Thromboxane Receptor Antagonism Combined with Thromboxane Synthase Inhibition. 1. (±)-(3-Pyridinylbicycloheptyl)alkanoic Acids

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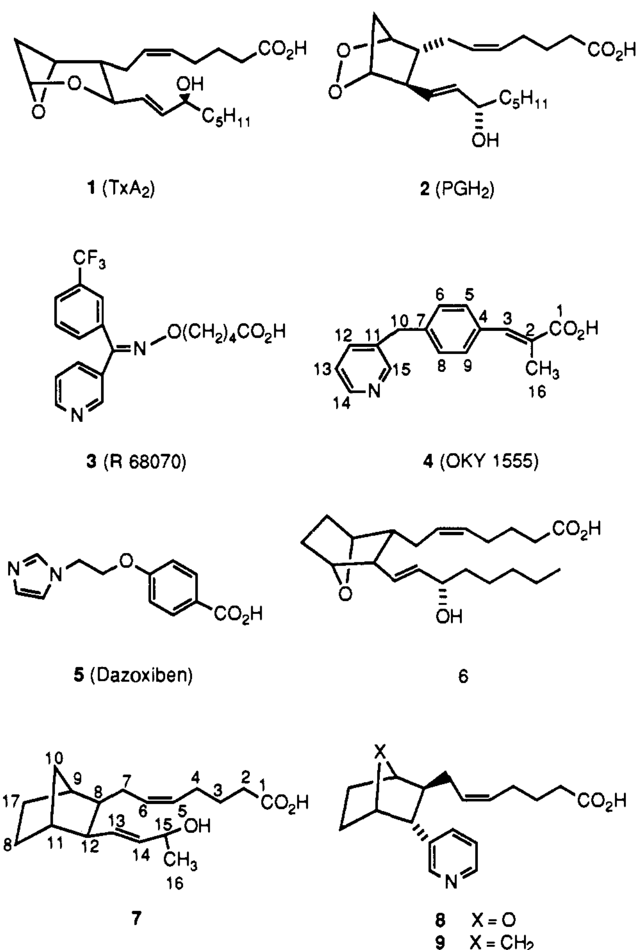
The design, synthesis, and in vitro pharmacology of a new class of compounds exerting both thromboxane receptor antagonist and thromboxane synthase inhibitory activities is described. [(3-Pyridinyl)bicycloheptyl]alkanoic acid 9 and its analogues, designed with the help of molecular modeling, were synthesized and found to be inhibitors of thromboxane A_2 (TxA_2) biosynthesis in a human platelet microsomal preparation. The compounds were also found to antagonize both platelet and vascular TxA_2 receptors. The compounds inhibited the U 46619 induced aggregation of human washed platelets and platelet-rich plasma and the U 46619 induced contraction of the dog saphenous vein.

Thromboxane A_2 (TXA_2, 1), an unstable metabolite of arachidonic acid, is an extremely potent vasoconstricting and platelet-aggregating agent.[1,2] The potent biological activity of TxA_2 may make an important contribution to the pathogenesis of various circulatory and certain renal disorders.[3,4] Thromboxane synthase inhibitors (TxSIs) and Thromboxane receptor antagonists (TxRAs) have been developed to treat these disorders.[5,6] A TxSI by itself has not shown efficacy in the treatment of various forms of angina and peripheral vascular disease.[5] One of the reasons cited[7] for this lack of efficacy is that the endoperoxide PGH_2 (2), which accumulates due to the inhibition of biosynthesis of TxA_2, itself is a potent platelet-aggregating and vasoconstricting agent[8,9] and this accumulation of PGH_2 may negate the beneficial effects of TxSI.

It has been proposed that use of a combination of TxSI and TxRA for the treatment of the clinical conditions cited above would be more beneficial than the use of either agent alone.[10,11,7] Use of a TxSI would prevent the biosynthesis of TxA_2 and lead to redirection of at least part of the accumulated PGH_2 to beneficial prostaglandins like PGI_2, PGD_2, and PGE_2, which would not be possible by the use of a TxRA. The TxRA, on the other hand, would antagonize the actions of TxA_2 and PGH_2. The studies on combination therapy in animals[12,13] and normal human volunteers[14] demonstrate that the two agents have greater therapeutic benefit in combination than when given individually.



Recently several compounds have been reported which possess both TxRA and TxSI properties in a single chemical entity.[15-19] One of these, R 68070 (3) is currently under clinical investigation.[20] In this paper we describe

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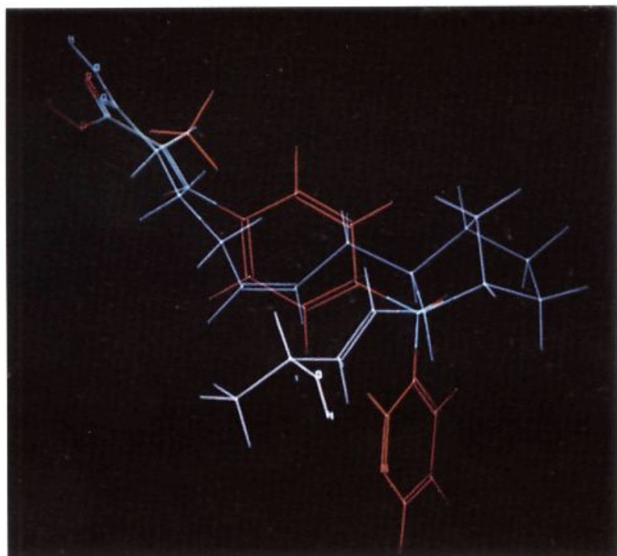

Figure 1. A plot of an overlap of conformation 63 (E_{63} = 53.35 kJ/mol) of **4** with conformation 10 (E_{10} = 126.99 kJ/mol) of **7** (overlap rms = 0.232), suggesting an endo orientation for the pyridine ring on C_{12} of **7**.

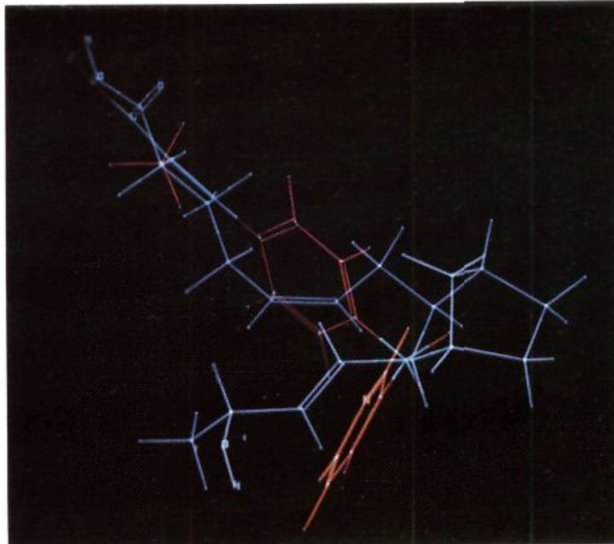

Figure 2. A plot of an overlap of conformation 29 (E_{29} = 49.88 kJ/mol) of **4** with conformation 10 of **7** (overlap rms = 0.237), suggesting an exo orientation for the pyridine ring on C_{12} of **7**.

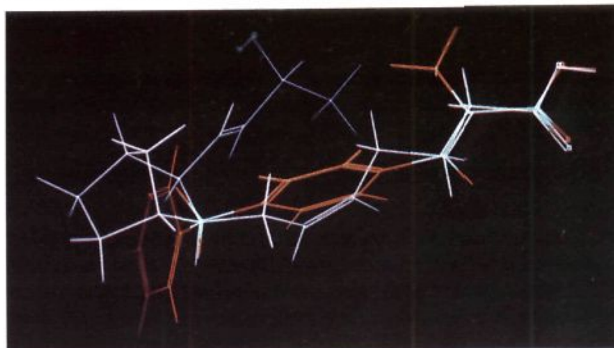

Figure 3. A plot of an overlap of conformation 61 (E_{61} = 52.86 kJ/mol) of **4** with conformation 32 (E_{32} = 128.77 kJ/mol) of **7** (overlap rms = 0.148), suggesting placement of the pyridine ring at C_8 of **7**.

the design, synthesis, and in vitro pharmacology of a series of compounds which possess both TxRA and TxSI properties (TxRA/TxSI).

Compound Design

The necessary structural feature of a TxSI like OKY-1555 (**4**)[21] or dazoxiben (**5**)[22] is the basic nitrogen atom of a 3-substituted pyridine or a 1-substituted imidazole ring and a carboxylic acid group separated by a distance of 9–10 Å.[23] We decided to incorporate these features into the bicyclo[2.2.1]heptane ring skeleton of several potent TxRA, one of which is **6**.[24] In this approach the carboxylic acid chain of **6** would also serve as the one in **4** or **5**.

In order to find an appropriate position to place the pyridine or imidazole ring a molecular modeling study was undertaken. For the purpose of energy minimization, compounds **4** and **7** were chosen to represent a TxSI and TxRA because they have fewer degrees of rotational freedom than **5** and **6**, respectively. A local minimum energy conformation of **4** was subjected to systematic conformational searching using the MULTIC[25] submode of the MacroModel/Batchmin program[26] to provide 81 low-energy conformations (see the Experimental Section for details). A similar procedure for **7** gave 941 conformations.

In the case of **4**, the desired distance of 9–10 Å between the pyridine nitrogen atom and C_1 is attained by attaching the pyridine ring to C_{10}. The distance between C_1 and C_{10} in the 81 low-energy conformations of **4** was between 8.13 and 8.17 Å. This distance was used as an important criterion for placement of the pyridine or imidazole ring on the TxRA. Consequently, all the atoms which were in the range of 7.65–8.65 Å from C_1 in the low-energy conformations of **7** were located. It was found that the C_1–C_{12} distance was in this range in about 50% of the conformations and C_1–C_9, C_1–C_{10}, C_1–C_{11}, and C_1–C_{13} in about 30%. Surprisingly, only 54 conformations were found to have the C_1–C_8 distance in the above range. Figures 1 and 2 depict the overlaps suggesting the placement of the pyridine ring at C_{12} with endo and exo stereochemistry, respectively. The overlap in Figure 3 suggests attachment of the pyridine ring at C_8. Placement of the pyridine ring at C_9, C_{10}, and C_{11} was not considered because of potential synthetic difficulties. The C_1–pyridine nitrogen distance was found to be in the desired 9–10 Å range in the low-energy conformations of **9**. Therefore, we decided to initiate our program by synthesizing compounds like **8**, **9**, and their stereoisomers and testing them for the dual (TxRA and TxSI) activities.

Chemistry

Synthesis of **9** began with the Diels–Alder reaction of methyl 3-(3-pyridinyl)propiolate (**10**)[27] with cyclopentadiene (Scheme I). Hydrogenation of adduct **11** at 1 atm reduced only one double bond to give **12** and hy-

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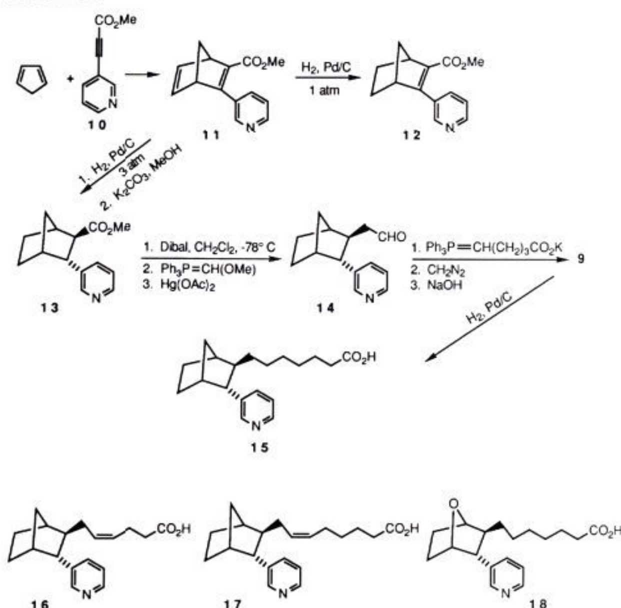
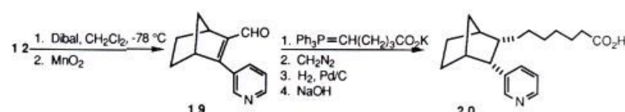
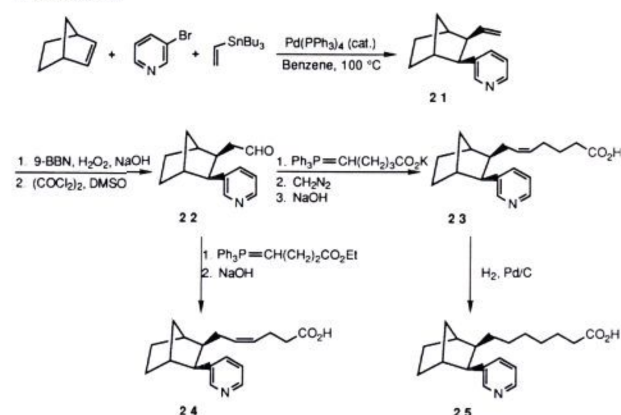
Scheme I





Scheme II

Scheme III



drogenation at 45 psi reduced both double bonds. The cis–endo product of complete hydrogenation was epimerized with anhydrous K_2CO_3 in dry MeOH to yield *trans*-ester **13**. The aldehyde obtained by the reduction of **13** with Dibal at −78 °C was subjected to Wittig reaction with methoxymethyltriphenylphosphorane and the resulting enol ether was hydrolyzed with $Hg(OAc)_2$ to give homologated aldehyde **14**. Chain extension using carboxybutyltriphenylphosphorane followed by esterification using diazomethane and hydrolysis gave **9**. Hydrogenation of **9** gave **15**, which was isolated as a crystalline solid. Compounds **16** and **17** were prepared analogously without the final hydrogenation. Compounds **8** and **18** were prepared as shown in Scheme I by starting with furan in place of cyclopentadiene. Compound **20** was prepared with a related strategy as shown in Scheme II.

The approach shown in Scheme I could not be used to prepare bicyclic compounds with cis–exo stereochemistry. These compounds were synthesized as shown in Scheme III. The palladium(0)-catalyzed coupling[28] of 3-bromopyridine and vinyltributyltin with norbornylene gave **21** in 68.5% yield. Hydroboration and oxidation of the borane gave the alcohol which upon Swern oxidation gave aldehyde **22** in 40% overall yield. Aldehyde **22** was then ela-

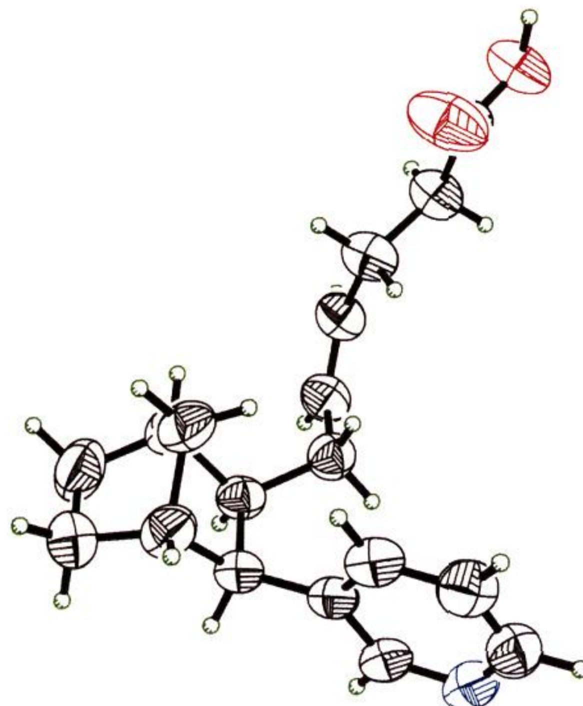


Figure 4. X-ray crystal structure (with thermal ellipsoids) showing the cis–exo stereochemistry of compound **24**.

Scheme IV

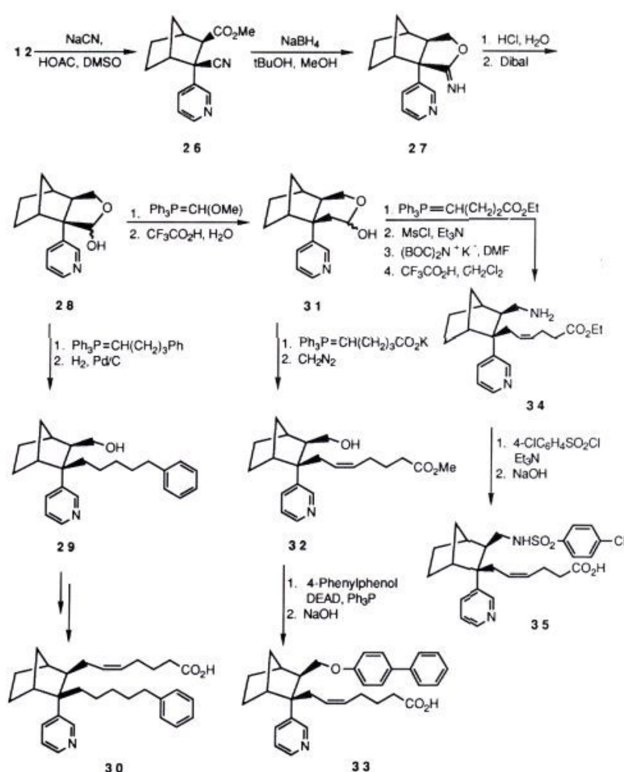


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Table I. In Vitro Activity of TxRA/TxSI

compound	formula[a]	mp, °C	IC_{50}, μM: thromboxane synthase inhibition[b]	IC_{50}, μM: inhibition of U 46619 induced aggregation of washed human platelets[c]
8	$C_{18}H_{23}NO_3$[d]	oil	>0.5	>10
9	$C_{19}H_{25}NO_2 \cdot 0.5H_2O$	oil	0.054	5.3
15	$C_{19}H_{27}NO_2$	91–92	0.125	9.77
16	$C_{18}H_{23}NO_2$	oil	0.078	3.85
17	$C_{20}H_{27}NO_2 \cdot 0.1H_2O$	oil	0.165	10.87
18	$C_{18}H_{25}NO_3$	88–90	>2.0	>10
20	$C_{19}H_{27}NO_2$	91–92	0.007	12.98
23	$C_{19}H_{25}NO_2$	109–117	0.050	4.29
24	$C_{18}H_{23}NO_2$	70–75	0.029	>10
25	$C_{19}H_{27}NO_2 \cdot 0.2H_2O$	88–90	0.054	7.25
30	$C_{30}H_{35}NO_3$	glass	0.79	1.5
33	$C_{32}H_{35}NO_2 \cdot 0.5H_2O$	foam	0.12	1.58
35	$C_{25}H_{29}ClN_2O_4S \cdot 0.5H_2O$	81–114	0.34	0.03
dazoxiben (**5**)			0.028	–
R 68070 (**3**)			0.003	1.28

[a] C, H, and N analyses were within ±0.4% of calculated values unless otherwise indicated. [b] Values represent average of two determinations. [c] Values represent single determinations. [d] Calcd: C, 71.72; H, 7.69; N, 4.65. Found: C, 70.23; H, 7.16; N, 4.62.

borated as before to prepare **23**. The cis–exo stereochemistry was confirmed by an X-ray analysis of **24**, which crystallized from methanol (see Figure 4).

The compounds described above do not have the second lipophilic side chain usually seen in thromboxane receptor antagonists with bicyclic structure such as **4**. Such compounds could be prepared as shown in Scheme IV. Addition of cyanide ion to the activated double bond of **12** gave **26**. The proton next to the carboxylic ester appears as a singlet at δ 2.87, indicative of endo stereochemistry. Furthermore, attempted reduction of the ester group using sodium borohydride in refluxing *tert*-butyl alcohol gave the cyclic imino ether **27**, substantiating the syn relationship of the nitrile and the ester group. Lactol **28**, obtained by the acid hydrolysis of **27** followed by Dibal reduction, served as a key intermediate for this series of compounds. Lactol **28** failed to undergo the Horner–Emmons reaction with the anion from dimethyl (2-oxoheptyl)phosphonate. However, Wittig reaction of **28** followed by hydrogenation gave **29**, which was further elaborated by the chemistry described in Scheme I to give **30**. Lactol **28** was homologated to **31** and then subjected to Wittig reaction conditions followed by esterification to give **32**. Ether formation under Mitsunobu conditions using 4-phenylphenol followed by hydrolysis gave **33**. Lactol **31** was converted to amino ester **34** as shown in the scheme. Formation of sulfonamide from **34** followed by hydrolysis gave **35**.

In Vitro Pharmacology and Discussion

The compounds described herein were initially tested for their thromboxane synthase inhibitory activity. Inhibition of TxB_2 formation from human microsomal platelet preparations, incubated with [^{14}C]arachidonic acid, was measured. The compounds were then tested for inhibition of aggregation of aspirinated, washed human platelets (WP) challenged with U 46619, a stable PGH_2/TxA_2 mimic. The platelet aggregation was measured on a Payton dual-channel aggregometer. The IC_{50} values for thromboxane synthase inhibition and thromboxane receptor antagonism are shown in Table I. Dazoxiben (**5**), a TxSI, and R 68070 (**3**), a TxRA/TxSI, were tested as reference compounds.

As evident from Table I, the TxSI activity of **9** and some of its analogues is of the same order of magnitude as that of **5** (IC_{50} = 7–60 nM). The TxRA activity is of the same order of magnitude as that of **3** (IC_{50} = 1.5–6 μM). All of the compounds in Table I with an all carbon bicycloheptane ring were found to have both TxSI and TxRA activities. Compounds **8** and **18** with a 7-oxabicycloheptane ring were surprisingly inactive both as a TxSI and TxRA.

Table II. Thromboxane Antagonist Activity on the Platelet and Vascular Receptors

compd	inhibition of U 46619 induced: aggregation of human PRP: IC_{50},[a] μM	inhibition of U 46619 induced: contraction of dog saphenous vein: pA_2[c]	
9	7.15	5.85[b]	(8)
16	12.1	6.48	(7)
23	2.72	6.29	(8)
33	>30	inactive	(8)
35	10.85	8.49	(12)
3	2.83	6.35	(11)

[a] Values represent single determinations. [b] Measured as the corresponding sodium salt. [c] Number of experiments is in parentheses.

Compounds **9** and **23**, which differ only in the stereochemistry at the 3-position of the bicycloheptane ring were found to be nearly equipotent as TxSI and TxRA. A similar change in stereochemistry between **16** and **24**, however, seems to affect the TxRA activity quite significantly. Compound **24** is much less active (IC_{50} > 10 μM) than **16** (IC_{50} = 3.85 μM) as a TxRA. The structurally similar analogues **15**, **20**, and **25** differ only in the stereochemistry at the 2- and 3-positions of the bicycloheptane ring. The change in stereochemistry of these compounds seems to affect the TxSI activity more significantly than the TxRA activity. Compound **20** with cis–endo stereochemistry is the most potent TxSI (IC_{50} = 0.007 μM) in this series of compounds.

Removal of the double bond leads to 2-fold loss in activities (compare **9**, **15**, **23**, and **25**). Shortening the carboxylic acid chain of **9** by one carbon atom (compound **16**) is better tolerated for both TxSI and TxRA activities than lengthening it by one (compound **17**). Introduction of a second chain (compounds **30**, **33**, and **35**) partially improves the TxRA activity; however, it reduced the TxSI activity significantly. The Sulfonamide **35** is an excellent TxRA (IC_{50} = 0.03 μM) but inactive as a TxSI. Therefore, it appears that increase in the bulk of these bicyclic structures is detrimental to the TxSI activity.

The best dual-acting compounds, **9**, **16**, **23**, **33**, and **35**, were tested further for their receptor antagonist properties in human platelet-rich plasma (PRP) and the dog saphenous vein (Table II). The activity in PRP represents

the functional antagonist property on the platelet receptor for TxA_2. The activity in the dog saphenous vein, on the other hand, represents antagonism on the vascular receptor for TxA_2. Testing in these two systems is important because it has been suggested that the platelet and vascular receptor for TxA_2 may be different in many species.[29]

The ability of **9** and **23** to inhibit U 46619 induced aggregation of WP (protein free) and PRP (protein rich) systems is similar, indicating that the compounds probably have little protein binding (see Tables I and II). In contrast, compound **35** with a sulfonamide group and **33** with a highly lipophilic side chain show >30-fold decrease in activity in PRP due to protein binding. The TxRA activity on the vascular receptor is of the same order of magnitude as that on the platelet receptor (WP). Compounds **9** (as its sodium salt), **16**, and **23** show pA_2 values in the range 6–6.5, which correlates well with the IC_{50} value of 3–5 μM obtained in washed platelets. Clearly these compounds interact with the two receptors similarly. Compound **33**, however, shows virtually no activity in the dog saphenous vein and such differences in activity of a TxRA in platelet versus vascular receptor are well-precedented.[29b] The pA_2 value (8.49) for **35** is consistent with its washed platelet activity (IC_{50} = 0.03 μM).

Conclusion

The bicyclic series of compounds described in this paper, designed with the help of molecular modeling, were found to exhibit two biological activities. Compounds **9**, **23**, and certain analogues inhibit TxA_2 biosynthesis in human platelets with good potency. These compounds also antagonize the TxA_2 receptor moderately by inhibiting U 46619 induced aggregation of human platelets and contraction of the dog saphenous vein. Changes in the stereochemistry on the bicyclic ring affect the TxSI activity more significantly than the TxRA activity. Introduction of a second chain into the structure of **9** improves the TxRA activity but adversely affects the TxSI activity. Compounds **9** and **23**, which differ only in their stereochemistry, are the best examples in a new series of compounds exhibiting both TxRA and TxSI activities.

Experimental Section

Molecular modeling studies using MacroModel[26] (version 2.5) were run on a VAX 8820 computer with an Evans and Sutherland PS 390 color graphics terminal. Infrared (IR) spectra were recorded on a Nicolet 5SXFT spectrometer. Proton NMR spectra were recorded on a Varian EM-390, XL-300, or XL-400 spectrometer. Chemical shifts are reported in ppm (δ) using tetramethylsilane, $CDCl_3$, or CD_3OD as internal standard. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Tetrahydrofuran (THF) was distilled from sodium benzophenone. Methylene chloride (CH_2Cl_2) was dried over 4-Å molecular sieves for 72 h before use. Organic solutions during workup were dried with anhydrous $MgSO_4$ or Na_2SO_4. Flash chromatography[30] was performed with silica gel 60 (0.04–0.06 mm) (Merck).

Molecular Modeling Studies. Structures **4** and **7** were subjected to energy minimization using the MM2 force field to obtain a local energy minimum (first derivative root mean square (rms) < 0.1). The energy minimized (E = −13.68 kJ/mol) structure of **4** was subjected to conformational analysis using the MULTIC (multiconformer) submode, using 60° increments for the dihedral angle about the C_1–C_2, C_3–C_4, C_7–C_{10}, and C_{10}–C_{11} bonds, to generate 720 starting conformations. Each of these was energy minimized to obtain 81 low-energy conformations. Although the energy window was set to 20 kJ/mol from the global minimum, E_{81} was only 8.00 kJ/mol higher than E_1 (E_1 = 47.80 kJ/mol). The energy minimizations in MacroModel using the MM2 force field uses a distance-dependent dielectric constant. In the energy minimizations of the starting conformations discussed in this paper, the electrostatic contribution was attenuated by adjusting the dielectric constant to $10 \times r$ where r is the interatomic separation. This was done to discourage the formation of an intramolecular hydrogen bond.

A similar process was performed on **7**. The bicyclic ring itself is reasonably rigid and was, therefore, assumed to have an optimal conformation. Increments of 90° (−120°, −60°, +60°, and +120°) were used for dihedral angles about the C_4–C_5, C_6–C_7, C_{12}–C_{13}, and C_{14}–C_{15} bonds, and 60° was used for C_7–C_8. An anti conformation was assumed for the C_2–C_3 and C_3–C_4 bonds. This generated 3550 starting conformations. Each of these was energy minimized to obtain 941 conformations spanning an energy window of 20 kJ/mol (E_1 = 110.55 kJ/mol; E_{941} = 130.49 kJ/mol).

A program designed to operate on a MacroModel multiple structures file was used to calculate the bond distances and represent them in a tabular form.[31] The C_1–C_{10} distance of **4** was 8.15 ± 0.02 Å in all the low-energy conformations. An examination of the structure–activity relationship of different TxSI reveals that the optimal distance between the pyridine nitrogen and the carboxylic acid carbon[23] is about 9.5 ± 0.5 Å. Therefore, for the purpose of locating an atom on the bicyclic ring to attach the pyridine ring, it was decided to consider all the atoms of the low-energy conformations of **7** which are at a distance of 8.15 ± 0.5 Å from C_1. The number of conformations found with C_8, C_9, C_{10}, C_{11}, C_{12}, and C_{13} in this distance range was 54, 287, 292, 313, 458, and 280, respectively. Clearly positioning pyridine at C_{12} could increase the probability of obtaining the right distance between C_1 and the pyridine nitrogen atom.

Overlaps of **4** and **7** were produced by matching C_1, C_2, C_3, and C_{10} of **4** with C_1, C_2, C_3, and either C_{12} or C_8 of **7**. Some of these overlaps (with superimposition rms <0.3) had the pyridine ring oriented into the bicyclic ring. Figures 1–3 show the pyridine ring in a desirable orientation.

Systematic conformational searching and energy minimization of **9** was done in order to confirm that C_1–pyridine nitrogen distance is in the desired range. More than 15% of the 484 low-energy conformations of **9** (E_1 = 124.12 kJ/mol, E_{484} = 143.66 kJ/mol) had the C_1–pyridine nitrogen distance in the 9–10 Å range.

2-Carbomethoxy-3-(3-pyridinyl)bicyclo[2.2.1]hepta-2,5-diene (11). A mixture of 1.35 g (8.4 mmol) of **10** and 10 mL of freshly distilled cyclopentadiene was heated at 80 °C in a sealed tube under nitrogen for 43 h. The reaction mixture was subjected to rotory evaporation and flash chromatography using 1:1 ether/hexane as eluant to obtain 0.52 g of recovered **10** and 1.08 g of **11** (92% based on recovered **10**) as a yellow oil. Purified **11** was used directly for the next step: IR (CH_2Cl_2) 1706, 1613, 1242, 1196 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.5–8.8 (m, 2 H), 7.9 (m, 1 H), 7.35 (dd, J = 8, 4.5 Hz, 1 H), 7.0 (m, 2 H), 4.07 (m, 1 H), 3.88 (m, 1 H), 3.7 (s, 3 H), 2.05–2.3 (m, 2 H).

2-*exo*-Carbomethoxy-3-*endo*-(3-pyridinyl)bicyclo[2.2.1]-heptane (13). A mixture of 3.27 g (14.4 mmol) of **11**, 70 mL of EtOH, and 0.34 g of 10% Pd/C was hydrogenated at 45 psi for 4 h. The catalyst was removed by filtration and washed with EtOH. The solvent was evaporated in vacuo to obtain 3.3 g of an oil.

To a solution of 2.3 g (9.9 mmol) of the oil obtained above in 55 mL of MeOH was added 1.46 g (10.6 mmol) of anhydrous K_2CO_3. After 4 h at room temperature, 5 mL of $SOCl_2$ was added dropwise and the mixture was allowed to stir for 15 h at room temperature. The volatiles were removed in vacuo and the residue taken up in water and adjusted to pH 8 with aqueous $NaHCO_3$. It was then extracted with CH_2Cl_2 (2 × 20 mL). The combined

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(31) The program was written by Dr. Regine Bohacek of Ciba Geigy, Summit, NJ. We would like to acknowledge the help of Drs. Regine Bohacek and Jeffrey Watthey in this regard.

organic extracts were dried, filtered, and concentrated in vacuo to give 2.23 g (67%) of **13** as a pale yellow oil, which was pure by TLC and ^{1}H NMR. Compound **13** was used directly for the next step: IR (CH_2Cl_2) 2962, 1730, 1196, 1175 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.48 (m, 2 H), 7.56 (br d, J = 8 Hz, 1 H), 7.28 (dd, J = 8, 4.5 Hz, 1 H), 3.7 (s, 3 H), 3.55 (br t, J = 5.5 Hz, 2 H), 2.6 (m, 3 H), 1.2–2.4 (m, 6 H).

2-*exo*-(Formylmethyl)-3-*endo*-(3-pyridinyl)bicyclo[2.2.1]heptane (14). A solution of 0.88 g (3.8 mmol) of **13** in CH_2Cl_2 (40 mL) under nitrogen was cooled to −78 °C and 5.0 mL (7.6 mmol) of a 1.53 M solution of diisobutylaluminum hydride in toluene was added slowly. The solution was stirred at −78 °C for 5 min and 3.8 mL MeOH was added. The mixture was warmed to 0 °C and 3.8 mL of saturated brine was added followed by 90 mL of Et_2O and 7.5 g of finely powdered anhydrous Na_2SO_4. The mixture was filtered off and washed with CH_2Cl_2 after being allowed to stir vigorously for 2 h at room temperature. The filtrate was evaporated in vacuo and subjected to flash chromatography using Et_2O as eluant to obtain 0.514 g (67%) of 3-*endo*-(3-pyridinyl)bicyclo[2.2.1]heptane-2-*exo*-carboxaldehyde as an oil, which was used as is: IR (CH_2Cl_2) 2962, 2715, 1719 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.9 (s, 1 H), 8.57 (m, 2 H), 7.55 (br d, J = 8 Hz, 1 H), 7.28 (dd, J = 8, 4.5 Hz, 1 H), 3.62 (br t, J = 6 Hz, 1 H), 2.6 (m, 3 H), 1.2–2.0 (m, 6 H).

To a suspension of 5.1 g (14.9 mmol) of methoxymethyltriphenylphosphonium chloride (dried at 80 °C and 0.1 mmHg for 24 h) in THF (40 mL) under nitrogen was added a solution of 1.78 M potassium *tert*-amylate (60 mL, 10.7 mmol) in toluene and the resulting red solution was allowed to stir for 1 h. A solution of 1.02 g (5.1 mmol) of the aldehyde prepared above in THF (5 mL) was added slowly. After allowing to stir for 3.5 h the reaction was quenched with saturated aqueous NH_4Cl. The aqueous phase was extracted with ether (2 × 20 mL). The combined organic extract was dried, filtered, and evaporated in vacuo to give an amber residue which was purified by flash chromatography using Et_2O as eluant to obtain 1.07 g (91.5%) of 2-*exo*-(2-methoxyvinyl)-3-*endo*-(3-pyridinyl)bicyclo[2.2.1]heptane as an oil, which was used as is: ^{1}H NMR ($CDCl_3$) δ 8.56 (m, 2 H), 7.6 (m, 1 H), 7.26 (dd, J = 8, 4.5 Hz, 1 H), 6.4 (d, J = 13 Hz, CH_3OCH trans), 5.82 (d, J = 6 Hz, CH_3OCH cis), 4.8 (dd, J = 13, 6 Hz, 1 H), 3.6, 3.52 (s, 3 H), 1.2–2.9 (m, 10 H).

To a solution of the above enol ether (1.07 g, 4.7 mmol) in THF (120 mL) and water (14 mL) was added 4.3 g (13.5 mmol) of $Hg(OAc)_2$ and the resulting yellow suspension was allowed to stir for 1 h. The mixture was poured into 600 mL of 10% potassium iodide. The aqueous phase was extracted with toluene (2 × 100 mL) and the combined organic extracts were washed with a 10% KI solution (2 × 500 mL) and brine. The organic phase was dried, filtered, and evaporated in vacuo to obtain an oil which was purified by flash chromatography using Et_2O as eluant to obtain 0.93 (92%) of **14** as an oil: IR (CH_2Cl_2) 2957, 2720, 1722 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.8 (br s, 1 H), 8.5 (m, 2 H), 7.56 (br d, J = 8 Hz, 1 H), 7.23 (dd, J = 8, 4.5 Hz, 1 H), 2.75 (br t, 4 Hz, 1 H), 1.2–2.5 (m, 11 H).

(5*Z*)-7-[3-*endo*-(3-Pyridinyl)bicyclo[2.2.1]hept-2-*exo*-yl]hept-5-enoic Acid (9). To a suspension of 5.99 g (13.5 mmol) of carboxybutyltriphenylphosphonium bromide (dried at 100 °C and 0.1 mmHg for 24 h) in THF (85 mL) under nitrogen was added slowly 15.0 mL (26.7 mmol) of a 1.78 M solution of potassium *tert*-amylate in toluene. The mixture was warmed to 50 °C for 45 min to complete the ylide formation. The red solution of the ylide was cooled to −10 °C and a solution of 0.94 g (4.4 mmol) of **14** in 5 mL of THF was added slowly. After allowing the reaction mixture to stir for 1 h, it was quenched with 1.54 mL of HOAc and then poured into brine. The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic extract was treated with an excess of a solution of diazomethane in ether. Excess diazomethane was destroyed with HOAc and the organic solution was washed with aqueous $NaHCO_3$, dried, filtered, and evaporated in vacuo to obtain an amber oil. Purification by flash chromatography 2:3 EtOAc/hexane as eluant gave 1.21 g (88%) of the methyl ester of **9** as an oil.

The ester (1.21 g, 3.9 mmol) was dissolved in MeOH (15 mL) and 4.4 mL (4.4 mmol) of 1 N NaOH was added. After 15 h at room temperature the mixture was evaporated in vacuo and the residue taken up in water and washed with 1:1 mixture of Et_2O and EtOAc. The aqueous phase was adjusted to pH 5 and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic layer was dried, filtered, and evaporated to obtain 1.1 g (95%) of **9** as a pale yellow oil: IR ($CDCl_3$) 3010, 2955, 2875, 1709, 1425, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.52 (br s, 1 H), 8.42 (br s, 1 H), 7.6 (d, J = 7 Hz, 1 H), 7.3 (dd, J = 8, 4.5 Hz, 1 H), 5.33 (m, 2 H), 2.7 (m, 1 H), 2.4 (m, 1 H), 2.32 (t, J = 7.5 Hz, 2 H), 2–2.2 (m, 5 H), 1.05–1.7 (m, 9 H). Anal. ($C_{19}H_{25}NO_2 \cdot 0.5H_2O$) C, H, N.

Acid **9** (0.64 g, 2.15 mmol) was dissolved in 1 mL of MeOH, and 0.11 N NaOH in MeOH (19.5 mL, 2.15 mmol) was added. The solvent was evaporated in vacuo and the residue triturated with hexane, filtered, and dried under vacuo to give 0.652 g (93%) of a pale yellow hygroscopic solid identified as the sodium salt of acid **9**: IR (KBr) 3004, 2947, 2872, 1570, 1417, 1025 cm^{-1}; ^{1}H NMR (D_2O) δ 8.45 (br s, 1 H), 8.38 (d, J = 4.5 Hz, 1 H), 7.75 (d, J = 8 Hz, 1 H), 7.4 (dd, J = 8, 4.5 Hz, 1 H), 5.4 (m, 2 H), 2.82 (br s, 1 H), 2.42 (br s, 1 H), 2.19 (t, J = 7.5 Hz, 2 H), 1.05–2.2 (m, 1 H). Anal. ($C_{19}H_{24}NO_2Na \cdot 0.5H_2O$) C, H, N.

3-*exo*-(3-Pyridinyl)-2-*exo*-vinylbicyclo[2.2.1]heptane (21). A mixture of 2.26 g (24 mmol) of norbornylene, 1.16 mL (12 mmol) of 3-bromopyridine, 3.52 mL (12 mmol) of vinyltributyltin, 0.188 g (0.12 mmol) of tetrakis(triphenylphosphine)palladium(0) and 12 mL of benzene under nitrogen was heated at 100 °C for 24 h. The reaction mixture was diluted with EtOAc and washed with 10% KF (2 × 100 mL) followed by aqueous $NaHCO_3$, water, and brine. The organic layer was dried, filtered, and evaporated under vacuo to obtain an amber oil, which, after flash chromatography using 7:3 EtOAc/petroleum ether, gave 1.63 g (68%) of **21** as a colorless oil which was used as is: IR ($CDCl_3$) 3080, 2958, 1636, 1576, 1481, 1423 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.3 (br s, 2 H), 7.37 (d, J = 8 Hz, 1 H), 7.11 (m, 1 H), 4.98 (td, J = 16.9, 9.6 Hz, 1 H), 4.78 (dd, J = 16.9, 2.3 Hz, 1 H), 4.6 (dd, J = 9.6, 2.3 Hz, 1 H), 2.91 (d, J = 9 Hz, 1 H), 2.6 (t, J = 9 Hz, 1 H), 2.52 (br s, 1 H), 2.16 (br s, 1 H), 1.79 (td, J = 10, 1.6 Hz, 1 H), 1.6 (br d, J = 8 Hz, 2 H), 1.33 (m, 3 H).

2-*exo*-(2-Formylmethyl)-3-*exo*-(3-pyridinyl)bicyclo[2.2.1]heptane (22). A mixture of 1.82 g (9.15 mmol) of **21** and 36.6 mL (18.3 mmol) of a solution of 0.5 M 9-BBN in THF was allowed to stir under nitrogen for 3 h. The reaction was quenched with 11 mL of 6 N NaOH followed by 11 mL of 30% H_2O_2. After allowing the mixture to stir for 3 h, it was diluted with EtOAc and washed with water and brine. The organic layer was dried, filtered, and evaporated in vacuo to obtain an oil which was flash chromatographed with EtOAc as eluant to give 0.9 g (45%) of a colorless oil identified as 2-*exo*-(2-hydroxyethyl)-3-*exo*-(3-pyridinyl)bicyclo[2.2.1]heptane: IR ($CDCl_3$) 3620, 2958, 2876, 1595, 1476, 1426 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.37 (br s, 2 H), 7.48 (br d, J = 8 Hz, 1 H), 7.18 (dd, J = 8, 4.5 Hz, 1 H), 3.46 (m, 2 H), 2.86 (d, J = 9 Hz, 1 H), 2.4 (br s, 1 H), 2.18 (br s, 1 H), 2.02 (q, J = 8 Hz, 2 H), 0.85–1.8 (m, 8 H).

To a solution of 0.44 mL (6.2 mmol) of DMSO in 20 mL of dry CH_2Cl_2 at −78 °C under nitrogen was added dropwise 0.44 mL (5.0 mmol) of oxalyl chloride. After 15 min, a solution of the alcohol prepared above (0.9 g, 4.1 mmol) in 3 mL of THF was added. The reaction was quenched after 15 min with 5.8 mL (41 mmol) of Et_3N. The mixture was warmed to 0 °C and diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, water, and brine. The organic layer was dried, filtered, and evaporated in vacuo to give 0.89 g (100%) of **22**, which was used as is: IR (CH_2Cl_2) 2958, 2875, 2721, 1722, 1425 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.47 (s, 1 H), 8.4 (br s, 2 H), 7.45 (d, J = 8 Hz, 1 H), 7.18 (m, 1 H), 2.96 (d, J = 9 Hz, 1 H), 2.56 (q, J = 9 Hz, 1 H), 2.48 (br s, 1 H) 2.09 (br s, 1 H), 1.88 (d, J = 9 Hz, 2 H), 1.3–1.8 (m, 6 H).

(4*Z*)-6-[3-*exo*-(3-Pyridinyl)bicyclo[2.2.1]hept-2-*exo*-yl]hex-4-enoic Acid (24). A solution of 1.78 M potassium *tert*-amylate in toluene (2 mL, 3.56 mmol) was added dropwise to a suspension of 1.9 g (4.18 mmol) of carbethoxypropyltriphenylphosphonium bromide (dried at 80 °C and 0.1 mmHg for 24 h) in 5 mL of THF under nitrogen. The orange solution was allowed to stir vigorously for 45 min and cooled down to 0 °C. A solution of 0.45 g (2.09 mmol) of **22** in 3 mL of THF was added and the mixture was allowed to stir for 1 h at room temperature. The reaction was quenched with aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and brine and then dried, filtered, and evaporated in vacuo to give an amber

oil which was purified by flash chromatography using 3:7 EtOAc/petroleum ether to give 0.39 g (60%) of ethyl 6-[3-*exo*-(3-pyridinyl)bicyclo[2.2.1]hept-2-*exo*-yl]hex-4-enoate, which was used without further purification: IR ($CDCl_3$) 2958, 2874, 1727 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.4 (m, 2 H), 7.53 (br d, J = 8 Hz, 1 H), 7.21 (dd, J = 8, 4.5 Hz, 1 H), 5.21 (m, 2 H), 4.05 (q, J = 7 Hz, 2 H), 2.86 (d, J = 9 Hz, 1 H), 2.41 (br s, 1 H), 1.2–2.3 (m, 17 H).

The ester prepared above was saponified in 2 mL of dioxane and 1.3 mL (1.3 mmol) of 1 N NaOH. After 3 h the reaction mixture was worked up by acidification to pH 5.7 followed by extraction with EtOAc. The organic layer was washed with brine, dried, filtered, and evaporated in vacuo to give an oil which was purified by flash chromatography using 5.5:4:0.5 EtOAc/petroleum ether/HOAc as eluant to obtain 0.26 g (73%) of **24** as an oil which solidified on standing. Recrystallization from MeOH gave crystals which melted at 70–75 °C. An X-ray analysis confirmed the cis–exo stereochemistry (see Figure 4): IR (KBr) 3414 (br), 2957, 2872, 2489 (br), 1714, 1425, 1185 cm^{-1}; ^1H NMR (CD_3OD) δ 8.34 (br s, 1 H), 8.29 (d, J = 4.5 Hz, 1 H), 7.72 (br d, J = 8 Hz, 1 H), 7.32 (dd, J = 8, 4.5 Hz, 1 H), 5.23 (m, 2 H), 2.96 (d, J = 9 Hz, 1 H), 2.43 (br s, 1 H), 1.2–2.25 (m, 14 H). Anal. ($C_{18}H_{23}NO_2$) C, H, N.

X-ray Structure Determination of 24. The X-ray structure determination was performed with a Siemans R3m/V diffractometer and the SHELLXTL PLUS software on a MicroVax II computer. The crystal was orthorhombic, space group *Pcan*, with cell constants a = 9.662 (1) Å, b = 15.515 (2) Å, and c = 22.316 (3), Å. A molecule of solvent, probably methanol, was located in the difference map with one of its atoms on a crystal axis. This molecule was not completely characterized.

2-Carbomethoxy-3-(3-pyridinyl)bicyclo[2.2.1]hept-2-ene (12). A mixture of 1.42 g (6.2 mmol) of **11**, 25 mL of EtOAc, and 0.15 g of 15% Rh/Al_2O_3 was hydrogenated at 1 atm of hydrogen until 160 mL (1 equiv) of H_2 was consumed. The catalyst was filtered off and washed with EtOAc. The solvent was evaporated in vacuo to give 1.42 g (99%) of **12** which was used without further purification: IR (CH_2Cl_2) 3053, 2971, 2952, 2876, 1705, 1607, 1435, 1260, 1229 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.8 (br s, 1 H), 8.6 (br d, J = 4.5 Hz, 1 H), 8.0 (br d, J = 8 Hz, 1 H), 7.33 (dd, J = 8, 4.5 Hz), 3.7 (s, 3 H), 3.42 (br s, 1 H), 3.28 (brs, 1 H), 1.2–2.0 (m, 6 H).

2-*exo*-Cyano-2-*endo*-(3-pyridinyl)-3-*exo*-carbomethoxybicyclo[2.2.1]heptane (26). To a solution of 9.62 g (42 mmol) of **12** in 210 mL of DMSO under nitrogen was added 2.5 mL (4.37 mmol) HOAc, followed by 6.31 g (97 mmol) of finely powdered KCN. The reaction mixture was heated at 60 °C for 23 h and then poured into saturated aqueous NH_4Cl solution. The mixture was extracted with EtOAc (4 × 100 mL), and the combined organic extracts were washed with water (3 × 100 mL) and brine. It was then dried, filtered, and evaporated in vacuo to give 9 g of an amber oil, which was flash chromatographed with 4:1 Et_2O/hexane as eluant to give 6.55 g (61%) of **26**, which crystallized upon standing: mp 70–72 °C; IR (CH_2Cl_2) 2974, 2226, 1738, 1280, 1178 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.7 (br s, 1 H), 8.5 (br s, 1 H), 7.8 (br d, J = 8 Hz, 1 H), 7.28 (dd, J = 8 Hz, 4.5 Hz, 1 H), 3.67 (s, 3 H), 2.94 (br s, 1 H), 2.86 (s, 1 H), 2.72 (br s, 1 H), 2.22 (d, J = 9 Hz, 1 H), 1.57 (d, J = 9 Hz, 1 H), 1.4–1.55 (m, 2 H), 1–1.1 (m, 2 H).

Octahydro-7a-(3-pyridinyl)-4,7-methanoisobenzofuran-1-ol (28). A mixture of 2.11 g (8.2 mmol) of **26**, 34 mL of *tert*-butyl alcohol, and 0.89 g (22 mmol) of $NaBH_4$ was heated to reflux and 6.6 mL of MeOH (distilled from magnesium methoxide) was added slowly over 1.25 h. The mixture was refluxed for an additional hour and 21 mL of 2 N HCl was added. After allowing to stir for 1 h the mixture was evaporated in vacuo and the residue was adjusted to pH 8 and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried, filtered, and evaporated in vacuo to give a residue which upon flash chromatography using 7:3 EtOAc/hexane as eluant gave 1.37 g (73%) of white crystals (mp 101–102 °C), which was identified as octahydro-7a-(3-pyridinyl)-4,7-methanoisobenzofuran-1-one: IR (CH_2Cl_2) 2963, 1761, 1606, 1509 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.72 (br s, 1 H), 8.55 (br s, 1 H), 7.86 (br d, J = 8 Hz, 1 H), 7.3 (dd, J = 8, 4.5 Hz, 1 H), 4.6 (t, J = 9 Hz, 1 H), 4.12 (dd, J = 9, 3 Hz, 1 H), 3.0 (br s, 1 H), 2.78 (br d, J = 9 Hz, 1 H), 2.38 (br s, 1 H), 1.05–1.8 (m, 6 H).

A solution of diisobutylaluminum hydride (4.4 mL, 6.7 mmol) in toluene was added dropwise at −78 °C to a solution of the lactone prepared above (1.37 g, 6 mmol) in 30 mL of CH_2Cl_2 under nitrogen. After allowing the reaction mixture to stir for 15 min it was quenched with 3.4 mL of MeOH and allowed to warm to 0 °C. Saturated brine (3.4 mL) was added followed by 75 mL of Et_2O and 7 g of anhydrous Na_2SO_4. The mixture was allowed to stir vigorously for 1 h and then filtered and washed with CH_2Cl_2. The solvent was evaporated in vacuo to obtain 1.27 g (67%) of **28** as a thick clear colorless oil (~2:1 mixture of two anomers by NMR) which solidified upon standing: mp 125–129 °C; IR (CH_2Cl_2) 3584, 2955, 2877, 1479, 1416, 1062 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.4–8.62 (m, 2 H), 7.62 (d, J = 8 Hz, 1 H), 7.3 (m, 1 H), 5.1–5.25 (2 s, anomeric proton of two anomers), 4.32 (t, J = 8 Hz), 4.03 (dd, J = 9, 8 Hz, 1 H), 3.83 (dd, J = 8, 4 Hz), 3.75 (dd, J = 8, 4 Hz) (signals at 4.32, 4.03, 3.83, 3.75 together integrate to 2 H), 1–3.2 (m, 10 H). Anal. ($C_{14}H_{17}NO_2$) C, H, N.

Octahydro-4a-(3-pyridinyl)-5,8-methano-2-benzopyran-3-ol (31). To a solution of 6.88 g (20 mmol) of methoxymethyltriphenylphosphonium chloride in 60 mL of THF under nitrogen was added 12.4 mL (20 mmol) of a 1.61 M solution of KOtBu in THF. After allowing the ylide formation to continue for 1 h, a solution of 1.34 g (5.8 mmol) of **28** in 5 mL of THF was added and the mixture stirred for 3 h at room temperature and then at 50 °C for 1 h. The reaction mixture was cooled and quenched with aqueous NH_4Cl. The mixture was extracted with Et_2O (2 × 50 mL) and then washed with brine, dried, filtered, and evaporated in vacuo to give an oil, which was partially purified by flash chromatography using EtOAc as eluant to obtain 4.9 g of a crude oil.

The crude oil was dissolved in dioxane and 40 mL of 25% CF_3CO_2H in water was added. After 60 h at room temperature, the solvent was evaporated in vacuo and the residue suspended in 10 mL of 1 N HCl. The aqueous phase was washed with ether and adjusted to pH 8 with aqueous $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried, filtered, and evaporated in vacuo to give a thick oil, which was flash chromatographed with 7:3 EtOAc/hexane as eluant to give 0.722 g (51%) of **31** as a thick colorless oil (~95% of one anomer): IR (CH_2Cl_2) 3579, 2956, 1569, 1415, 1281, 1028 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.62 (br s, 1 H), 8.43 (d, J = 4.5 Hz, 1 H), 7.75 (br s, J = 8 Hz, 1 H), 7.27 (dd, J = 8, 4.5 Hz), 4.73 (dd, J = 9, 6 Hz, 1 H), 3.89 (dd, J = 11.3, 8 Hz, 1 H), 3.6 (t, J = 11.3 Hz, 1 H), 1–3.0 (m, 12 H).

(5*Z*)-Methyl 7-[3-*exo*-(Hydroxymethyl)-2-*endo*-(3-pyridinyl)bicyclo[2.2.1]hept-2-*exo*-yl]hept-5-enoate (32). Lactol **31** (0.94 g, 3.92 mmol) was subjected to Wittig reaction with the ylide derived from carboxybutyltriphenylphosphonium bromide followed by esterification with diazomethane according to the procedure for **9** to obtain 0.304 g (23%) of **32** after purification using 7:3 EtOAc/hexane as eluant: IR (CH_2Cl_2) 3610, 2955, 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.68 (br s, 1 H), 8.47 (br d, J = 4.5 Hz, 1 H), 7.78 (br d, J = 8 Hz), 7.26 (dd, J = 8, 4.5 Hz, 1 H), 5.25 (m, 2 H), 3.8–4.3 (m, 2 H), 3.7 (s, 3 H), 1–2.8 (m, 17 H).

(5*Z*)-7-[3-*exo*-[(4-Phenylphenoxy)methyl]-2-*endo*-(3-pyridinyl)bicyclo[2.2.1]hept-2-*exo*-yl]hept-5-enoic Acid (33). To a mixture of 0.14 g (0.4 mmol) **32**, 6 mL of CH_2Cl_2, 0.077 g (0.45 mmol) of 4-phenylphenol, and 0.16 g (0.61 mmol) of triphenylphosphine under nitrogen was added dropwise 0.073 mL (0.46 mmol) of diethyl azodicarboxylate. After allowing the reaction to stir for 15 h, the solvent was evaporated in vacuo and preparative thin-layer chromatography using 3:7 EtOAc/hexane as eluant was performed to obtain 0.23 g of partially purified methyl 7-[3-*exo*-[(4-phenylphenoxy)methyl]-2-*endo*-(3-pyridinyl)bicyclo[2.2.1]hept-2-*exo*-yl]hept-5-enoate in a crude form which was saponified as is.

A solution of 0.23 g of the partially purified ester prepared above in 5 mL of MeOH was saponified with 1 mL of 1 N NaOH. After the saponification was complete (15 h), the reaction mixture was washed with CH_2Cl_2. The aqueous phase was adjusted to pH 5 and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phase was dried, filtered, and evaporated to give a thick oil which was purified by preparative thin-layer chromatography using 95:5:1 CH_2Cl_2/MeOH/HOAc to obtain 0.126 g (65%) of **33** as a white foam: IR ($CDCl_3$) 2762, 2877, 1709, 1608, 1519, 1486 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.75 (br s, 1 H), 8.48 (br s, 1 H), 7.82 (d, J = 8 Hz, 1 H), 7.53 (d, J = 8 Hz, 4 H), 7.37 (t, J = 8 Hz, 3 H), 7.29

(dd, $J = 8, 4.5$ Hz, 1 H), 7.04 (d, $J = 8$ Hz, 2 H), 5.22 (m, 2 H), 4.24 (m, 2 H), 2.7 (br s, 1 H), 1.1–2.5 (m, 15 H). Anal. ($C_{32}H_{35}NO_3 \cdot 0.5H_2O$) C, H, N.

Measurement of Thromboxane Synthase Inhibition. The method as described previously[32] was used to measure IC_{50} values for the TxSI activity of the compounds.

Measurement of Thromboxane Receptor Antagonism. 1. Inhibition of U 46619 Induced Aggregation of Washed Human Platelets. Approximately 60 mL of venous blood was withdrawn via an antecubital vein puncture from human volunteers free of medication for 2 weeks. The blood was collected in an acid–citrate–dextrose (ACD) anticoagulant at a ratio of 8.5 parts blood:1.5 part ACD. The blood was centrifuged at 150*g* for 20 min at room temperature to obtain platelet-rich plasma. The platelets were pelleted by centrifugation of the platelet-rich plasma at 2000*g* for 20 min. The supernatant (platelet-poor plasma, PPP) was set aside and the pellet was resuspended in a buffer (pH 6.8) consisting of 10.0 mmol PIPES, 135 mmol NaCl, 5.0 mmol KCl, 5.5 mmol dextrose, and 0.2 mmol EGTA, 0.25 mmol of acetylsalicylic acid (aspirin) was added, and the suspension was allowed to incubate for 15 min at room temperature. At the end of the incubation, the platelets were repelleted by centrifugation at 1500*g* for 10 min. The resultant pellet was resuspended in a HEPES dextrose buffer (pH 7.4) with 50 ng/mL prostacyclin. The suspension was centrifuged at 1500*g* for 10 min and the pellet resuspended in the same HEPES buffer (without prostacyclin) and 0.125–0.25% autologous PPP containing 1.0 mmol $CaCl_2$ and 1.0 mmol $MgCl_2$. The platelet count was adjusted to 2.5×10^8 cells/mL with an incubation buffer (containing 0.125–0.25% PPP) and a Coulter Model ZBI particle counter (Coulter Electronics Inc., Hialeah, Fl).

Washed platelet aggregation was performed on a Payton dual-channel aggregometer (Payton Associates Inc., Buffalo, NY) attached to a Compaq Deskpro 286 Personal Computer (Compaq Computer Corp., Houston, TX). Aliquots (0.5 mL) of the washed-platelet suspension were incubated with either compound or its appropriate vehicle under stirring (900 rpm) at 37 °C for 2 min. At the conclusion of the incubation U 46619 (4,11-dideoxy-11α,9α-(epoxymethano)prostagandin $F_{2\alpha}$) (Upjohn Diagnostics, Kalamazoo, MI) was added (1.25–4.0 μM). Aggregation was allowed to proceed for 6 min. Data was compiled and calculated with the area under the curve by using a least-squares fit. IC_{50} values were calculated by linear-regression analysis. Stock concentrations of compounds were dissolved in DMSO and diluted to the appropriate working concentrations with HEPES buffer (pH 7.4) or distilled water.

2. Inhibition of U 46619 Induced Aggregation of Human Platelet-Rich Plasma. Venous blood (50–100 mL), anticoagulated in 0.0129 M (final) trisodium citrate, was withdrawn from human volunteers free of medication for 2 weeks, via antecubital vein puncture. The blood was centrifuged at 150*g* at room temperature for 20 min. The platelet-rich plasma supernatant was removed and the remaining infranatant was centrifuged at 2000*g* for 20 min to yield PPP. The platelet count was determined with a Coulter Model ZBI particle counter and adjusted to 2.5×10^8 cells/mL with autologous PPP.

Platelet aggregation was performed on a Payton dual-channel aggregometer attached to a Compaq Deskpro 286 Personal Computer. Aliquots (0.5 mL) of platelet rich plasma were incubated with either compound or its appropriate vehicle under stirring (900 rpm) at 37 °C for 2 min. Following this incubation, collagen (1–3 μg/mL) (hormon-Chemie, Munchen GMBH) or U 46619 (1.25–4.0 μM) (Upjohn Diagnostics, Kalamazoo, MI) was added and aggregation was allowed to proceed for 6 min. Data was compiled and calculated with the area under the curve by using a least-squares fit. IC_{50} values were calculated by linear-regression analysis. Stock solutions of compounds were dissolved in DMSO and diluted to their appropriate working concentrations with HEPES buffer (pH 7.4) or distilled water.

3. Inhibition of U 46619 Induced Contraction of Dog Saphenous Vein. Saphenous veins excised from anesthetized, colony bred, mongrel dogs (10–18 kg, Bartons West End Farms, Oxford, NJ) were placed in modified Krebs buffer (112 mmol NaCl, 5.0 mmol KCl, 1.0 mmol KH_2PO_4, 1.2 mmol $MgSO_4$, 2.5 mmol $CaCl_2$, 25 mmol $NaHCO_3$, and 11.5 nmol of *d*-glucose, pH 7.4) and aerated with a 95% O_2/5% CO_2 gas mixture. The saphenous veins were cleaned, trimmed, and kept refrigerated up to 48 h prior to use. The veins were cut into 2–3 mm rings and mounted vertically in a 20-mL bath aerated with 95% O_2/5% CO_2 at 37 °C. The bathing solution was a modified Krebs buffer with 1.0 μM indomethacin added (to prevent endogenous prostaglandin formation). The tissues were attached to an FT.03 isometric force transducer (Grass Instruments, Quincy, MA), and the Buxco T120B automated in vitro bath system (Sharon, CT) was used in all experiments. The preload tension was 2.0 g. The vessels were allowed to equilibrate for 1.5 h.

Compounds were dissolved in DMSO, stored at −20 °C, and diluted with DMSO prior to use. DMSO vehicle was also used for parallel control vessels. U 46619 was prepared in 100% ethanol, stored at −20 °C, and diluted with distilled water prior to each assay. Following tissue equilibration, a contractile response to 10^{-9} M U 46619 was elicited. The tissues were then washed four times and rested. The contractile responses to 10^{-9} M U 46619 were repeated until consistent contractions were obtained. After subsequent washes and the return of tension to baseline levels, either test compound in vehicle or vehicle alone was added to the bath. Ten minutes after addition of the compound or vehicle control dose–response curves to U 46619 (5×10^{-11}–10^{-5} M) were obtained. Data were analyzed with the Branch Technology SM-STAT system (Dexter, MI) to give pA_2 estimates derived from the parallel dose–response curves obtained above.

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Supplementary Material Available: Details of the structure determination of **24**, the numbering system, tables of atomic coordinates and bond distances and angles (8 pages). Ordering information is given on any current masthead page.

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