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DISCOVERY OF DUAL ACTIVITY MOLECULES WITH THROMBOXANE ANTAGONIST AND THROMBOXANE SYNTHASE INHIBITORY ACTIVITY

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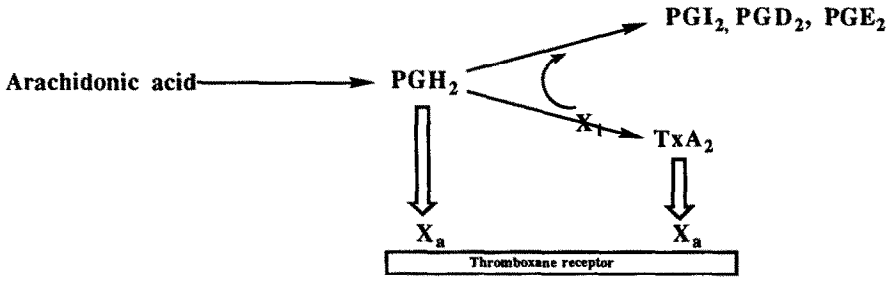
Abstract: A modification of a known thromboxane antagonist by the incorporation of a tethered pyridine moiety which gave a series of dual acting thromboxane antagonist/thromboxane synthase inhibitors is described. Compound **9** inhibits human thromboxane synthase with an IC_{50} of 50nM and is an antagonist of human platelet aggregation with a pA_2 of 6.0. This compound also demonstrates *ex vivo* activity when dosed orally to rats at 10mg/kg. p.o.

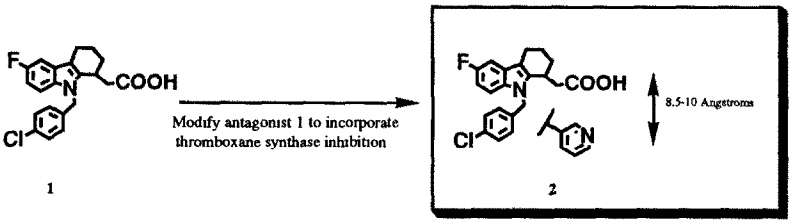
Thromboxane A_2 (TXA_2) is known to be a potent vasoconstrictor, bronchoconstrictor, and platelet aggregation agent.[1] Elevated levels of TXA_2 have been implicated in a wide range of pathological conditions including occlusive vascular disease and asthma.[2] A therapeutic approach to such diseases has been to reduce the effects of TXA_2 by selective TXA_2 receptor antagonism.[3] An alternative approach, particularly for occlusive vascular diseases, is to increase the blood levels of Prostacyclin (PGI_2), a vasodilator produced by endothelial cells which also inhibits platelet aggregation. A therapeutic approach to occlusive vascular disease using a stable mimetic of PGI_2 has also been explored.[4]

A strategy which combines the potential benefits from both of the above uses a dual acting thromboxane antagonist/thromboxane synthase inhibitor.[5] Such a compound would be expected to increase the levels of PGI_2, as well as other beneficial prostaglandins such as PGD_2 and PGE_2, by virtue of redirection of the biosynthetic route (Scheme 1) and decrease the levels of TXA_2. Elevated levels of PGH_2, which is itself a thromboxane agonist, are blocked by the antagonist activity of the compound. Studies in animals and in normal human volunteers with mixtures of TXA_2 antagonists and TXA_2 synthase inhibitors have demonstrated that the two agents have greater therapeutic benefits when given in combination than when given individually.[6]



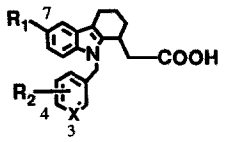
X1 =Thromboxane synthase inhibition
Xa =Thromboxane antagonism

We describe herein our search for such a dual acting agent by the structure modification of a known TXA_2 antagonist (1).[7]



Our approach was based on the idea that it might be possible to find a way of modifying **1** which would incorporate thromboxane synthase inhibition without loosing thromboxane antagonist activity. Heme-binding heterocycles such as pyridine or imidazole are reported to confer good inhibitory activity for thromboxane synthase when combined with a carboxylic acid using an appropriate linker.[8] It has also been shown previously that the heterocyclic nitrogen to carboxylate distance was in the range of 8.5 -10 angstroms in the most active thromboxane synthase inhibitor.[9] It was suggested that the 8.5 - 10 angstrom distance represented the Fe-Heme to carboxylate binding site separation in the enzyme. Recognizing that **1** contains a carboxylic acid we decided to explore the incorporation of a pyridine unit into **1** in the hope that it might be possible to pick up thromboxane synthase inhibitory properties and not lose thromboxane antagonist activity. We used the geometric information cited above to direct our initial efforts to find a suitable position for incorporation of the pyridyl substituent in the tetrahydrocarbazole nucleus. Initial attempts to replace the benzylic group in **1** with a 3-pyridylmethyl moiety led to compound **3** (Table 1) which was only weakly active as a thromboxane antagonist, and was inactive opposite thromboxane synthase. Since a 8.5 - 10 angstrom distance between the carboxylate and the pyridine nitrogen could not be attained in **3**, we than tried extension of the carboxylate chain to attain this separation between the functional groups. This gave a compound, which was found to be devoid of either thromboxane antagonist or thromboxane synthase inhibition.

We next turned to replacement of the 7-fluoro substituent with pyridine containing side chains. Although substitution at this position gave compounds which showed weak thromboxane synthase inhibition we were discouraged by the loss of thromboxane antagonist activity. In fact simple replacement of the 7-fluoro substituent in **1** with methoxy led to an order of magnitude decrease in the thromboxane antagonist activity of this molecule. Thromboxane antagonist activity is clearly sensitive to changes at this site.



Compound	R 1 *	R 2 *	X	Thromboxane antagonism (pA_2)#	Thromboxane synthase inhibition (IC_{50}, μM)♦
1	F	Cl	H	7.1	NA-
3	F	H	N	5.5	NA
4	F	3-[-$CH_2CH_2(CH_2)_3$Py]	C-H	5.4	0.5
5	F	3-[-$OCH_2(CH_2)_3$Py]	C-H	6.3	0.1
6	F	3-[-$O(CH_2)_5$-Py]	C-H	5.5	0.3
7	F	4-[-$O(CH_2)_3$—≡—Py]	C-H	6.8	0.6
8	F	4-[-$O(CH_2)_4$—≡—Py]	C-H	6.7	0.4
9	**F**	**3-[-$O(CH_2)_3$—≡—Py]**	**C-H**	**6.0**	**0.05**
10	F	3-[-$O(CH_2)_4$—≡—Py]	C-H	5.6	0.04
11	F	3-[-$O(CH_2)_3$—═—Py]	C-H	6.2	0.2

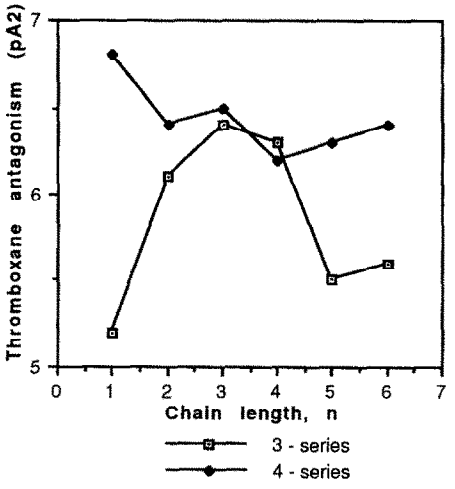
* Py = 3-pyridyl. - NA = not active. # Thromboxane antagonist potency against U 44619 induced platelet aggregation using human platelet rich plasma. ♦ Thromboxane synthase inhibition using human microsomal TXA_2 synthase; results are expressed as IC_{50}s for the inhibition of TxB_2 formation from ^{14}C labelled arachidonic acid.[13]

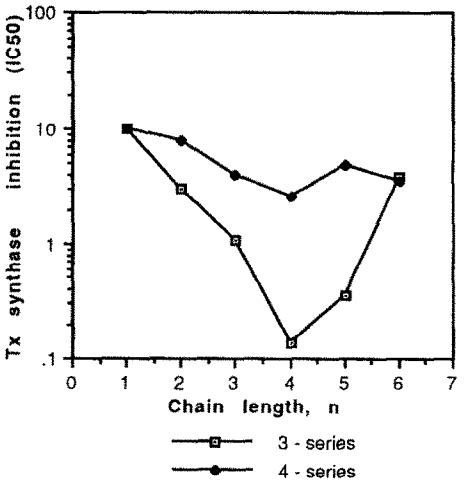
Table 1

Surprisingly substitution on the chlorobenzyl moiety proved to be more profitable. The activity of a series of compounds substituted in the 3- or 4- position of the phenyl group are shown in Fig. 1 and Table 1. It can be seen that in general thromboxane antagonist potency is retained with both substitution patterns although the most potent compounds are found with the pyridine - containing substituent in the 4- position. Inhibiting thromboxane synthase, however, appears to be more effective in compounds with the pyridine - containing substituent in the 3- position, with chain lengths of 4 to 5 (Fig 1).

Of course these compounds are all very flexible and they must pay an entropic penalty for this when they bind to the enzyme or receptor in terms of the loss of internal conformational freedom.[10] However, for each methylene unit in the sidechain we gain an increment of binding energy by virtue of an increased hydrophobic effect.[11] These two binding energy components may largely cancel out. The advantage of having such a flexible linking unit is that the terminal pyridine unit can more easily adopt the correct three dimensional position relative to the rest of the molecule. Therefore, we viewed the linker groups as not contributing to the binding energy in a major way but merely serving as a means of holding the pyridine moiety so that it could bind to the heme iron center. Thus these initial studies gave us important information relating to the feasibility of achieving good TXA_2 synthase inhibition while maintaining TXA_2 antagonism. Our strategy from this point was to try to reduce the conformational flexibility of the side chains by the incorporation of geometrically rigid elements in the hope that greater potency would result. We were aware,

however, that the conformation adopted by the compound when it binds to the enzyme may be different from that which binds to the receptor.





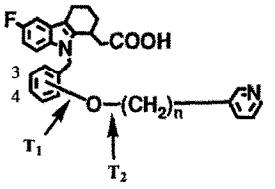
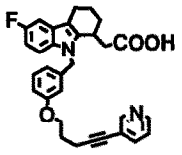


Fig 1

The phenolic link in the side chain is thought to introduce one element of rigidity with T_1 and T_2 prefering torsions of 0 degrees and 180 degrees respectively.[12] Replacement of the CH_2-CH_2 unit with the less conformationally flexible O-CH_2 leads to an increase in both activities (see **4** and **5**). Incorporation of an acetylene unit in place of a CH_2-CH_2 in **6** provided another increase in potency to give **9** . In the 4-substituted series compounds **7** and **8** showed good antagonist activity and a reasonable level of thromboxane synthase inhibition. Potent thromboxane synthase inhibition is seen in the 3- series with **9** and **10** with the former being a reasonably good antagonist. Reduction of the acetylene yielded the *cis*-olefin **11** which although slightly more potent than **9** was a poorer enzyme inhibitor.



9

Compound **9** was found to exhibit the dual properties of thromboxane synthase inhibition and receptor antagonism. When dosed orally (10mg/kg) to conscious rats compound **9** caused inhibition (86%, 77%, and 71%) of platelet TXA_2 receptors at 1, 3 and 5 hours after dosing, respectively.[13]

In conclusion we have demonstrated the successful modification of a known TXA_2 antagonist structure to obtain a dual action compound which involved appending a heme-binding pyridyl moiety. The synthesis and testing of a series of flexibly linked pyridines allowed us to evaluate the feasibility of the general approach. Increased potency was obtained by incorporation of an acetylene unit which restricted the conformational flexibility of the linking chain. The SAR to date has demonstrated that it is difficult to match high antagonist potency with potent TXA_2 inhibition in this system and may be suggestive of different conformational requirements for the TXA_2 receptor and TXA_2 synthase. Oral activity was demonstrated by **9** in an *ex vivo* rat model.[13]

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13. Thromboxane antagonism was assessed using a blood platelet aggregation test based on that described by Born [*Nature*, **194**, 927-929 (1962)]. Human citrated, platelet-rich plasma was aggregated by the addition of the TXA_2 mimetic agent U46619 so that a dose- response curve was generated. This was repeated in the presence of increasing amounts of test compound (generally in the range 10^{-5}M to 10^{-10}M) and a K_b was calculated. An apparent pA_2 , indicating antagonist potency, was thus generated (where $pA_2 = -\log K_b$). In *ex vivo* rat experiments antagonism was measured as a dose ratio (that is the ratio of the concentration of U46619 required to cause a 50% of platelet aggregation in the presence and absence of the test compound)
Thromboxane synthase inhibition was demonstrated using the standard *in vitro* test procedure used by Howarth [*Biochem. Soc. Transactions*, **10**, 239-240 (1982)] using a human platelet microsomal TXA_2 synthase preparation and using a quantitative thin layer radiochromatographic method to assess the conversion of [1-^{14}C] arachidonic acid to the TXA_2 metabolite thromboxane B_2.