# A Proposed Common Spatial Pharmacophore and the Corresponding Active Conformations of Some $TxA_2$ Receptor Antagonists

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Received March 8, 1994*

Four pharmacophore recognition sites have been proposed for active thromboxane $A_2$ ($TxA_2$) antagonists. We have sought to define the corresponding spatial pharmacophore for these four sites by performing conformational analysis and molecular superposition studies on five known antagonists: SQ 29,548, SQ 28,668, SQ 27,427, BM 13.505, and a Merck Frosst compound. The strategy was to identify a low-intramolecular-energy conformer state for each antagonist for which the relative locations and orientations of the corresponding recognition site groups were in common when all five antagonists were superimposed. The conformations used in the successful molecular superpositions were then postulated to be the active conformations of each antagonist. Since SQ 29,548 is the most potent of the five antagonists, it was considered the reference structure in the molecular superposition. A unique spatial pharmacophore was identified and may be a useful template in designing a new $TxA_2$ antagonists.

## INTRODUCTION

Thromboxane $A_2$ ($TxA_2$), a metabolite of arachidonic acids, is an extremely potent inducer of platelet aggregation and vascular pulmonary smooth muscle contraction. It is involved in a variety of thrombosis, asthma, ischemic, and myocardial infarction diseases.[1-3] The actions of $TxA_2$ are mediated via a specific thromboxane/prostaglandin $H_2$ (TP) receptor in the membrane of target cells.[4] Therefore, antagonists of the $TxA_2$ receptor are expected to have therapeutic importance. Some compounds which block the $TxA_2$ receptor have already been in clinical trials.[5]

Since the middle of the 1970s, many receptor antagonists have been synthesized. Hall has summarized the structural classes of $TxA_2$ receptor antagonists.[5] The structural diversity of these antagonists make rational design based upon structure–activity relationships in this class of therapeutic agents difficult. Nevertheless, some rational drug design studies have been performed.[6-9]

Some investigators suggest that an antagonist may share the same "active" conformation as an agonist. Ezumi et al.[6] used molecular mechanics and molecular orbital calculations to study some $TxA_2$ agonists and antagonists: $TxA_2$ (I), U-46619 (II), S145 (III), and BM 13.177 (IV). These structures are shown in Figure 1. A common binding conformation was postulated for all of these compounds in which the molecules adopt a hairpin conformation. However, the conformational searching was done at 120° resolution in torsion angle space which may be too coarse and overlook plausible conformational models. Yamamoto and co-workers[7] have reported molecular modeling of the human $TxA_2$ receptor. The $TxA_2$ receptor is a member of the G-protein-coupled receptor family. Its amino acid sequence has been determined.[10] Yamamoto and co-workers built their $TxA_2$ receptor model on the basis of this sequence. They docked TCV-144, a $TxA_2$ receptor antagonist, into the protein model and generated an explanation of the structure–activity relationship for this series of antagonists. It was pointed out that their ligand–receptor model needed refinement, so that the proposed active ligand conformation might also need to be re-evaluated.

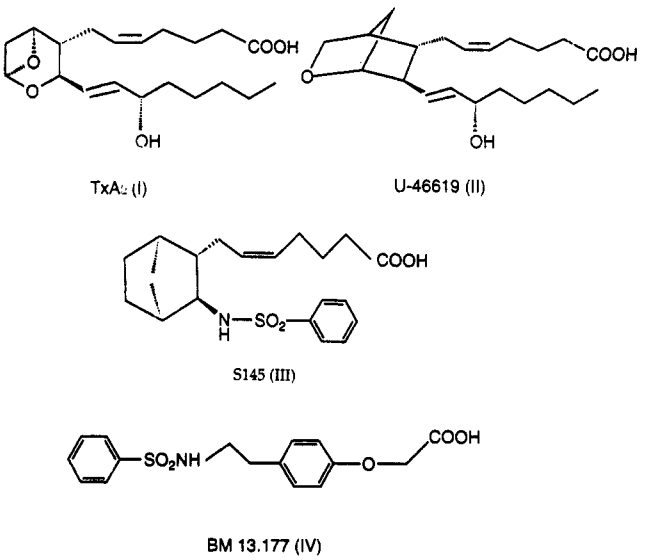


**Figure 1.** Structures of $TxA_2$, U-46619, BM 13.177, and S145.

The goal of the work reported in this paper has been to use molecular modeling methods, specifically conformational analysis and molecular superposition techniques, to map out and hypothesize an active conformation for some classes of $TxA_2$ receptor antagonists.

## METHODS

**A. Selection of Antagonists.** Analogs in the 7-oxabicyclo-[2.2.1]heptane series, namely, compounds SQ 29,548 (**1**),[11] SQ 28,668 (**2**),[12] and SQ 27,427 (**3**),[13] as well as a sulfonamide compound BM 13.505 (**4**)[14] and a relatively rigid Merck Frosst compound (**5**),[15] were selected. The structures of these antagonists are shown in Figure 2. All of these compounds are potent $TxA_2$ receptor antagonists. They competitively bind to the $TxA_2$ receptor with high affinity. SQ 29,548 is one of the most well characterized and most potent $TxA_2$ receptor antagonists ($K_d$ = 5.2 nm) known.

**B. Proposed Pharmacophore.** According to Farmer's three-site-ligand concept,[16] high affinity requires that an antagonist

* Abstract published in *Advance ACS Abstracts,* June 1, 1994.

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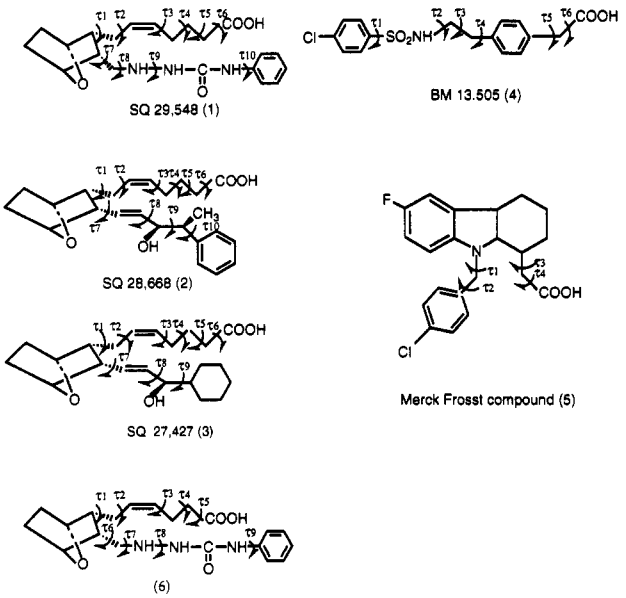


**Figure 2.** Structures of SQ 29,548, SQ 28,668, SQ 27,427, BM 13.505, the Merck Frosst compound (**5**), and the test compound (**6**).

possess at least three recognition sites. In general, almost all $TxA_2$ receptor antagonists possess a carboxylic acid group which is hypothesized to act as a primary recognition site. Some studies support this hypothesis. Hirata and co-workers[10] found that the $TxA_2$ receptor has an Arg295 in the seventh transmembrane domain. The side chain of this residue may interact with the carboxyl group of $TxA_2$ receptor antagonists. Nicolar et al.[17] report that an acidic group at the extremity of the side chain of most of $TxA_2$ receptor antagonists is necessary to obtain high binding affinity. The replacement of the terminal carboxylic acid of an antagonist (compound **37a** in ref 17) with a carboxamide or carbonitrile leads to a significant loss of its activity. Misra et al.[18] report that the interphenylene 7-oxabicyclo[2.2.1]heptane oxazole series of $TxA_2$ receptor antagonists also require an acidic group to maintain potent antagonistic activity. S1 is used to define this pharmacophore site.

Some studies also suggest that a carbonyl or carbonyl-like hydrogen bond acceptor group in the $\omega$-chain is required for activity. Misra et al.[18] pointed out that removal of the hydrogen bond acceptor group in the $\omega$-chain results in more than a 150-fold loss in inhibition potency. This functional requirement is further supported by the poor antagonistic activity of the carbinol analog whch is more than 700-fold less potent than the unsubstituted 4-amide analog. These structure–activity findings demonstrate that a carbonyl, or a carbonyl-like hydrogen bond acceptor group, in the $\omega$-chain is important to antagonist activity. The symbol S2 is used to indicate this site.

The third recognition site (S3) is assumed to be the head of the SQ series of compounds, that is the 7-oxabicyclo[2.2.1]-heptane ring. Many different heads have been synthesized.[19] The effect on the overall biological profiles of these structural variations is mixed, but it is clear that the head structure influences $TxA_2$ receptor antagonist activity. Although the oxygen in the head can be replaced by a carbon, no compound with a head other than 7-oxabicyclo[2.2.1]heptane ring is reported to be more potent than, or as potent as, the corresponding antagonist with the 7-oxabicyclo[2.2.1]heptane ring as the head.

**C. Conformational Analysis and Distance Matching.** If the proposed pharmacophore is correct, then for an active conformation the distances between the three recognition sites should be nearly the same for each potent antagonist. For active analogs of the same antagonist class, some low-energy conformational states should possess these common distances between the pharmacophore sites. Since many antagonists are flexible molecules, there are many low-energy conformations with different molecular shapes and corresponding interatmoic atom-pair distances. It is virtually impossible but, fortunately, also not necessary to consider all of the possible conformations. Only conformations within 5 kcal/mol of the lowest identified intramolecular energy state (the global minimum) are assumed to be possible during the ligand–receptor interaction. For Boltzmann statistics these conformations constitute almost 90% of the conformer population. Systematic conformational searching is used to find these low-energy conformations. Moreover, only apparent intramolecular energy minima with the low-energy conformer population are initially considered as pharmacophore candidates.

The distances between the three recognition sites are determined for the set of low-energy, apparent minima of each compound. SQ 29,548 is one of the most potent $TxA_2$ antagonists. It binds to the receptor with high affinity, and it also has a relatively rigid $\omega$-chain. Thus, the low-energy conformations of SQ 29,548 have been used as candidate reference active conformations. Low-energy conformations of SQ 28,668 and SQ 27,427 have been sought such that each superimposes on the candidate active conformations of SQ 29,548 with respect to the postulated pharmacophore distances. Further, if any resulting active conformation candidate has a distinct pharmacophore that is also consistent with the pharmacophore distances of both the sulfonamide compound, BM 13.505, and the Merck Frosst compound (compound **5**) in their respective low-energy conformations, then it is reasonable to conclude that all of these $TxA_2$ receptor antagonists can adopt low-energy conformations containing the common pharmacophore and, in composite, map the binding site.

**D. Molecular Modeling.** The CHEMLAB-II (version 11.0)[20] and QUANTA (version 3.3)[21] molecular modeling programs were used to perform the molecular modeling. Specifically all work was done in CHEMLAB-II except for the molecular superposition and alignment studies. The $\alpha$-chains and $\omega$-chains of SQ 29,548, SQ 28,668, and SQ 27,427 were built separately, and their respective geometries were optimized using the MM2 force field[22] with some force field parameters generated according to the method developed by Hopfinger.[23] A reasonable geometry of 7-oxabicyclo[2.2.1]-heptane could not be constructed using the MM2 force field. The structure of this fragment was optimized using the MNDO semi-empirical molecular orbital method.[24]

After the initial structure was optimized, a strategy was employed to systematically scan the torsion angle space of each antagonist. The definition of each torsion angle is given in Figure 2. Some of these molecules contain up to 10 torsion angles, and if each angle is scanned at reasonable resolution, such as 30-deg increments, almost 62 billion conformations will have to be explored. In order to reduce the size of the conformational search, the molecular decomposition and recomposition technique was used.[25] Compounds SQ 29,-548, SQ 28,668, and SQ 27,427 were decomposed into two fragments. One fragment possesses the head with the $\alpha$-chain, and the other possesses the head with the $\omega$-chain. The decomposition fragment structures are shown in Figure 3. The scanning resolution for the torsion angles are given in Table 1. The apparent local minima (the energy cutoff is 10

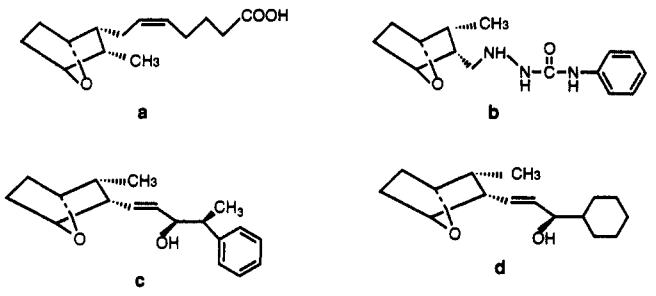


**Figure 3.** Fragments used in the decomposition of SQ 29,548, SQ 28,668, and SQ 27,427.

**Table 1.** Resolution of the Grid Scans for Compounds SQ 29,548, SQ 28,668, SQ 27,427, BM 13,505, and Compound **5**

| torsion angle | SQ 29,548 | SQ 28,668 | SQ 27,427 | BM 13.505 | compound **5** |
|---|---|---|---|---|---|
| t1 | 30 | 30 | 30 | 30 | 10 |
| t2 | 30 | 30 | 30 | 30 | 10 |
| t3 | 60 | 60 | 60 | 30 | 10 |
| t4 | 60 | 60 | 60 | 30 | 10 |
| t5 | 120 | 120 | 120 | 30 | |
| t6 | 180 | 180 | 180 | 30 | |
| t7 | 30 | 30 | 30 | | |
| t8 | 30 | 30 | 30 | | |
| t9 | 30 | 30 | 30 | | |
| t10 | 60 | 60 | | | |

kcal/mol from the lowest energy conformation) of the two fragments were used to generate initial whole molecule conformations. Each of the combined whole molecule conformations was optimized with respect to the torsion angle degrees of freedom, and all conformations within 5 kcal/mol of the lowest energy conformations of each complete antagonist were selected as active conformation candidates.

BM 13.505 was built and its geometry was optimized by MM2 using the sulfonamide force field parameters reported by Nicholas et al.[26] The ring geometry of compound **5** was optimized by MNDO, and the remainder of the valence geometry was optimized using the MM2 force field. The conformational scan resolution is given in Table 1.

Molecular superpositions were performed using QUANTA. Pairs of molecules and corresponding pharmacophore sites were specified. One antagonist was defined as a reference template which retained a rigid structure. The other "test" molecule was oriented in order to match its pharmacophore to that of the template. The fitting procedure in which the torsion angles of the oriented test molecule are not changed is referred to as rigid body fitting. Changing the torsion angles of the oriented molecule in the molecular superposition is called flexible torsion angle fitting. Both rigid body fitting and flexible torsion angle fitting were carried out in this study.

## RESULT AND DISCUSSION

The conformational energy minima of fragment **a** with fragment **b**, **a** with **c**, and **a** with **d** (see Figure 3) were combined to form trial conformations of the parent molecules SQ 29,548, SQ 28,668, and SQ 27,427 respectively. Energy minimization of each of these trial conformations led to four low-energy conformations for SQ 29,548, eleven low-energy conformations for SQ 28,668, and twelve low-energy conformations for SQ 27,427 using the 5 kcal/mol energy cutoff constraint. The conformational energy and corresponding torsion angles of each of these conformations are listed in Tables 2–4, respectively.

**Table 2.** Energy and Torsion Angle Data of the Low-Energy, Apparent Minima Conformers of SQ 29,548

| conformer | energy (kcal/mol) | $\tau$1 | $\tau$2 | $\tau$3 | $\tau$4 | $\tau$5 | $\tau$6 | $\tau$7 | $\tau$8 | $\tau$9 | $\tau$10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C27 | -63.1 | -22.0 | 144.3 | 62.6 | 30.9 | 58.4 | 136.3 | 64.8 | 126.0 | 174.2 | 43.5 |
| C16 | -59.4 | 82.4 | 132.0 | 65.1 | 14.1 | 38.9 | 37.1 | 74.9 | 123.6 | 180.4 | 44.1 |
| C18 | -58.4 | 97.4 | 156.1 | 62.4 | 61.8 | 119.6 | 151.0 | 74.1 | 129.3 | -176.5 | 45.0 |
| C8 | -57.2 | 75.6 | 153.5 | 68.7 | 54.1 | 146.2 | 126.7 | 73.4 | 135.7 | 173.2 | 142.6 |

**Table 3.** Energies and Torsion Angle Data of the Low-Energy, Apparent Minima Conformers of SQ 28,668

| conformer | energy (kcal/mol) | $\tau$1 | $\tau$2 | $\tau$3 | $\tau$4 | $\tau$5 | $\tau$6 | $\tau$7 | $\tau$8 | $\tau$9 | $\tau$10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C25 | -64.7 | 130.5 | 76.4 | 77.7 | 144.6 | 63.8 | -23.9 | 42.4 | 47.4 | 1.2 | 54.4 |
| C23 | -62.6 | -45.0 | 116.6 | 162.5 | -177.3 | 64.7 | 12.6 | -177.5 | 93.9 | 25.7 | 46.7 |
| C5 | -61.7 | 56.8 | 167.7 | 61.5 | 33.2 | -115.9 | 117.6 | 42.4 | 47.7 | 8.9 | 44.6 |
| C10 | -61.3 | 141.9 | 79.1 | 167.1 | 127.7 | 55.2 | 101.8 | 45.9 | 36.6 | 24.9 | 30.3 |
| C13 | -61.2 | 119.3 | 76.7 | 82.5 | 155.5 | 74.5 | 146.7 | 38.4 | 33.5 | 21.0 | 35.3 |
| C16 | -60.6 | 133.7 | 116.4 | 179.0 | 150.8 | 25.0 | 75.4 | 47.8 | 62.6 | -28.1 | 70.8 |
| C12 | -60.1 | 123.3 | 77.8 | 70.6 | 18.2 | 45.2 | 21.0 | 50.3 | 59.8 | 25.4 | 70.3 |
| C11 | -60.0 | 129.3 | 77.8 | 70.6 | 18.2 | 45.2 | 21.0 | 50.3 | 59.8 | 25.4 | 70.3 |
| C35 | -59.8 | 69.3 | 75.7 | 142.5 | 53.0 | 157.1 | 124.6 | -171.0 | 94.1 | 22.6 | 53.4 |

**Table 4.** Energies and Torsion Angle Data of the Low-Energy, Apparent Minima Conformers of SQ 27,427

| conformer | energy (kcal/mol) | $\tau$1 | $\tau$2 | $\tau$3 | $\tau$4 | $\tau$5 | $\tau$6 | $\tau$7 | $\tau$8 | $\tau$9 |
|---|---|---|---|---|---|---|---|---|---|---|
| C28 | -57.2 | 129.5 | 81.2 | 71.4 | 16.6 | 46.9 | 29.0 | 47.6 | 27.3 | 49.7 |
| C8 | -55.7 | -172.2 | 135.7 | 73.1 | 16.1 | 48.7 | 24.6 | 27.9 | -173.0 | 51.5 |
| C2 | -55.4 | 116.2 | 76.1 | 66.4 | 23.3 | 39.0 | 31.9 | 53.6 | 66.4 | -46.9 |
| C4 | -55.4 | -54.6 | 155.8 | -179.8 | 59.9 | 163.9 | 103.6 | -177.0 | 79.1 | 53.7 |
| C20 | -54.9 | 147.4 | 100.7 | 129.9 | 92.0 | 79.0 | 131.2 | 38.9 | 18.4 | 54.0 |
| C9 | -54.5 | 105.5 | 65.9 | 55.8 | 34.7 | 58.2 | 106.3 | 53.1 | -77.8 | 175.6 |
| C10 | -54.0 | 131.2 | 75.7 | 77.5 | 70.8 | 78.2 | 83.4 | 48.3 | 37.2 | 49.7 |
| C27 | -54.0 | 74.4 | 153.8 | 58.1 | 35.0 | 31.2 | 48.2 | 53.1 | 72.0 | -48.4 |
| C17 | -53.6 | 34.3 | 57.3 | 60.4 | 33.6 | 31.4 | 48.2 | 170.4 | 34.8 | 44.6 |
| C30 | -53.5 | 126.1 | 62.8 | 95.7 | 156.0 | 98.9 | 106.6 | 34.4 | 75.2 | 177.1 |
| C24 | -52.4 | -178.8 | 150.3 | 57.7 | 35.9 | 162.1 | 104.5 | 50.5 | 81.6 | 176.5 |
| C3 | -52.4 | 49.6 | 76.8 | 65.6 | 24.8 | 39.9 | 33.5 | 40.7 | 32.1 | 47.6 |

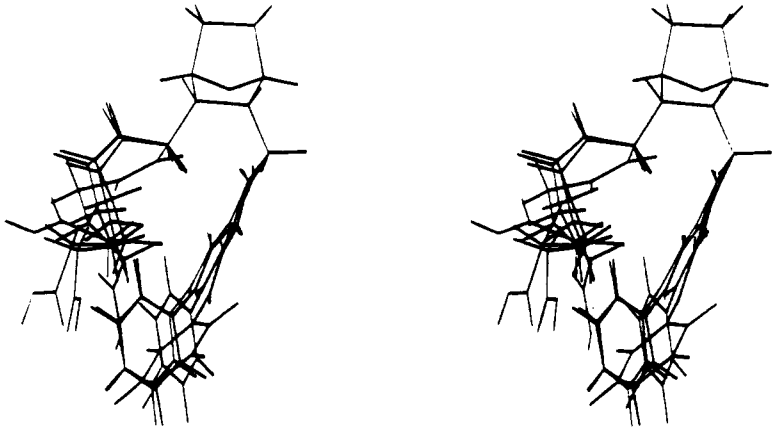

**Figure 4.** Low-energy, apparent conformer minima of SQ 29,548 (within the 5 kcal/mol energy cutoff).

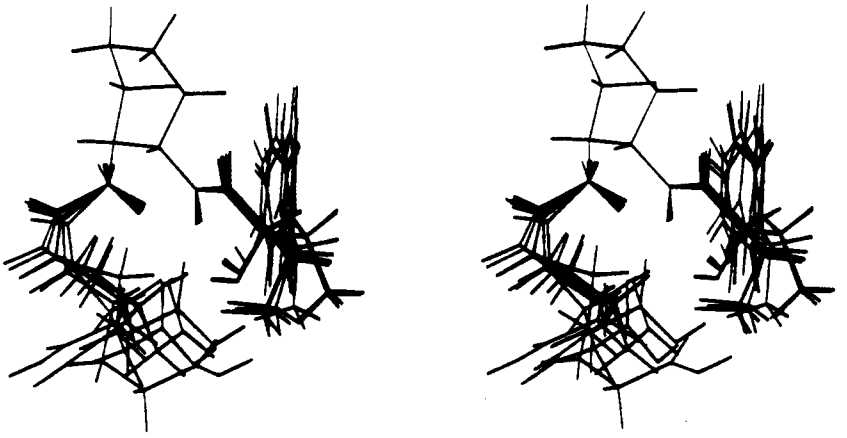

**Figure 5.** Low-energy, apparent conformer minima of SQ 28,668 (within the 5 kcal/mol energy cutoff).

The simultaneous superposition of all four conformations of SQ 29,548, using the head ring as the alignment criterium, is shown in Figure 4. These four conformations can be classified into two groups according to their pharmacophore site similarity match. Group 1 contains conformations C27, C18, and C8, and group 2 contains conformation C16 using the conformational state numbering scheme of Table 2. The same analysis was done for SQ 28,668, and the eleven conformations were classified into the four groups reported in Table 3. Unfortunately, it is not obvious how to classify the low-energy conformations of SQ 27,427 into specific groups according to pharmacophore site similarity matching.

The search for conformations which all of these antagonists can adopt, for a common pharmacophore site matching under the low-energy constraint, was initiated with rigid fits of all low-energy conformations of SQ 28,668 and SQ 27,427 to conformations C27 and C16 of SQ 29,548. SQ 28,668 and SQ 27,427 each have three conformations which fit conformation C27 of SQ 29,548. These are conformations C25, C10, and C13 of SQ 28,668 (see Table 3) and conformations C28, C2, and C9 of SQ 27,427 (see Table 4). Conformations C27 of SQ 29,548, C25 of SQ 28,668, and C28 of SQ 27,427 were selected as candidates for the active conformation of each antagonist since these are the lowest energy conformational states of each antagonist which also satisfy the common pharmacophore fit.

The distances between the three recognition sites for each of the three antagonists are listed in Tables 5–7. The corresponding distances between the postulated pharmacophore sites for each low-energy conformation are very nearly identical to one another. For each candidate active conformation, the distance between the oxygen in the carboxylic acid (S1) and the oxygen in the 7-oxabicyclo[2.2.1] ring (S3) is 7.4–8.4 Å. The second binding site (S2) for the compound SQ 29,548 was initially found to be either the 14 position nitrogen, or the oxygen in the ω-chain, since the distances from either of these atoms to the oxygen in the ring (S3) are very close to one another (4.2–5.2 Å). However, the oxygen in the ω-chain fits the corresponding pharmacophore site in SQ 28,668, SQ 27,427, BM 13.505, and compound **5** better than the nitrogen in the SQ 29,548 ω-chain on the basis of the molecular superpositions. Therefore, the hydrogen binding

**Table 5.** Distances between Pharmacophore Sites for Active Conformation Candidates of SQ 29,548

| conformer | energy (kcal/mol) | distance (Å) | | |
|---|---|---|---|---|
| | | S1-S3 | S2-S3 | S1-S2 |
| C27 | −63.1 | 7.9 | 4.9 | 4.3 |
| C16 | −59.4 | 7.6 | 6.2 | 4.4 |
| C18 | −58.4 | 8.3 | 5.5 | 4.3 |
| C8 | −57.2 | 7.6 | 4.6 | 4.4 |

**Table 6.** Distances between Pharmacophore Sites for Active Conformation Candidates of SQ 28,668

| conformer | energy (kcal/mol) | distance (Å) | | |
|---|---|---|---|---|
| | | S1-S3 | S2-S3 | S1-S2 |
| C25 | −62.7 | 6.2 | 5.2 | 4.6 |
| C23 | −62.6 | 7.5 | 5.4 | 8.9 |
| C5 | −61.7 | 8.4 | 5.1 | 6.5 |
| C10 | −61.3 | 8.0 | 5.2 | 5.5 |
| C13 | −61.1 | 8.1 | 5.1 | 5.5 |
| C16 | −60.6 | 7.5 | 5.3 | 2.4 |
| C12 | −60.1 | 8.0 | 5.3 | 5.6 |
| C11 | −60.0 | 8.1 | 5.2 | 5.3 |
| C35 | −59.8 | 10.4 | 5.4 | 11.7 |

**Figure 6.** Pharmacophore site similarity matching for SQ 29,548, SQ 28,668, and SQ 27,427.

**Table 7.** Distances between Pharmacophore Sites for Active Conformation Candidates of SQ 27,427

| conformer | energy (kcal/mol) | distance (Å) | | |
|---|---|---|---|---|
| | | S1-S3 | S2-S3 | S1-S2 |
| C28 | −57.2 | 8.1 | 5.3 | 5.5 |
| C8 | −55.7 | 7.9 | 5.4 | 6.8 |
| C2 | −55.4 | 8.1 | 5.5 | 5.7 |
| C4 | −55.4 | 8.5 | 5.7 | 9.7 |
| C20 | −54.9 | 9.7 | 5.1 | 6.0 |
| C9 | −54.5 | 8.7 | 4.9 | 7.8 |
| C10 | −54.0 | 8.9 | 5.3 | 5.0 |
| C27 | −54.0 | 7.7 | 5.5 | 7.1 |
| C17 | −53.6 | 7.5 | 6.3 | 9.8 |
| C30 | −53.5 | 7.9 | 5.4 | 4.2 |
| C24 | −52.4 | 7.1 | 5.5 | 7.8 |
| C3 | −52.4 | 7.9 | 5.2 | 8.1 |

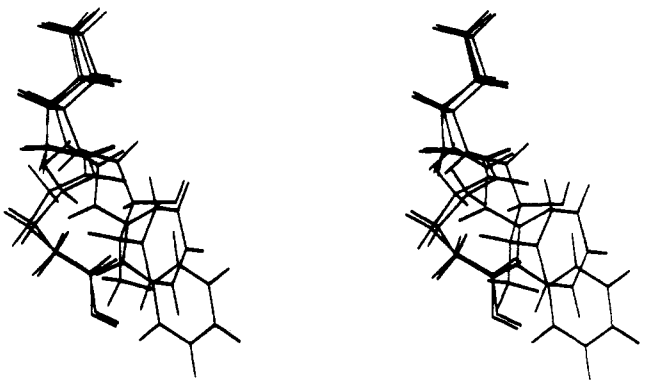

**Figure 7.** C25 adjusted fit conformation of SQ 28,668 superimposed on the C27 conformation of SQ 29,548.

acceptor atom is assumed to be the oxygen in the ω-chain of SQ 29,548. The distance between recognition site 1 (S1) and recognition site 2 (S2) is 4.6–5.6 Å.

The pharmacophores of SQ 29,548, SQ 28,668, and SQ 27,427 are nearly identical as can be seen in Figure 6. The molecular shapes of these three antagonist α-chains are somewhat different even though their chemical structures are identical. To test if it is possible for all three compounds to adopt a common conformation (shape), it is necessary to determine how much energy is required for SQ 28,668 and for SQ 27,427 to adopt a common conformation of the α-chain, and for the oxygens in the ω-chains to match the corresponding oxygen in the ω-chain of SQ 29,548. Conformation C25 of SQ 28,668 and conformation C28 of SQ 27,427 were used to perform flexible torsion angle fittings. The torsion angles of the α-chains of conformation C25 and conformation C28 were forced to rotate in order to adopt the same shape as conformation C27 of the SQ 29,548 α-chain with the constraint of also matching the pharmacophore sites. These superimposed structures are shown in Figure 7 and Figure 8, respectively. The energy of the new conformation of SQ 28,668 is 0.2 kcal/mol higher than C25 of SQ 28,668. There is almost no energy difference between the new conformation of SQ 27,427 and C28. Therefore, the C27 conformation of SQ 29,548 is selected as the active conformation of this series of antagonists.

Since the entire torsion angle space of each of these flexible molecules is too large to systematically scan, the corresponding global minimum energy conformation is unknown. However, it is important to know the global minimum energy conformation because the energy difference between the global minimum, and a proposed active conformation is a critical criterion to test whether or not a proposed active conformation is a reasonable choice. A strategy was developed to find the global minimum energy conformation of SQ 29,548. The regions of torsion angle space having low-energy conformations are not randomly distributed. Plots of conformational energy versus torsion angles show that low-energy conformations are located in limited torsion angle spaces. The global minimum energy conformation is assumed to be in one of the low-energy regions. Consequently, only limited torsion angle subspaces were investigated instead of total torsion angle space. The torsion angle space scanning resolution was 30°. The energy of the global minimum energy conformation is 3.1 kcal/mol lower than the proposed active conformation.

The global minimum energy conformation is not considered to be a candidate for the active conformation for two reasons. First, there is a partial intramolecular hydrogen bond in the global minimum energy conformation. This hydrogen bond is formed by the hydrogen in the carboxylic acid and the oxygen in the 7-oxabicyclo[2.2.1] ring. The O···HO hydrogen bond distance is 2.3 Å and the angle is 102.6°. Available structure–activity data[18] indicate that the carboxylic group interacts with the $TxA_2$ receptor rather than forming an intramolecular hydrogen bond. Second, some reasonably active antagonists do not have the 7-oxabicyclo[2.2.1]heptane ring or an equivalent atom which can serve as a hydrogen bond acceptor. Therefore, it is difficult to form an intramolecular hydrogen bond.

Figures 6 and 7 illustrate the shapes of the proposed active conformations of SQ 29,548, SQ 28,668, and SQ 27,427, which are almost identical except for the space occupied by the benzene ring at the end of the SQ 29,548 ω-chain. This space cannot be occupied by the benzene ring in SQ 28,668 or by the cyclohexane ring in SQ 27,427. Since SQ 29,548 is more potent than SQ 28,668 and SQ 27,427, the space occpied by

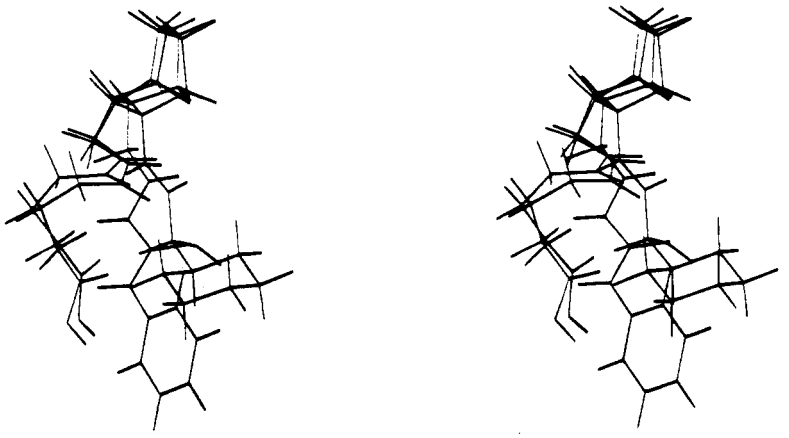

**Figure 8.** C28 adjusted fit conformation of SQ 27,427 superimposed on the C27 conformation of SQ 29,548.

the benzene ring may permit intermolecular interactions which are responsible for the potency differences among these three antagonists. It is reasonable to hypothesize that the hydrophobic benzene ring of SQ 29,548 may interact with a hydrophobic pocket in the TxA$_2$ receptor to enhance potency. This hypothesis is consistent with the structure–activity study which shows that a hydrophobic end for an $\omega$-chain, especially the benzene or the *p*-chlorobenzene ring, is important to retain potency.[27] S4 defines this pharmacophore site. Still, SQ 28,-668 and SQ 27,427 occupy nearly all of the sites which are postulated to be important for bioactivity. Hence, their observed similar potencies are also consistent with our model.

Some antagonists have a bicyclo[2.2.1]heptane ring as a head instead of a 7-oxabicyclo[2.2.1]heptane ring. Other antagonists do not possess a ring as a head but are still potent antagonists. This might be explained by the specific nature of the hydrophobic head group interaction with a hydrophobic pocket in the TxA$_2$ receptor. The head, 7-oxabicyclo[2.2.1]-heptane ring may fit the hydrophobic pocket in the TxA$_2$ receptor much better than any other head. The oxygen may provide the optimum steric bulk to the head so that there is almost no gap between the antagonist and the receptor wall. It is believed that this interaction is not dominant in the process of an antagonist binding to the TxA$_2$ receptor. Therefore, if a compound can interact strongly with other sites, but not realize this hydrophobic steric-matching interaction, the compound still might be a potent antagonist. BM 13.505 is an example of such a compound and is discussed below.

BM 13.505 and compound **5** were studied to test whether the proposed active conformation for the SQ series is a common active conformation for other classes of TxA$_2$ receptor antagonists. The torsion angle space of BM 13.505 was systematically scanned at the resolution listed in Table 1. The low-energy conformations (with an energy cutoff of 10 kcal/mol) of BM 13.505 were jointly superimposed, and it was found that these low-energy conformations (the energy within 5 kcal/mol of the global minimum energy conformation) were almost identical in their shapes except for the space occupied by the benzene ring. Consequently, the lowest energy conformation was selected as the representative for the active conformation of this compound. This lowest energy conformation was superimposed on conformation C27 of SQ 29,548 using the rigid body fitting method to match their pharmacophores. The carboxylic group in BM 13.505 fits its counterpart, the carboxylic group (S1), in SQ 29,548. One of the oxygens of the sulfonate group fits the oxygen (S2) in the SQ 29,548 $\omega$-chain. The *p*-chlorobenzene fits the benzene ring in SQ 29,548. The superimposed structures of these two antagonists are shown in Figure 9. Although there is no

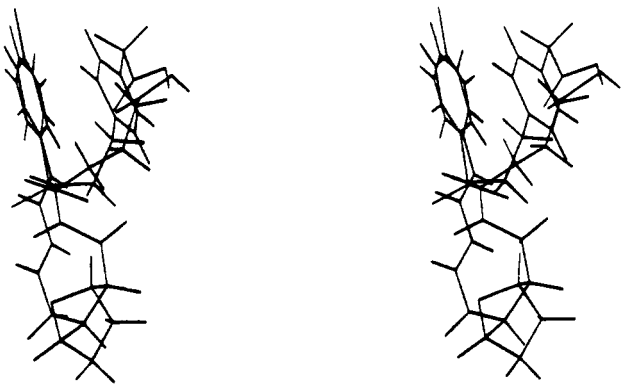

**Figure 9.** Conformation of BM 13.505 superimposed on the C27 conformation of SQ 29,548.

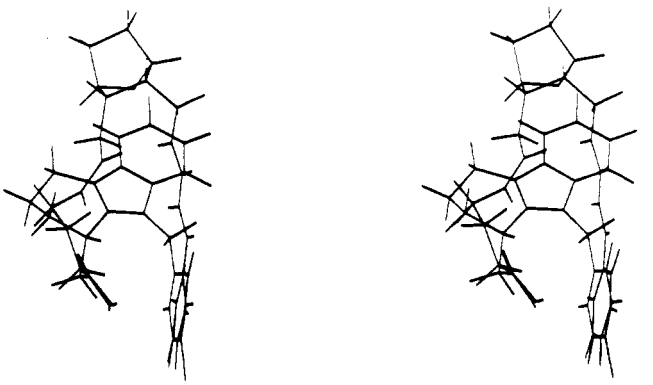

**Figure 10.** C27 conformation of SQ 29,548 superimposed on compound **5**.

counterpart head group in BM 13.505, a good fitting of the pharmacophore sites S1, S2, and S4 may demonstrate why this compound is a potent antagonist. The absence of a head group in BM 13.505 may also explain why it is less potent than SQ 29,548.

Compound **5** is a potent TxA$_2$ receptor antagonist. It has a rigid structure, but also exhibits a high binding affinity ($k_i$ = 15.3 nm). Its minimum energy conformation is a good template choice to test the proposed active conformation. All of the torsion angles of this compound were systematically scanned at high resolution (10-deg increments). The lowest energy conformation of this compound was superimposed on C27 of SQ 29,548 using rigid body fitting to match pharmacophores. The F in the fluorobenzene was positioned to fit the oxygen in the 7-oxabicyclo[2.2.1] ring on the basis of the assumption that the fluorobenzene ring interacts with the hydrophobic pocket in the TxA$_2$ receptor. Reported structure–

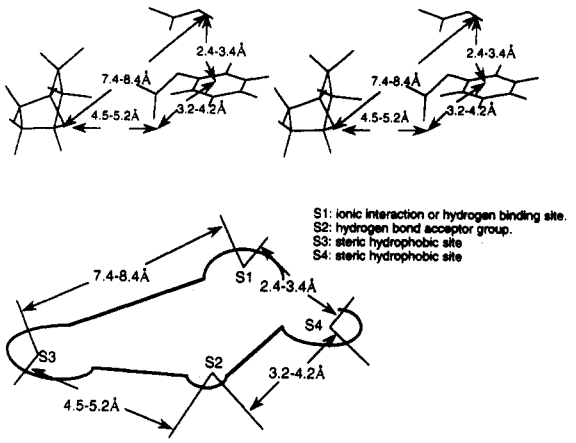


**Figure 11.** $TxA_2$ receptor antagonist spatial pharmacophore.

**Table 8.** Distances between Pharmacophore Sites of Active Conformation Candidates for Compound **6**

| conformer | energy (kcal/mol) | distance (Å) | | |
|---|---|---|---|---|
| | | S1-S3 | S2-S3 | S1-S2 |
| C18 | -64.0 | 8.6 | 7.0 | 4.7 |
| C19 | -64.1 | 8.6 | 7.0 | 4.7 |
| C9 | -61.9 | 6.0 | 7.2 | 4.1 |
| C1 | -61.5 | 8.5 | 7.5 | 2.7 |
| C17 | -60.9 | 7.3 | 6.8 | 6.0 |
| C11 | -60.8 | 7.3 | 6.8 | 2.9 |
| C6 | -60.7 | 7.3 | 6.8 | 6.1 |
| C7 | -60.7 | 7.3 | 6.8 | 6.1 |
| C8 | -60.7 | 7.3 | 6.8 | 6.0 |
| C15 | -60.5 | 4.9 | 6.7 | 6.1 |
| C5 | -60.4 | 7.7 | 6.8 | 5.0 |
| C10 | -60.3 | 5.9 | 6.7 | 4.1 |
| C21 | -60.2 | 8.0 | 7.2 | 5.1 |
| C2 | -60.0 | 7.3 | 6.8 | 5.2 |
| C14 | -60.0 | 9.5 | 7.4 | 5.7 |
| C12 | -59.7 | 7.6 | 6.8 | 4.9 |
| C26 | -59.7 | 5.3 | 3.2 | 6.2 |
| C13 | -59.7 | 7.6 | 6.8 | 4.9 |
| C20 | -59.6 | 6.8 | 7.0 | 3.4 |
| C3 | -59.5 | 9.2 | 7.5 | 4.4 |
| C4 | -59.5 | 9.2 | 7.5 | 4.4 |
| C23 | -59.2 | 4.8 | 6.9 | 5.3 |
| C27 | -59.1 | 5.0 | 7.2 | 4.1 |

activity data indicate that potency decreases if this F is removed.[17] The nitrogen in compound **5** may function as a hydrogen bond acceptor to fit its counterpart which is the oxygen in the SQ 29,548 $\omega$-chain. The *p*-chlorobenzene, as mentioned before, may interact with the hydrophobic pocket in the $TxA_2$ receptor. These two molecules align well, as is shown in Figure 9. Compound **5** is rigid but still shares the same conformation and shape and matches all of the pharmacophore sites of SQ 29,548. Hence, the observed high affinity is expected from our model.

It has often been pointed out that the distance from the carboxylic group of the $\alpha$-chain to the head of SQ series compounds is important to realize maximum activity.[5,17,18] For example, there is a 7-fold decrease in potency if there is one less carbon in the SQ 29,548 $\alpha$-chain. Since all members of this class of antagonists have the same $\omega$-chain and the same pharmacophore sites, the potency differences may be due to distances between pharmacophore sites which do not match those of the proposed active conformation. This hypothesis seems to be true for compound **6**. In the conformational analysis of compound **6** it was found that the distances (shown in Table 8) S1 to S3 and S1 to S2 are longer than the distances in the proposed active conformation. These distance differences are consequently assumed to be responsible for compound **6** being 7-fold less potent than SQ 29,548.

Overall, the proposed $TxA_2$ spatial pharmacophore, common to five antagonists studied, is schematically illustrated in Figure 11.

## CONCLUSION

Many $TxA_2$ antagonists possess a common spatial pharmacophore embedded in an active conformation which is exemplified by conformation C27 of SQ 29,548. There are four recognition sites in the spatial pharmacophore. It is believed that the carboxylic acid group may interact with the $TxA_2$ receptor by an ionic interaction or forming a hydrogen bond. This interaction is the key factor for high potency. Without this carboxylic group, a compound is almost inactive. A hydrogen bond acceptor group in the $\omega$-chain of the SQ series of compounds is also important for activity. There are two hydrophobic pharmacophore sites and both are required for high potency. The distances between these pharmacophore sites have been postulated from conformational analysis and molecular superposition. The spatial pharmacophore may be helpful in designing more specific and potent $TxA_2$ receptor antagonists.

## ACKNOWLEDGMENT

Resources of the Laboratory of Molecular Modeling and Design were used over the course of this study.

## APPENDIX

The pharmacophore coordinates of each of the compounds studied are available upon request from the authors.

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