

TABLE 1. *IN VITRO* TXA_2 RECEPTOR ANTAGONIST/ SYNTHASE INHIBITORY ACTIVITIES OF COMPOUNDS RELATED TO CV4151

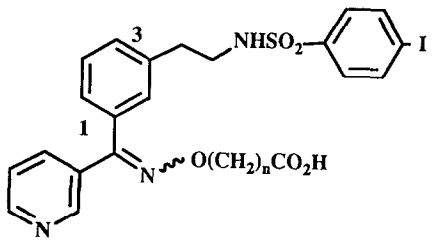


m	n[a]	X	TxA_2 ANTAGONIST ACTIVITY pA_2[b] HWB[c]	pA_2[b] Rat Aorta[d]	TxA_2 SYNTHASE ACTIVITY pIC_{50}
2	2	H	5.1	7.5	7.1
3	2	H	nse	NT	NT
2	3	H	6.1	7.8	7.3
3	3	H	6.0 (7.2)	6.9	7.3
2	3	4-I **(GR 85305)**	6.2 (7.4)	9.0	7.5
2	3	3,4-diCl	6.5 (7.5)	7.2	7.2
2	3	4-NO_2	6.2 (7.2)	8.4	7.4
2	3	2-NO_2	6.0	6.7	7.3
2	3	4-F	6.1 (6.9)	7.6	7.5
2	4	4-I	6.1	8.0	7.7
2	3	4-I[f] **(GR 108774)**	6.7 (7.6)	9.2	7.5
CV4151			4.8	5.2	7.0

a. Although compounds were generally tested as mixtures of E:Z isomers *ca* 4:1, **GR 85305** and **GR 108774** were pure E-derivatives. The corresponding Z-isomers were at least ten fold less active as synthase inhibitors. b. pA_2 values are a mean of at least two experiments. Schild analysis gave slopes not significantly different from unity. c. Inhibition of U-46619-induced platelet aggregation in human whole blood. Figures in parenthesis refer to a resuspended platelet preparation. 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. This compound has a $-C(Me)_2CH_2-$ link at position 3[8].

Despite the encouraging activity demonstrated *in vitro* by the compounds related to **CV4151**, derivatives with greater potency as thromboxane receptor antagonists were desired. A limited number of analogues derived from **R68070** which incorporated an oxime side chain were therefore investigated. Table 2 shows that thromboxane receptor blocking activity is highly dependant on the length of the oxime side chain and is optimised with a two methylene spacer group with **GR 103237** being the most potent antagonist synthesised. However, the overall profile of activity was less favourable since these oximes are an order of magnitude less active as synthase inhibitors. Furthermore, when tested *in vivo* their biological effects were extremely short-lived ($t_{1/2}$ <30min).

TABLE 2. *IN VITRO* TXA_2 RECEPTOR ANTAGONIST/ SYNTHASE INHIBITORY ACTIVITIES OF COMPOUNDS RELATED TO R68070



	TxA_2 ANTAGONIST ACTIVITY		TxA_2 SYNTHASE ACTIVITY
n[a]	pA_2 HWB	pA_2 Rat Aorta	pIC_{50}
1	6.7	8.8	<6.0
2 **(GR 103237)**	7.0 (8.3)	9.2	6.3
3	5.8 (6.9)	7.9	6.7
4	5.7 (6.9)	6.7	6.7
2[b]	6.5	8.7	6.7
R68070	5.4	5.9	7.5

a. The oximes were tested as mixtures of E:Z isomers *ca.* 1:1. b. This compound has a $-C(Me)_2CH_2-$ link at position 3.

An overall assessment of these hybrid compounds suggested that **GR 85305** and **GR 108774** had the best profile of activity and were therefore evaluated in more detail. As indicated in Table 1 both compounds antagonised U-46619-induced platelet aggregation in human whole blood (pA_2 values of 6.2 and 6.7 respectively). This antagonism was shown to be competitive, surmountable and also specific since the primary phase of ADP-induced aggregation was unaffected by both drugs (1mM). A specific effect was also seen on the thromboxane synthase enzyme with these drugs (0.01-10μM) with increases in PGE_2 and PGD_2 being observed during inhibition of serum thromboxane production in human clotting whole blood. However, at concentrations in a 100 fold excess of those required to completely inhibit TxA_2 formation, the levels of PGE_2 and PGD_2 began to decline, indicating an effect at the cyclooxygenase enzyme[10].

Since inhibition of collagen-induced platelet aggregation is a useful *in vitro* model for evaluating the metabolic re-direction of prostaglandin endoperoxides[1], these new agents were compared to some standard drugs in this sytem. Inhibition of aggregation by **GR 85305** and **GR 108774** (1μM) was at least equivalent to a maximal TxA_2 inhibiting concentration of aspirin (2mM). At higher concentrations (10-100μM), a greater effect was seen which was equivalent to that achieved with maximally effective concentrations of the thromboxane receptor blocking drug, **GR 32191** (10μM) and the synthase inhibitor, dazoxiben (10μM)[1].

In the anaesthetised dog, **GR 85305** or **GR 108774** (10μg/kg/min) produced >98% inhibition of serum TxA_2 production with collagen-induced platelet aggregation *ex vivo* also being markedly inhibited. Oral administration of either compound to the conscious dog (3 or 10mg/kg) produced similar maximum effects with **GR 108774** being the longer-acting. Specifically, **GR 108774** (10mg/kg) significantly inhibited collagen-induced platelet aggregation *ex vivo* for 12-24h and markedly inhibited (>90%) serum TxA_2 formation for 12h and partially (>30%) for 24-48h. At a dose of 3mg/kg effects persisted for 7-12h and 12-24h respectively.

The above data shows that **GR 108774** is an orally effective and long-acting combined thromboxane receptor antagonist/synthase inhibitor which may be of value in the treatment of thromboembolic disease.

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