Bioorganic & Medicinal Chemistry Letters, Vol.1, No.3, pp. 173-178, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00
© 1991 Pergamon Press plc

STRUCTURAL HOMOLOGIES AMONG THROMBOXANE (TXA_2) RECEPTOR ANTAGONISTS: MINIMAL PHARMACOPHORIC REQUIREMENTS FOR HIGH AFFINITY INTERACTION WITH TXA_2 RECEPTORS

Gaétan Ladouceur,* Dale E. Mais, Joseph A. Jakubowski, Barbara G. Utterback, and David W. Robertson.
Departments of Chemistry and Cardiovascular Pharmacology, Lilly Research Laboratories,
Lilly Corporate Center, Indianapolis, IN 46285

(*Received* 11 *February* 1991)

Abstract: Several sulfonamide derivatives related to S-145 were synthesized and tested as thromboxane receptor antagonists. The results reveal the importance of the orientation between the sulfonamide moiety and the carboxyclic acid group of the alpha-side chain in order to obtain high affinity interaction with TXA_2 receptors.

Thromboxane (TXA_2, **1**) appears to mediate a variety of pathophysiological states.[1] This molecule plays a major role in platelet aggregation and vasoconstriction.[2] The regulation of its biological activity may have important applications in the treatment of thrombosis and other vascular disorders. Accordingly, many TXA_2 receptor antagonists have been studied, including S-145 (**2**).[1-3] This molecule, and other potent sulfonamide-derived TXA_2 receptor antagonists, possesses a carboxylic acid group, tethered by 9 atoms to a sulfonamide moiety.

CO_2H O O OH NHSO$_2$Ph CO_2H

1 **2**

In an effort to deduce the minimal pharmacophoric requirements for high affinity interaction with TXA_2 receptors, we synthesized and evaluated the receptor affinities of simplified versions of S-145. For example, the ethano bridge or the entire bicyclic system of S-145 were consecutively removed, leading to simple cyclopentane (**3**)[4] and, straight alkyl chain (**4**) analogues (Figure 1)[5].

Figure 1:

NHSO$_2$Ph CO_2H

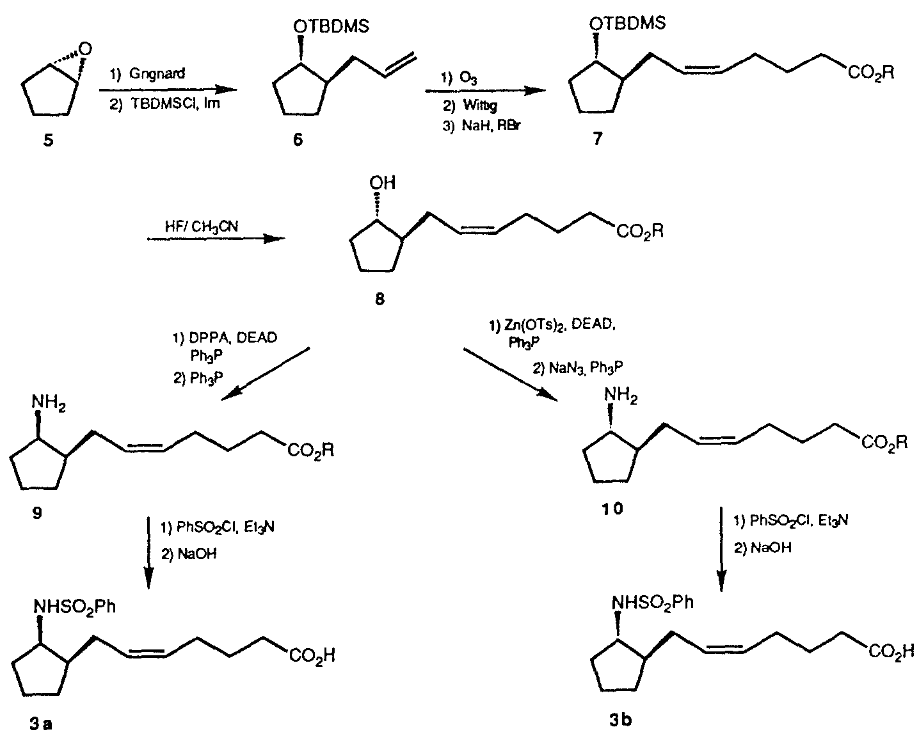
2

NHSO$_2$Ph CO_2H NHSO$_2$Ph CO_2H

3 **4**

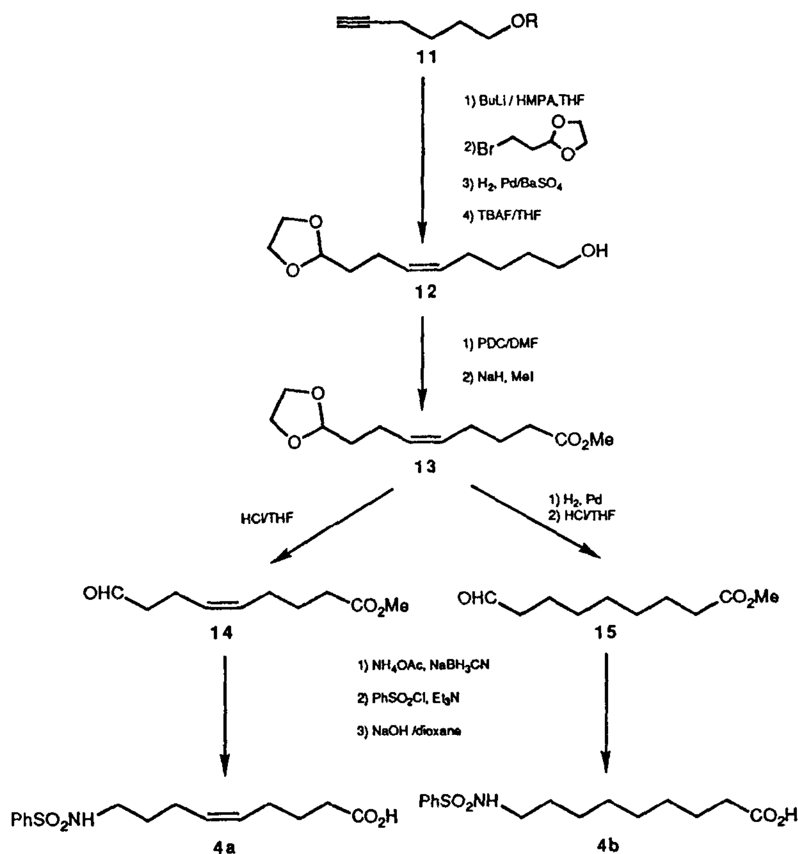
The synthesis of the analogues **3a** and **3b** (scheme 1) began with cyclopentane oxide **5**, which undergoes a nucleophilic attack by allyl magnesium bromide[6] to give, after protection, the silylether **6**. An oxidative cleavage of the olefin (78%), followed by a Wittig reaction using 4-carboxybutyltriphenylphosphonium bromide and t-butoxide[7] gave the carboxylic acid which was converted to the corresponding ester **7**[8] (73%, 2 steps). After deprotection of the silylether group, alcohol **8** was treated with DPPA under Mitsunobu conditions[9] to obtain, after reduction of the azide,[10] amine **9** (62%). Formation of the sulfonamide and a careful saponification of the ester, gave the *cis* thromboxane receptor antagonist **3a** (71%). The *trans* isomer **3b** was obtained in a similar way: a tosylation of **8** with inversion of configuration[11] (55%), followed by a SN_2 displacement with sodium azide, afforded the *trans*-azide (74%). Reduction (80%), followed by coupling with benzenesulfonylchloride and saponification (74%), gave the pure *trans* sulfonamide **3b**.

Scheme 1:



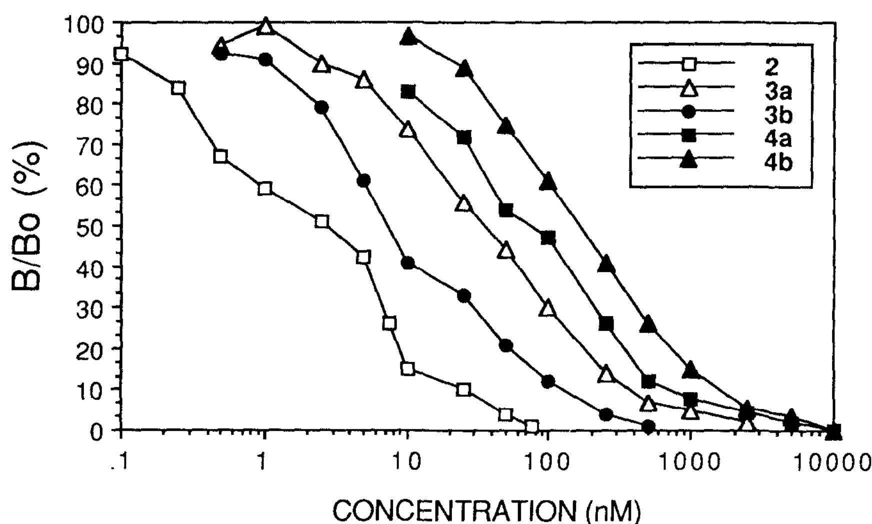
Analogues **4a** and **4b**, were synthesized from alkyne **11**, which was treated with n-BuLi in a mixture of THF and HMPA (3:2), then alkylated with 2-(2-bromoethyl)-1,3-dioxolane (38 %) (scheme 2). Hydrogenation with Lindlar catalyst followed by a deprotection of the silylether, gave the alcohol **12** (76 %). A further oxidation of the alcohol and esterification afforded the ester **13** (40 %). Hydrolysis of the ketal gave the aldehyde **14** (~90 %), which underwent a reductive amination,[12] followed by a sulfonylation of the amine. Saponification of the ester group gave the sulfonamide **4a** (overall 20 %). We were also interested in examining the fully saturated compound **4b**, which was prepared from the common intermediate **13**, by hydrogenation of the olefin in ethyl acetate (95 %), and then following the same sequence as described for the preparation of sulfonamide **4a**.

Scheme 2:



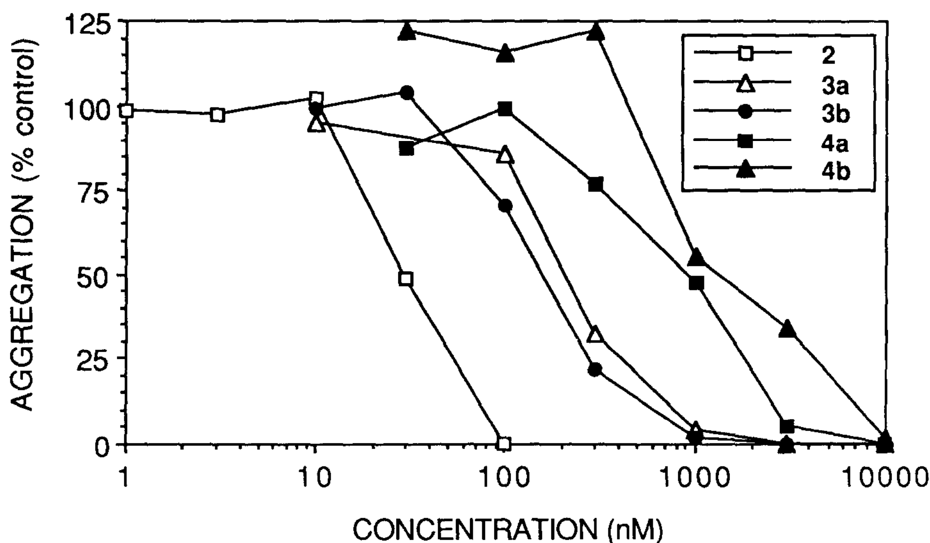
Biological Results and Discusion: In order to assess the affinities of compounds **2-4b** for platelet TXA_2 receptors, radioligand binding assays were performed in washed human platelets using the radioiodinated ligand [^{125}I]IBOP, which has been shown to bind with high affinity to TXA_2 receptors in human platelets.[13] Washed human platelets were incubated with [^{125}I]IBOP and increasing concentrations of the various antagonists at 37°C for 30 min.[13] Unbound ligand was removed by rapid filtration and specific binding was determined. The results of these binding assays are shown in the series of competition curves in Figure 2. Compound **2**, *(d,l)* S-145, is also shown for reference. The K_d values for the analogues were 2.9 ± 0.9 nM, 36.0 ± 4.1 nM, 7.5 ± 0.3 nM, 67 ± 6 nM and 172 ± 12 nM for compounds **2**, **3a**, **3b**, **4a** and **4b**, respectively (mean, n = 3 for all compounds).

Figure 2: Inhibiton of [^{125}I]IBOP Binding to Washed Human Platelets by Analogs **2-4b**



The above data indicate that the analogs bind to the platelet TXA_2 receptor; however, the data do not indicate the nature of the analog-receptor interaction, i.e., antagonism, agonism or partial agonism. In order to determine the precise nature of the interaction, functional platelet aggregation studies were performed. Human platelet aggregation studies in platelet-rich plasma were performed. Platelets were incubated with **2-4b** for 30 min at 25°C and then challenged with the stable thromboxane mimetic U46619. The platelet aggregation response in stirred suspensions was followed at 37°C turbidometrically as previously descibed.[14] Analogs **3-4b** alone did not induce platelet aggregation. However, as illustrated in Figure 3 analogs **3-4b** did antagonize the platelet aggregatory activity of U46619 in a dose-dependent manner, S-145 (**2**) was included for comparison. The rank order of antagonism matched exactly that found in the radioligand-receptor binding studies (Figure 2) with a calculated correlation coefficient (r) of 0.95. The IC_{50} values (concentration required to inhibit platelet aggregation by 50%) were 32 nM, 250 nM, 150 nM, 1000 nM, and 1500 nM for **2**, **3a**, **3b**, **4a**, **4b**, respectively. The overall higher IC_{50} values compared to the Kd values from binding studies no doubt reflect the plasma binding of the analogs in the plasma-based aggregation studies.

Figure 3: Antagonism of U46619-Induced Human Platelet Aggregaion by Analogs **2-4b**



These results indicate that the rigid bicyclic nucleus is not absolutely necessary for antagonist activity at the TXA_2 receptor. In the cyclopentyl series only a modest loss in activity was observed for the *trans* cyclopentyl analogue (**3b**), when compared to S-145 (**2**). However, the orientation of the two side chains was more critical as reflected in the 12-fold loss in receptor binding activity of the *cis* cyclopentyl analogue (**3a**), compared to S-145 (**2**). The loss of the cyclopentyl group produces a further decrease in affinity as seen in compound **4a** compared to **3a** and **3b**. This indicates that the retention of some rigidity in maintaining the two side groups is important to maintain optimal interaction with the receptor. Finally, saturation of the double bond (**4b**) leads to a further 3-fold loss in receptor binding activity compared to the olefin **4a**.

In conclusion, these data demonstrate that the minimum pharmacophoric requirements for high affinity interaction with TXA_2 receptors include the carboxylic acid and sulfonamide moieties, held in appropriate spatial orientation relative to one another. The nature of the tether between these two groups, including the rigid bicyclic framework of S-145 (**2**), does not appear to be an absolute determinant of biological activity within this class of sulfonamide-derived TXA_2 receptor antagonists. Since our studies were limited to the platelet TXA_2 receptor, we cannot address the specificity of these antagonists or there relative activities toward TXA_2 receptors on other cell types. Such questions will be the subject of future studies.

References

1) C. Patrono, G. Ciabattoni, and G. Davi. *Stroke*, **1990**, *suppl. iv*, 130.

2) E.W. Collington and H. Finch, *Annual Reports in Medicinal Chemistry*, **1990**, *25*, 99.

3) M. Narisada, M. Ohtani, F. Watanabe, K. Uchida, H. Arita, M. Doteuchi, K. Hanasaki, H. Kakushi, K.Otani and S. Hara, *J. Med. Chem.*, **1988**, *31*, 1847; M. Nakajima and M. Ueda, *J. Cardiovasc. Pharmacol.*, **1989**, *14*, 502; Y. Hori, H. Hatakeyama, K. Yamada and A. Kurosawa, *Japan J. Pharmacol.*, **1989**, *50*, 195; K. Ezumi, M. Yamakawa, and M. Narisada, *J. Med. Chem.*, **1990**, *33*, 1117.

4) A different synthesis of compounds 3a & 3b have been reported elsewhere; K. Kawada and T. Tsushima, *Heterocycles*, **1989**, *28*, 573.

5) Satisfactory spectroscopic and analatical data were obtained on each of the products reported herein.

6) L.S. Hegedus, M.S. Holden, and J.M. McKearin, *Org. Synth.*, **1984**, *62*, 48.

7) E. J. Corey and G. Moinet, *J. Am. Chem. Soc.*, **1973**, *95*, 6831.

8) Approximately 5% of the trans olefin could be detected by nmr.

9) B. Lal, Birenda N. Pramanik, M.S. Manhas, and A.K. Bose, *Tet. Lett.*, **1977**, *23*, 1977.

10) M. Vaultier, N. Knouzi and R. Carrié, *Tet. Lett.*, **1983**, *24*, 763.

11) I. Galynker and W.C. Still, *Tet. Lett.*, **1982**, *23*, 4461.

12) R.F. Borch, M.D. Bernstein, and H.D. Durst, *J.Am.Chem.Soc.*, **1971**, *93*, 2897.

13) T.A. Morinelli, J.E. Oatis Jr., A.K. Okwu, D.E. Mais, P.R. Mayeux, A. Masuda, D.R. Knapp, and P.V. Halushka, *J. Pharm. Exp. Ther.*, **1989**, *251*, 557.

14) J.A. Jakubowski, M.J. Stampfer, R. Vaillancourt, D. Faigel and D. Deykin. *J. Lab. Clin.Med.*, **1986**, *108*, 616.