Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 3, pp. 273-278, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00004-2

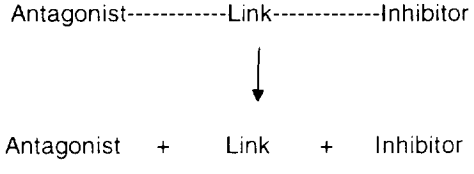
DESIGN OF DUAL-ACTING THROMBOXANE ANTAGONIST-SYNTHASE INHIBITORS BY A MUTUAL PRODRUG APPROACH

G.R. Brown*, D.S. Clarke, A.W. Faull, A.J. Foubister, M.J. Smithers.

Cardiovascular and Metabolism Department,
Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK

Abstract: A mutual prodrug approach to dual acting thromboxane receptor antagonist - thromboxane synthase inhibitor compounds is reported in which TXA_2 antagonist and inhibitory 1,3-dioxanes with hexenoic acid side chains, were linked by diester and diamide groups. When linking of the components was achieved via di O-alkyl carboxylic esters of catechol, both TXA_2 receptor antagonist activity and TXA_2 synthase inhibition were observed for a single enantiomer (**16**) in *ex vivo* tests following oral dosing to dogs at 5 mg/kg.

The hypothesis that the unstable arachidonic acid metabolite thromboxane A_2 (TXA_2) may be involved in the pathogenesis of cardiovascular disease, is now generally accepted[1]. Clinical trials of TXA_2 receptor antagonists or TXA_2 synthase inhibitors for these indications, have given equivocal results[2] and it has been postulated[3] that a dual acting TXA_2 receptor antagonist-synthase inhibitor compound might show positive clinical results. The design of these dual acting compounds could be envisaged in a number of ways, but herein the feasibility of a mutual prodrug approach is examined. Firstly this requires that a compound acting only as a TXA_2 receptor antagonist is linked to a TXA_2 synthase inhibitor. Secondly the link between these compounds has to be designed in such a way that following oral dosing, it will be cleaved *in vivo* to give the antagonist and inhibitor components.



We have previously described[4] a potent series of 1,3-dioxane TXA_2 receptor antagonist compounds and two typical examples of this series (**1**,**2**) were used as the antagonist components of the putative dual acting compounds. Further structural modification of these dioxanes led[5] to a novel series of dioxanes with antagonist and inhibitory properties. One of these compounds (**3**) which was a very potent TXA_2 synthase inhibitor, but only a very weak receptor antagonist, was selected as the inhibitor component for the dual acting compounds. Test results for the antagonist and inhibitor components are displayed in Tables 1 and 2.

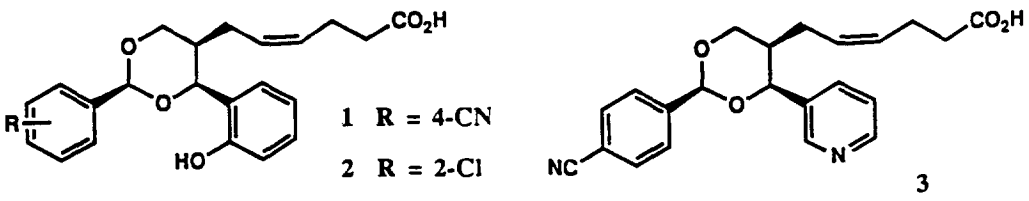


TABLE 1: Pharmacological Activities of Antagonist and Inhibitor Components for Human Platelet *In Vitro*

Compound	TXA_2 Antagonist Activity pA_2	TXA_2 Inhibitory Activity IC_{50} micromolar
1 (±)	8.3	>10
2 (±)	8.5	>10
3 (±)	5.9	0.02

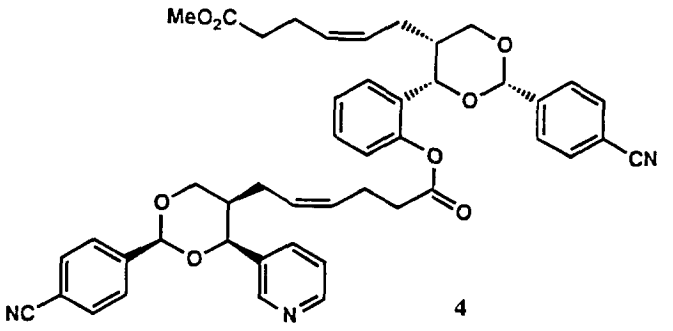
TABLE 2 : Dog *Ex Vivo* Test Results for Components

Compound	Dose mg/kg	TXA_2 Concentrarion Ratio at		TXA_2 Synthase % Inhibition at	
		2h.	8h.	2h.	8h.
1	0.05	60	35	<50	<50
2	0.05	93	76	<50	<50
3	1.0	-	-	84	84
3	1.0	1.4	1.3	97	93

Compounds were tested[6,7] *in vitro* for receptor antagonist and enzyme inhibitory properties using human platelets. Receptor antagonism was determined from concentration response curves following platelet aggregation with the thromboxane mimetic U46619 in the presence and absence of test compound. Agonist IC_{50} values were calculated and results expressed as pA_2 values. To determine TXA_2 synthase inhibition, human platelet microsomes were incubated with 1-^{14}C arachidonic acid in the absence and presence of test compound. The percentage conversion of labelled arachidonic acid to TXB_2 and $PGE_2/PGF_{2\alpha}$ was measured and potency expressed as an IC_{50} value. The potential mutual prodrugs described below (**5,6,7,8,13,14,15,16**), were all inactive (antagonist pA_2 <5.0, IC_{50} >10 μM) in these *in vitro* tests.

Ex vivo testing was carried out in conscious dogs (n= 4) which were orally dosed with 3 or 5 mg/kg of test compound in soft gelatin capsules. Blood was drawn from the jugular vein at 2 and 8 hours after dosing. Platelet rich plasma was prepared by differential centrifugation and antagonist activity measured by the method

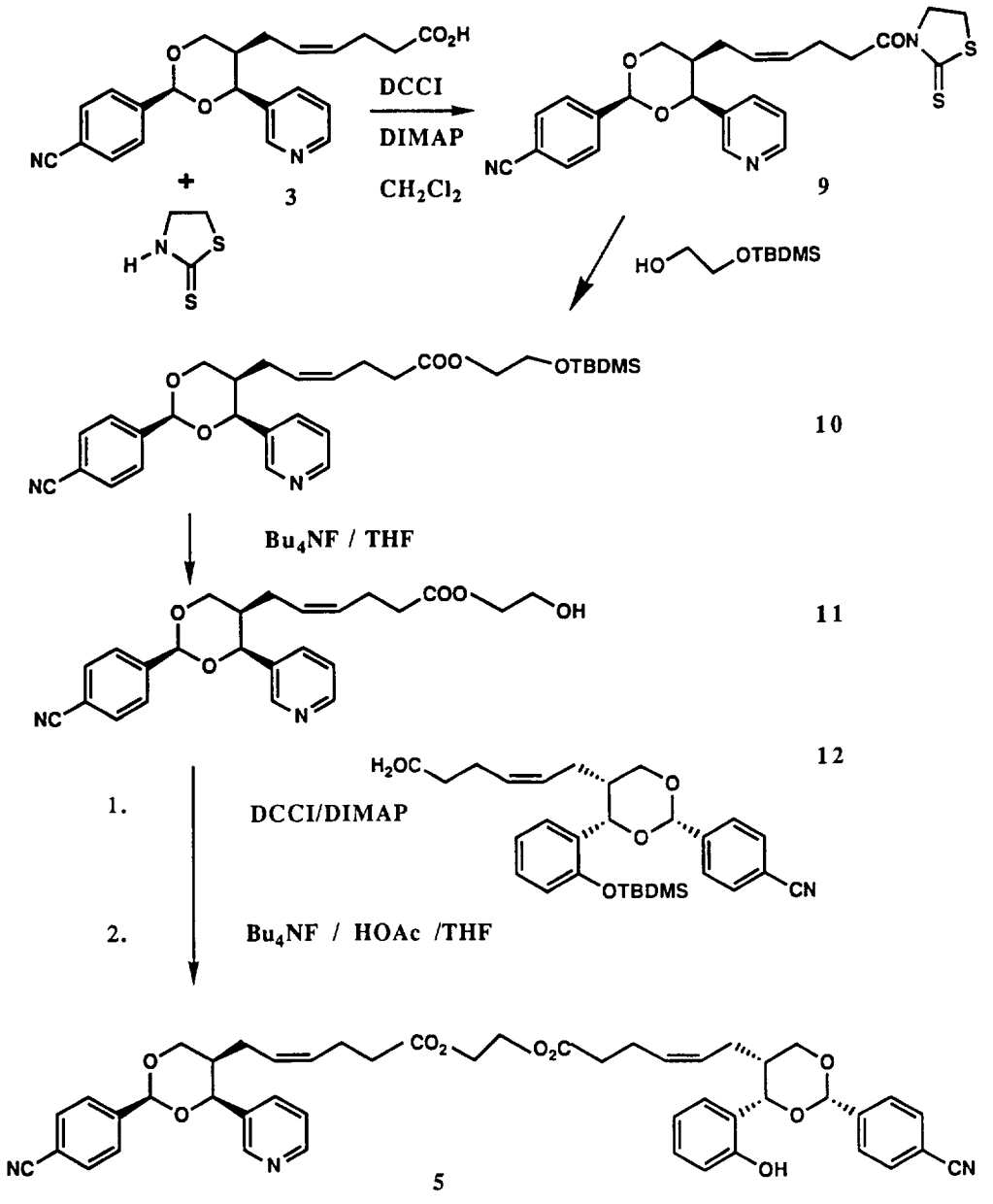
described above and expressed as concentration ratios. TXA_2 synthase inhibition was determined by measuring collagen stimulated TXB_2 production in the blood samples and reported as % inhibitions.



4

The mutual prodrugs were designed by linking the inhibitor component at two different points of attachment to dioxane **1**. Firstly the ester **4** was examined, where the phenolic hydroxyl of **1** was acylated by the carboxyl group of dioxane **3**. Compound **4** (mp., 42-44^{0}C) was prepared in 89% yield by reaction of the methyl ester of dioxane **1** with dioxane **3** in methylene chloride solution for 2 hours in the presence of dicyclohexylcarbodiimide (DCCI) and 4-dimethylaminopyridine (DIMAP). Compound **4** displayed neither antagonist nor enzyme inhibitory properties following an oral dose to dogs of 3 mg/kg. There can be a number of reasons why a compound does not display biological activity *in vivo*, but the object of this work was to express the activity of known active components of a mutual prodrug. Therefore analysis of the biological test results was restricted to consideration of the extent of cleavage of the prodrug to give the active components (**1**,**3**). The inactivity of **4** may thereby be due to the poor accessibility of the ester link for hydrolysis. In the second approach, diester and diamide linking groups were used to join the carboxyl groups of **1** and **3**. These groups were selected as they are commonly used in the design of conventional prodrugs[8]. Four links were examined $O(CH_2)_2O$ (**5**), $O(CH_2)_6O$ (**6**), $NH(CH_2)_2NH$ (**7**) and $NH(CH_2)_6NH$ (**8**) . Compounds **5** (m.p., 58-61^{0}C) and **6** (m.p., 49-50^{0}C) were prepared by reaction of dioxane **3** with 2-mercaptothiazoline in the presence of DCCI and DIMAP to give an activated ester (eg **9**, Scheme 1). The activated ester **9** was allowed to react with the appropriate mono t-butyldimethylsilyl protected diol to afford **10**. The silyl group was removed either with THF/ aqueous acetic acid or n-tetrabutyl ammonium fluoride (TBAF) in THF to give **11**. Compound **1** was treated with t-butyldimethylsilyl chloride in the presence of imidazole and DMF to yield the acid intermediate **12**. To obtain the mutual prodrugs (eg **5**), **11** was coupled with **12** using DCCI and DIMAP in THF, followed by de-silylation with TBAF/HOAc in THF . The diamide derivatives (**7**, m.p., 47-48^{0}C, **8**, m.p., 83-86^{0}C)) were synthesized by reaction of excess of the appropriate diamine with the methyl ester of **1** in methanol at the boiling point and the resulting primary amine treated with the dioxane **3** in the presence of 1-hydroxybenzotriazole and DCCI.

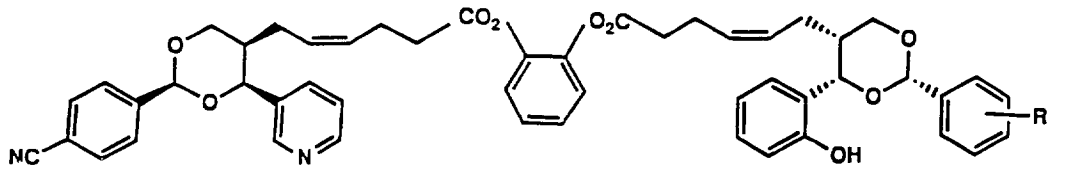
Surprisingly no *ex vivo* antagonist or inhibitory activity was observed in the dog after a 3 mg/kg oral dose of test compounds (**5**,**6**,**7**,**8**). It was postulated that hydrolysis of these mutual prodrugs had not proceeded and potentially more easily cleaved[9,10] aryl ester derivatives were examined. The quinol derivative (**13**, m.p., 79-82^{0}C) and the catechol diesters (**14**, m.p., 64-69^{0}C, **15**, m.p..71-73^{0}C **16**. m.p., 62-66^{0}C. Table 3) were also


DCCI
DIMAP
CH_2Cl_2
3
9
10
11
12
Bu_4NF / THF
1. DCCI/DIMAP
2. Bu_4NF / HOAc /THF
5

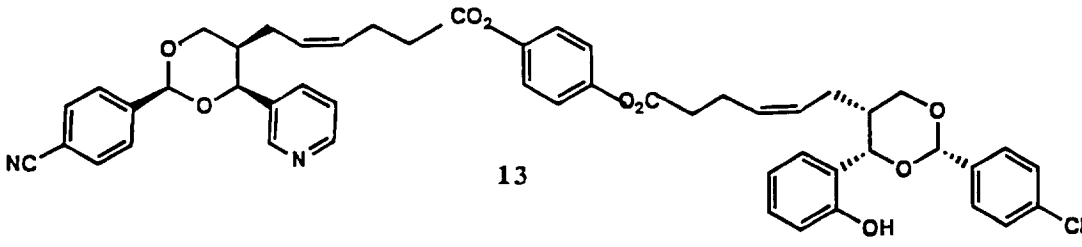
Scheme 1

prepared by the above synthetic method (Scheme 1). The diester (**13**) formed between components **1** and **3** and quinol, afforded *ex vivo* receptor antagonist concentration ratios against the thromboxane mimetic U46619 (of 50 at 2 hours and 57 at 8 hours) in tests in dogs dosed orally at 3 mg/kg.

Table 3 : Dog Ex Vivo Test Results for Catechol Esters



Compound	R	Dose mg/kg	Concentration Ratio		% Inhibition	
			at 2h	8h	at 2h	8h
14	4-CN	3	587	283	68	<50
15	2-Cl	5	140	170	54	57
16	2-Cl (-)	5	35	150	31	64



13

However no inhibition of TXA_2 synthase was found in these *ex vivo* tests. A possible explanation of this result is that slow breakdown of the quinol diester had occured. In these circumstances there might be too low a concentration of the inhibitor component (**3**) to demonstrate inhibition, whereas there may be sufficient of the very potent antagonist component (**1**) (Table 2), for receptor antagonism to be observed. It was anticipated[11] that breakdown might be even more facile if the components were linked as a catechol diester. Results of *ex vivo* tests in dogs of catechol esters are displayed in Table 3. These results show that both receptor antagonism and synthase inhibition were observed for compounds **14** and **15**. Compounds **14** and **15** were prepared from racemic dioxane starting materials (**1**,**2**,**3**) and are thus mixtures of diastereoisomers. To avoid the uncertainties associated with the administration of mixtures, a mutual prodrug was prepared as a single enantiomer. Thus **16** ($[\alpha]^{25}_D$ = -113.9^0 , c = 1.98, EtOH)was prepared from the active (-) enantiomers of **2** and **3** by the method in Scheme 1. Results of *ex vivo* dog tests for compound **16** dosed at 5 mg/kg (Table 3) showed that both activities were present at the 2 hour test point although the inhibitory activity was weak. At 8 hours following dosing, both properties (Table3) were well expressed.

Pharmaceutical stability data was not determined for all compounds, but the catechol linked compound **14** had a half life for chemical hydrolysis[4] of 17 days in the range pH 4 - 7 at room temperature. At pH = 2

however, the half life was 1 day and it was concluded that prodrugs linked as catechol esters might possibly be cleaved to some extent before intestinal absorption took place.

In conclusion we have shown that application of the mutual prodrug approach has led to the identification of a single enantiomer with TXA_2 receptor antagonist - TXA_2 synthase inhibitory properties *in vivo*, when the separate 1,3 dioxane components were linked together as catechol diesters.

Acknowledgements We thank our bioscience colleagues, Mr R. Jessup and Mr M. Wayne, for the bioscience results. Stability work was done by Mr S. Nicholson and Dr J. L. Longridge.

References

1 Gresele, P.; Deckmyn, H.; Nenci, G.G.; Vermylen, J. *Trends Pharmacol. Sci.* **1991**, *12*, 158-163.
2 Collington, E.W.; Finch, H. *Ann. Rep. Med.Chem.*, Bristol, J.A. Ed., Academic Press Inc, San Diego, **1990**, *25*, 99-108.
3 Patscheke, H. *Blut* **1990**, *60*, 261-268.
4 Brewster, A.G.; Brown, G.R.; Foubister, A.J.; Jessup, R.; Smithers, M.J. *Prostaglandins*, **1988**, *36*, 173-178.
5 Faull, A.W.; Brewster, A.G.; Brown, G.R.; Smithers, M.J.; Jackson, R. *J. Med. Chem.* **1995**, *38*, 686-694.
6 Jessup, C. L.; Jessup, R.; Wayne, M. *J. Pharm. Pharmacol.* **1986**, *38*, 754-757.
7 Carey, F.; Fisher, R.W.; Haworth, D. *Biochem. Soc. Trans.* **1982**, *10*, 239-240.
8 Krogsgaard-Larsen, P.; Bundgaard, H. *A Textbook of Drug Design and Development*, Harwood Academic Publishers, Philadelphia, **1991**, p153.
9 Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, John Wiley and Sons, New York, **1990**, p 162.
10 Krogsgaard-Larsen, P.; Bundgaard, H. *A Textbook of Drug Design and Development*, Harwood Academic Publishers, Philadelphia, **1991**, p156.
11 Fuller, E.J. *J.Am.Chem.Soc.* **1963**, 85, 1777-1780.

(*Received in Belgium* 7 *November* 1995; *accepted* 22 *December* 1995)