

Short communication

Hydrogen bond interactions of a series of *N*-substituted TXA₂ receptor antagonistsDawne M. Taylor^a, Perry V. Halushka^b, G. Patrick Meier^{a,b,*}^a Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC 29425, USA^b Departments of Cell and Molecular Pharmacology and Experimental Therapeutics and Medicine, Medical University of South Carolina, Charleston, SC 29425, USA

Received 23 December 2001; received in revised form 30 June 2003; accepted 10 July 2003

Abstract

A series of *N*-substituted sulphonamide based thromboxane A₂ (TXA₂) receptor antagonists were synthesised with the objective to explore the role of hydrogen bond donation properties in the binding of these ligands to the TXA₂ receptor. Pharmacological evaluation of these compounds revealed that the binding affinity decreased significantly with the removal of the hydrogen bond donor. This indicates that a hydrogen bond donor is important for the binding of these antagonists to the TXA₂ receptor.

© 2003 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: thromboxane A₂; TXA₂; receptor antagonists; hydrogen bonding; sulphonamide; platelets

1. Introduction

Thromboxane A₂ (TXA₂) is produced enzymatically from arachidonic acid via the cyclooxygenase pathway and has been implicated as a pathophysiological mediator in a wide variety of cardiovascular and renal diseases [1–4]. TXA₂ induces platelet aggregation as well as vascular and respiratory smooth muscle contraction via its receptor, which belongs to the G-protein coupled receptor family. Receptors in this family are highly lipophilic and consist of seven transmembrane regions [5]. Considerable work in the areas of molecular modelling [6–8], site-directed mutagenesis [9–12], study of naturally occurring mutations [13,14] and structure activity relationship (SAR) studies [15–19], have been done to understand the mechanism by which TXA₂ binds to its receptor.

TXA₂ has two side chains (α and ω) attached to an oxobicyclic ring system containing two oxygen atoms. Early efforts in the synthesis of TXA₂ analogues focused on alterations of this ring system. It was found that

almost every combination of nitrogen, oxygen, sulphur and carbon atoms that led to a stable [2.2.1] or [3.1.1] bicyclic ring system and that possessed the α - and ω -side chains, maintained activity [17]. This indicates that the bicyclic ring is important for optimal activity by maintaining the relative stereochemistry of the α - and ω -side chains. It is important to note that the α - and ω -side chain stereochemistry necessary for binding to the receptor varies between different heterocyclic ring systems [20]. SAR studies of early analogues established that the stereochemistry of the side chain C-15 hydroxyl group is important for effective ligand receptor binding [21].

Receptor sequence studies have shown that Arg295 in the seventh transmembrane domain (TM7) is conserved in all prostanoid receptors. An Arg295Gln point mutation resulted in a loss of binding of I-BOP, a TXA₂ receptor agonist, to the receptor. From these results, it is clear that Arg295 is important for ligand-receptor binding. It is hypothesised that Arg295 interacts with the TXA₂ via formation of a salt bridge with the C-1 carboxylate on the α -side [10].

We are interested in studying the importance of hydrogen bonding between the sulphonamide group on the ω -side chain of pinane based TXA₂ antagonists and the TXA₂ receptor. Takasuka et al. proposed that

* Corresponding author. Department of Chemistry, 402 Fulmer Hall, PO Box 643140, Washington State University; Pullman, WA 99164-4630, USA.

E-mail address: meiergp@wsu.edu (G.P. Meier).

these prostanoid based ligands underwent intramolecular hydrogen bonding between the carboxylate of the α -side chain and the hydrogen bond donor/acceptor of the ω -side chain to achieve their active conformation (Fig. 1) [22–24]. Non-prostanoid derivatives such as sulotroban (Fig. 2) are unable to form this conformation, yet still have good binding affinity to the TXA₂ receptor. Therefore, these ligands must interact with the receptor via a different mechanism than the prostanoid based ligands. Recently, Langenhan et al. reported that secondary sulphonamides can form hydrogen bonds with the N–H and C=O of a proximal α -amino acid residue [25]. This latter suggests the possibility of the ligand sulphonamide acting either, or both as a hydrogen bond donor or acceptor to the receptor amino acid residues.

A molecular modelling study of the TXA₂ receptor suggests that Ser201 in the fifth transmembrane domain (TM5) of the receptor is capable of hydrogen bonding with the C-15 hydroxyl group of TXA₂ [6]. Point mutations of the human TXA₂ receptor at Ser201 and a conserved Ser255 did not affect binding affinity for [³H]SQ29548, a TXA₂ receptor antagonist, but significantly altered binding affinity for [¹²⁵I]BOP. It is thought that the difference in binding affinity is due to the C-15 hydroxyl group in I-BOP that is not present in SQ29548 and that Ser201/Ser205 may interact with I-BOP via hydrogen bonding with the C-15 hydroxyl group. It is also thought that other serines residues in TM5–TM7 may be intimately involved in hydrogen bonding with TXA₂ analogues [9].

The role of intermolecular hydrogen bonding between the ligand and the TXA₂ receptor has not been explored fully. Hall et al. synthesised a series of 7-oxabicyclo[2.2.1]heptane TXA₂ receptor antagonists where a simple thioether made up the ω -side chain. They determined through SAR studies that the sulphur at the C-14 position gave the greatest binding affinity. Thioethers (Fig. 2) can serve as a hydrogen bond acceptor only. While these thioethers exhibited excellent binding, they all displayed partial agonist activity and were subsequently not pursued [26]. In a similar finding, Misra and coworkers have reported that a hydrogen bond acceptor in the ω -side chain was important for the

high affinity of their interphenylene 7-oxabicyclo[2.2.1]heptane oxazole TXA₂ receptor antagonists (Fig. 2) [18]. However, these ligands still possessed a potential hydrogen bond donor in the form of the secondary amide and the results do not indicate whether a hydrogen bond donor or acceptor is important for binding. Sato et al. reported in a SAR study of the nonprostanoid derived sulphonamide based TP receptor antagonists Sulotroban (Fig. 2) that binding affinity decreased significantly when the N–H was replaced with an alkyl group [19].

Based on these observations, our study was designed to explore the importance of hydrogen bond donors and acceptors with respect to the affinity and pharmacological activity within a series of TXA₂ receptor ligands. Our approach was to synthesise a series of TXA₂ receptor antagonists based on the bicyclo[3.1.1]heptyl ring system employing a sulphonamide with varying hydrogen bonding ability as the ω -side chain. Radiolabelled competition binding assays and platelet aggregation studies were performed to determine the pharmacological properties of the ligands. The importance of hydrogen bond donors and acceptors with respect to binding affinity and pharmacological activity are discussed.

2. Chemistry

Sulphonamides **1–3** were synthesised using modifications of the work by Seno and Hagishita [27] and as shown in Fig. 3. Their method for the reduction of ketone **4** used sodium borohydride and was reported to give a mixture of α and β alcohols **5** in a 1:1 ratio. While exploring the use of more sterically hindered reducing agents, it was discovered that DIBAL-H at -78°C gave a 98% yield of a 20:1 mixture of α : β alcohols. Reaction of the α alcohol with methanesulphonyl chloride followed by displacement with sodium azide resulted in azide **6** with an overall yield of 50%. Reduction of this azide to amine **7** was accomplished with 10% palladium on carbon under an atmosphere of H₂, and was followed by the addition of toluenesulphonyl chloride to generate sulphonamide **8** in a 75% yield. Sulphonamides **9** and **10** were obtained by alkylation of **8** using KH and iodomethane or iodoethane, respectively, in good yields. Debenzylation of the alcohols **11–13** using 10% palladium on carbon under an atmosphere of H₂, followed by oxidation with TPAP/NMO [28] readily generated aldehydes **14–16** in 75–95% yield. The α -side chain was extended using the Wittig olefination reaction. As seen previously [29], this procedure produced both *cis* and *trans* isomers. These isomers were not separated by normal phase and argentified silica gel chromatography, crystallisation of the dicyclohexylammonium salt, and both normal and reverse phase

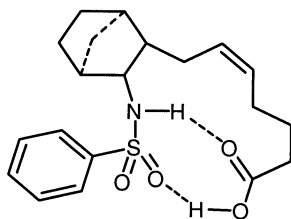


Fig. 1. Proposed conformation of S-145 in a non-aqueous environment. This conformation enables intermolecular hydrogen bonding between the carboxylic acid of the α -side chain and the sulphonamide of the ω -side chain [22].

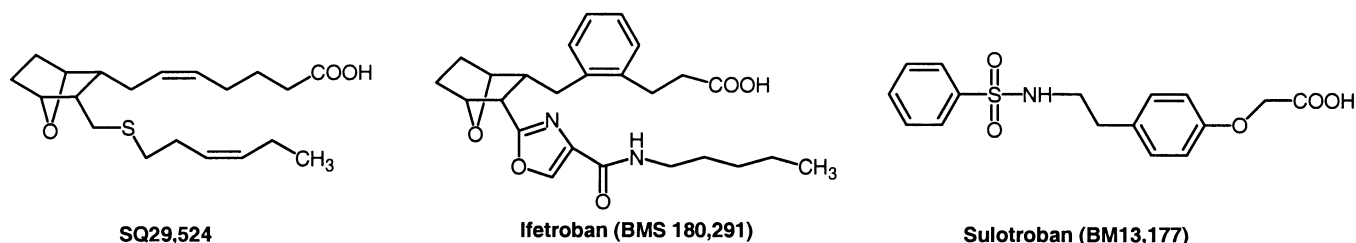


Fig. 2. Chemical structures of SQ29,524, Ifetroban and Sulotroban. SQ29,548 [26] is an example of a thioether, Ifetroban [18] is an example of an interphenyle 7-oxabicyclo[2.2.1]heptane oxazole that was suggested to require a hydrogen bond donor for high binding affinity and Sulotroban [19] in which Sato et al. reports a decrease in binding affinity when the N–H was replaced with an alkyl group.

HPLC. Two peaks were observed using reverse phase HPLC but, since these peaks largely overlapped, the compounds were carried forward as the mixture. The percentage of the *trans* isomer was between 5 and 20% for the three analogues.

3. Pharmacology

3.1. Radiolabelled competition binding assay

Crude platelet membranes were prepared as described previously [30] and incubated in silanized (12 × 75 mm) glass tubes at 30 °C for 30 min. The incubation buffer consisted of 50 mM Tris–HCl buffer at pH 7.4 and between 0.02 and 0.2 nM [¹²⁵I]BOP (50,000 cpm) per tube. Sulphonamides **1**–**3** were tested at final concentrations of 10^{−11} to 10^{−5} M. The reaction was terminated by the addition of 4 mL of ice-cold buffer followed by rapid filtration through Whatman GF/C glass fiber filters. The filters were washed three more times with 4

mL of the ice-cold buffer. The filtration procedure was complete within 10 s. Non-specific binding was defined as the amount of radioactivity bound in the presence of 10 μM SQ29548, a specific TP receptor antagonist. Specific binding was approximately 90% of the total binding.

3.2. Platelet aggregation

Blood (50–100 mL) was drawn via venipuncture from healthy human volunteers, who had not taken any medication for at least 10 days, into syringes containing indomethacin (10 μM) and EDTA (5 mM) (final concentrations) and prepared as previously described [31]. The study was approved by the Medical University of SC IRB for human research and informed consent was obtained from all subjects. The washed human platelets (2.5 × 10⁸ mL^{−1}) were suspended in a 50 mM Tris–HCl/100 mM NaCl buffer containing glucose (5 mM) and indomethacin (10 μM) at pH 7.4. Before initiating the aggregation, fibrinogen (0.28 mg mL^{−1})

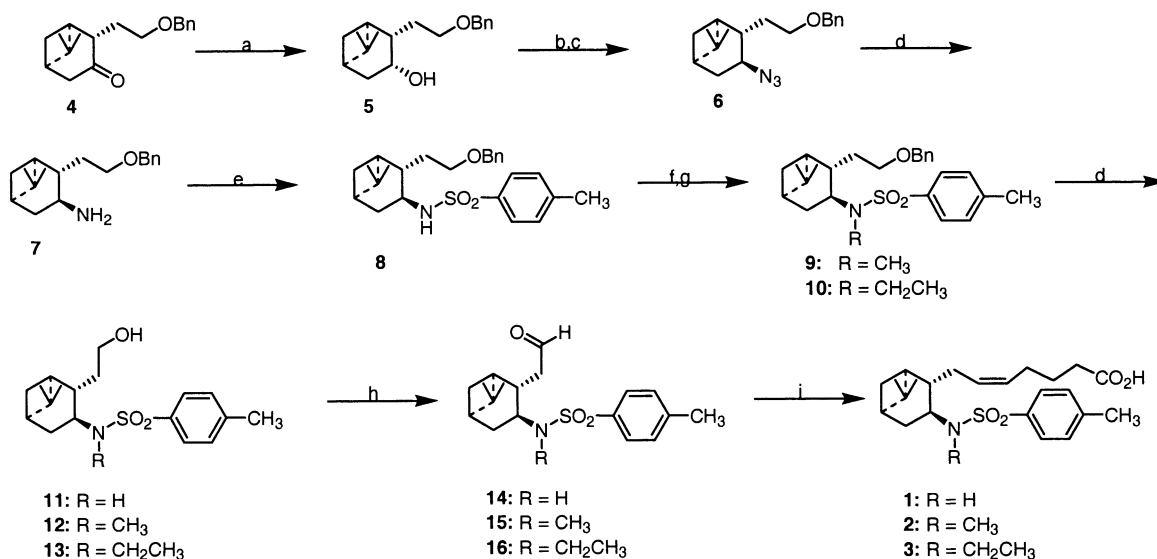


Fig. 3. The synthetic scheme for the synthesis of the sulphonamide based TP receptor antagonists. (a) DIBALH, −78 °C; (b) MsCl, TEA; (c) NaN₃, HMPA; (d) H₂, 10% Pd–C; (e) TsCl, pyridine; (f) KH, THF; (g) CH₃I or CH₃CH₂I; (h) NMO, TPAP; (i) *tert*-BuOK, Ph₃P⁺(CH₂)₄COOH Br[−].

and CaCl_2 (250 μM) (final concentrations) were added to the platelet suspension. The washed human platelets (450 μL) were added to individual silanised glass cuvettes containing a stir bar and preincubated for 1 min at 37 $^\circ\text{C}$ with either the vehicle or varying concentrations of sulphonamides 1–3. Addition of 100 nM U46619, a TP receptor agonist, initiated the aggregation response which was recorded using a Chronolog model 300 aggregometer (Havertown, PA) [31] for 1 min. Concentration response curves were constructed for the analogues and the IC_{50} values were calculated directly from the log–logit transformation of the data. The IC_{50} value was defined as the concentration needed to inhibit 50% of the maximum aggregation response 1 min after the addition of the aggregating agent.

3.3. Statistics

The equilibrium binding data were corrected for non-specific binding and fitted to a two parameter model identifying one binding site using PRISM software for nonlinear regression. All values are expressed as mean \pm SEM. ANOVA was used to ascertain the statistical significance at the $P < 0.05$ level.

4. Results and discussion

Compounds 1–3 bind to the TP receptor in the same manner. However, the *N*-alkylated sulphonamides 2 and 3, which are not able to participate in hydrogen bond donation, have significantly higher K_d values than that of the non-alkylated sulphonamide 1 ($P = 0.027$) (Table 1) as determined by radiolabelled competition binding assays (Fig. 4) against [^{125}I]BOP, a known TP receptor agonist. All three sulphonamides were found to be competitive antagonists by virtue of their ability to completely block the agonist action of 100 nM U46619 in platelet aggregation assays (Fig. 5). Compounds 2 and 3 had significantly higher K_d values as compared to

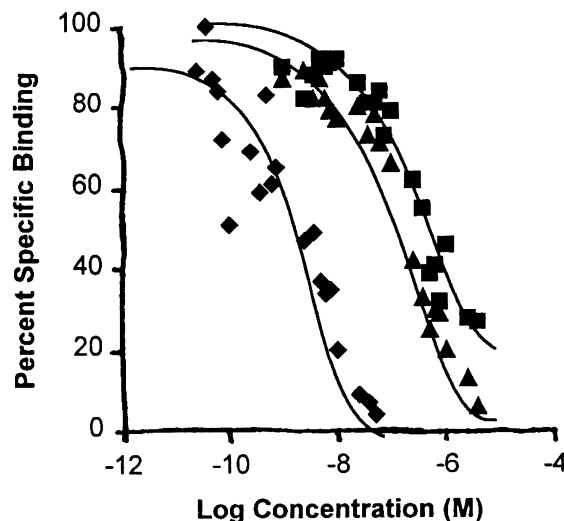


Fig. 4. Representative competition binding curves. Percent specific binding vs log concentration as obtained from radiolabelled competition binding assays for the \blacklozenge N-H, \blacktriangle N-methyl, and \blacksquare N-ethyl sulphonamide antagonists (compounds 1–3) against [^{125}I]BOP.

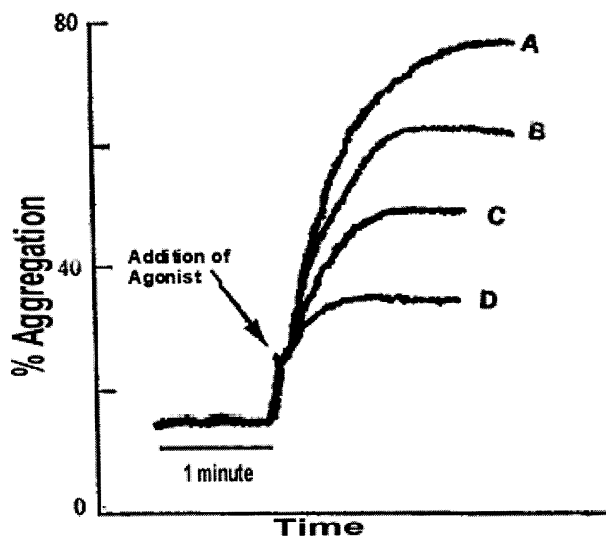


Fig. 5. Representative platelet aggregation responses to the following concentrations of the non-alkylated sulphonamide 1: A, control (100 nM of the agonist U46619); B, 0.5 nM; C, 5.0 nM and D, 25 nM.

Table 1
Results of the radioligand competition binding and aggregation assays

Compound	Binding K_d (nM) ^a Crude platelet membranes	Aggregation K_d (nM) ^b Washed human platelets
1	4.3 \pm 0.1	9.5 \pm 1.5
2	227 \pm 19	2602 \pm 796
3	272 \pm 8	1804 \pm 353

^a Radiolabelled competition binding assays were performed against [^{125}I]BOP in crude platelet membranes.

^b Aggregation assays using U46619 (100 nM) as the agonist were performed in washed human platelets. IC_{50} values for the antagonists were obtained and were converted to K_d values using the Cheng–Prusoff equation. The K_d values are reported as the mean \pm SEM of 3–4 experiments. The K_d values for compound 1 are significantly different from those of 2 and 3, $P < 0.05$ (ANOVA).

compound 1 ($P = 0.0002$) (Table 1). The IC_{50} values obtained from the aggregation studies were transformed to K_d values using the Cheng–Prusoff equation [32].

It was determined that all three of these compounds are TP receptor antagonists as determined by platelet aggregation assays. The *N*-methyl sulphonamide 2 and the *N*-ethyl sulphonamide 3 both have significantly higher K_d values than the non-alkylated sulphonamide 1, but are not significantly different from each other. These results suggest that the increased K_d values of 2 and 3 compared to 1 is not a result of steric effects, but rather reflects a loss in the ability to act as a hydrogen

bond donor. If steric interactions played a significant role in the loss of biological activity of compounds **2** and **3**, one might expect there to be a significant increase in the K_d values of *N*-ethyl sulphonamide over *N*-methyl sulphonamide, which was not seen.

It is interesting to note that all the K_d values determined from the aggregation assays are substantially higher than the K_d as determined by radiolabelled competition binding assays. It is thought that this increase in binding affinity is due to the activation of receptor reserves in platelets from the agonists, U46619 [33]. Also, alkylation of the sulphonamides creates a more lipophilic molecule than the parent sulphonamide and can result in non-specific binding.

5. Conclusion

These results indicate that the decrease in binding affinity for compounds **2** and **3** is most likely due to the loss of hydrogen bond donation and not from steric hindrance. Furthermore, it was determined that these compounds are TP receptor antagonists as determined by platelet aggregation assays. Therefore, our results indicate that a hydrogen bond donor in the ω -side chain is necessary for high affinity binding in this class of antagonists to the TP receptor.

6. Experimental protocols

6.1. General

All solvents were HPLC grade purchased from Fischer Scientific and all chemicals were purchased from Sigma-Aldrich unless otherwise noted. THF was distilled from Na/benzophenone. HMPA was distilled at 5 mmHg from CaH_2 , TFA was straight distilled, ether was distilled from LiAlH_4 , pyridine CH_2Cl_2 , toluene and CH_3CN were distilled from CaH_2 prior to use. Solutions were evaporated under reduced pressure with a rotary evaporator and products were purified by chromatography on silica gel columns (60 Å, 200–425 mesh) using ethyl acetate/hexanes as the eluent. Analytical thin-layer chromatography was performed on precoated silica gel plates (0.25 mm thickness 60F 254 Merck) and was visualised under a UV lamp and by staining with either iodine vapour, *p*-anisaldehyde, potassium permanganate, ninhydrin, or ammonium molybdate. Preparative thin-layer chromatography was performed on precoated silica gel plates (1.0 mm thickness 60F 254 Merck). The ^1H -NMR spectra were obtained on a Varian Unity INOVA 400 MHz or a Varian 300 MHz Mercury-VXR spectrometer at ambient temperature. The ^{13}C -NMR spectra were obtained on a Varian Unity INOVA or Varian Mercury-VXR spectrometer at 100 and 75 MHz,

respectively. Melting points were determined on a Mel-Temp capillary melting point apparatus and are uncorrected. Infrared absorption spectra were obtained on a Mattson Genesis II with a Golden-gate ATR.

6.2. [2,3 α]-2-Benzylloxymethyl-6,6-dimethyl-bicyclo[3.1.1]heptan-3-ol (**5**)

To a stirred solution of 1.0 g (3.68 mmol) of ketone **4** in 12.0 mL of ether at -78°C was added 2.6 mL (7.36 mmol) of a 2.8 M DIBALH solution in toluene and the mixture was allowed to stir at -78°C for 24 h. The reaction mixture was quenched by the slow addition of 1 N HCl while at -78°C and then warmed to rt. At this time, additional 1 N HCl was added until the solution became clear. The mixture was extracted with ethyl acetate and the combined organic layers were washed with 1 N HCl (3×10 mL) and a saturated NaCl solution (3×10 mL). The solvent was removed under reduced pressure to afford 0.96 g (98%) of a colourless oil in a 20:1 mixture of α : β alcohols. No further purification was done. TLC (5% THF/hexanes, $R_f = 0.22$): IR (neat) 3456, 2912, 1494, 1460, 1364, 1102, and 1054 cm^{-1} ; ^1H -NMR (CDCl_3 , 400 MHz) δ 1.02 (s, 3H), 1.05 (d, 1H, $J = 10.0$ Hz), 1.15 (s, 3H), 1.24–1.31 (m, 1H), 1.77–1.83 (m, 1H), 1.86–1.93 (m, 2H), 2.16–2.25 (m, 1H), 2.30–2.36 (m, 1H), 2.44 (ddd, 1H, $J = 13.6, 10.0, 3.6$ Hz), 2.49–2.60 (m, 1H), 3.35 (ddd, 1H, $J = 10.7, 8.4, 2.8$ Hz), 3.67 (ddd, 1H, $J = 8.4, 4.0, 2.8$ Hz), 3.87 (s, 1H), 4.42 (ddd, 1H, $J = 10.0, 10.0, 5.2$ Hz), 4.52 (d, 1H, $J_{AB} = 12.0$ Hz), 4.56 (d, 1H, $J_{AB} = 12.0$ Hz) and 7.28–7.37 (m, 5H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 22.5, 27.8, 30.1, 30.9, 36.6, 38.6, 40.6, 47.4, 48.9, 62.8, 72.6, 73.5, 127.7, 127.9, 128.6 and 137.6.

6.3. [2 α ,3 β]-3-Azido-2-(2-benzylloxyethyl)-6,6-dimethylbicyclo[3.1.1]heptane (**6**)

To a stirred solution of 0.21 g (0.77 mmol) of alcohol **5** in 2.0 mL of CH_2Cl_2 at -24°C was added 83.0 μL (1.1 mmol) of mesyl chloride dropwise and the mixture was allowed to stir for 30 min at -24°C . The progress of the reaction was followed by TLC (5% THF/hexanes, $R_f = 0.11$) and upon completion, quenched by the addition of ice. Note: maintaining the temperature of the work-up procedure at, or below, 0°C reduces decomposition of the mesylate. The reaction mixture was extracted with cold ethyl acetate, washed with cold 1 N HCl (3×10 mL) and cold saturated NaCl solution (3×10 mL), dried over MgSO_4 and the solvent was removed under reduced pressure. The crude mesylate was carried onto the next reaction without further purification to limit decomposition.

To a stirred solution of 0.25 g (3.8 mmol) of NaN_3 in 1.5 mL of HMPA was added via cannula to the crude mesylate dissolved in 0.5 mL HMPA. The mixture was

heated at 53 °C for 30 min and allowed to cool to rt. The reaction mixture was diluted with ethyl acetate, washed with H₂O (3 × 10 mL) and a saturated NaCl solution (3 × 10 mL), dried over MgSO₄ and the solvent was removed under reduced pressure. The crude residue was subjected to flash chromatography (5% THF/hexanes, *R_f* = 0.15) to afford 0.11 g (49%) of azide **6** as a colourless oil: IR (neat) 2919, 2814, 2093, 1639, 1494, 1453, 1253, 1102 cm⁻¹; ¹H-NMR (CDCl₃, 400 MHz) δ 0.91 (s, 3H), 0.94 (d, 1H, *J* = 10.0 Hz), 1.18 (s, 3H), 1.72 (ddt, 1H, *J* = 14.0, 14.0, 6.8 Hz), 1.69–1.77 (m, 4H), 2.01–2.08 (m, 1H), 2.33–2.40 (m, 1H), 2.44–2.51 (m, 1H), 3.49–3.54 (m, 2H), 3.67 (ddd, 1H, *J* = 10.4, 5.2, 5.2 Hz), 4.47 (d, 1H, *J_{AB}* = 12.0 Hz), 4.50 (d, 1H, *J_{AB}* = 12.0 Hz) and 7.25–7.36 (m, 5H); ¹³C-NMR (CDCl₃, 100 MHz) δ 23.8, 27.7, 33.7, 35.0, 36.2, 38.4, 41.3, 45.5, 46.1, 59.9, 68.8, 73.2, 127.8, 127.9, 128.6 and 138.6.

6.4. [2α,3β]-2-Benzylloxymethyl-6,6-dimethylbicyclo[3.1.1]hept-3-ylamine (**7**)

To a slurry of 11.0 mg of palladium on carbon (10% wt on activated carbon) in 4.0 mL of ethanol was added a solution of 0.11 g (0.37 mmol) of azide **6** in 1.0 mL of ethanol via cannula under an atmosphere of N₂. The reaction was allowed to stir under an atmosphere of H₂ for 40 min, and then purged with N₂ before the mixture was passed through a layer of celite. The ethanol was removed under reduced pressure and the crude mixture was purified by preparative TLC (5% NH₄OH/10% methanol/CHCl₃, *R_f* = 0.28) to afford 87.0 mg (98%) of the amine as a white solid: mp = 35–36 °C; IR (neat) 2926, 1577, 1494, 1453, 1364, 1102 and 1026 cm⁻¹; ¹H-NMR (CDCl₃, 400 MHz) δ 0.92 (s, 3H), 0.97 (d, 1H, *J* = 9.6 Hz), 1.18 (s, 3H), 1.52 (ddd, 1H, *J* = 13.6, 5.2, 2.4 Hz), 1.64–1.92 (m, 7H), 2.29–2.39 (m, 1H), 2.39–2.46 (m, 1H), 3.16 (ddd, 1H, *J* = 10.0, 5.2, 5.2 Hz), 3.48–3.59 (m, 2H), 4.50 (s, 2H), and 7.24–7.34 (m, 5H); ¹³C-NMR (CDCl₃, 100 MHz) δ 23.6, 27.9, 34.0, 36.2, 38.2, 39.2, 41.9, 46.4, 49.5, 50.8, 69.5, 73.2, 127.8, 127.9, 128.6 and 138.5.

6.5. [2α,3β]-N-[2-(2-Benzylloxyethyl)-6,6-dimethylbicyclo[3.1.1]hept-3-yl]-p-toluene sulphonamide (**8**)

To a stirred solution of 64.0 mg (0.23 mmol) of amine **7** in 2.0 mL of pyridine was added 90.0 mg (0.47 mmol) of tosyl chloride. The reaction was allowed to stir for 12 h at rt before 9 N HCl was added until the solution was a pH 1 as determined by pH paper. The mixture was extracted with ethyl acetate (3 × 10 mL) and the combined organic layers were washed with 1 N HCl (3 × 10 mL) and a saturated NaHCO₃ solution (3 × 10 mL), dried over MgSO₄ and the solvent was removed under reduced pressure. The crude mixture was purified

by flash chromatography (20% ethyl acetate/hexanes, *R_f* = 0.59) and recrystallised from hexanes to afford 87.0 mg (87%) of sulphonamide **8** as white needles: mp = 126–128 °C; IR (neat) 3268, 2912, 1598, 1453, 1329, 1159, 1039, and 814 cm⁻¹; ¹H-NMR (CDCl₃, 400 MHz) δ 0.84 (d, 1H, *J* = 10.0 Hz), 0.88 (s, 3H), 1.15 (s, 3H), 1.55–1.70 (m, 3H), 1.83–1.89 (m, 3H), 2.22–2.34 (m, 2H), 2.42 (s, 3H), 3.39 (t, 2H, *J* = 6.4 Hz), 3.57 (dddd, 1H, *J* = 12.8, 12.8, 8.9, 6.4 Hz), 4.42 (d, 1H, *J_{AB}* = 12.0 Hz), 4.48 (d, 1H, *J_{AB}* = 12.0 Hz), 4.89 (d, 1H, *J* = 6.8 Hz), 7.29 (d, 1H, *J* = 8.0 Hz), 7.24–7.34 (m, 5H) and 7.78 (d, 2H, *J* = 8.0 Hz); ¹³C-NMR (CDCl₃, 100 MHz) δ 21.8, 23.6, 27.9, 34.6, 35.1, 37.8, 38.3, 41.6, 45.7, 47.3, 51.8, 68.1, 72.9, 127.4, 127.8, 128.0, 128.6, 129.8, 138.3, 138.5 and 143.4; HRMS (direct probe) 427.2179 observed (427.2181 calculated).

6.6. [2α,3β]-N-[2-(2-Benzylloxyethyl)-6,6-dimethylbicyclo[3.1.1]hept-3-yl]-N-methyl-p-toluene sulphonamide (**9**)

To a slurry of 72.0 mg (1.8 mmol) of KH in 4.0 mL of THF was added a solution 0.26 g (0.6 mmol) of sulphonamide **8** dissolved in 2.0 mL THF via cannula. The reaction was allowed to stir for 1 h at rt after which 0.51 g (3.6 mmol) of iodomethane was added. The mixture was monitored by TLC (20% ethyl acetate/hexanes, *R_f* = 0.49). The reaction mixture was quenched with 1 N HCl and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with 1 N HCl (3 × 10 mL) and a saturated NaHCO₃ solution (3 × 10 mL), dried over MgSO₄ and the solvent removed under reduced pressure. The crude mixture was purified by flash chromatography (20% ethyl acetate/hexanes), and recrystallised from hexanes to afford 0.26 g (97%) of the N-methyl sulphonamide **9** as a white solid: mp = 94–96 °C; IR (neat) 2924, 1617, 1494, 1454, 1337, 1153, 1090, 971, 861, 742 and 663 cm⁻¹; ¹H-NMR (CDCl₃, 400 MHz) 80.78 (d, 1H, *J* = 10.0 Hz), 1.02 (s, 3H), 1.17 (s, 3H), 1.38 (ddd, 1H, *J* = 13.6, 7.2, 2.4 Hz), 1.63–1.73 (m, 1H), 1.77–1.89 (m, 2H), 1.95–2.13 (m, 3H), 2.25–2.32 (m, 1H), 2.41 (s, 3H), 2.80 (s, 3H), 3.57 (t, 2H, *J* = 6.4 Hz), 4.43–4.56 (m, 3H), 7.28 (d, 2H, *J* = 8.2 Hz), 7.30–7.35 (m, 5H) and 7.73 (d, 2H, *J* = 8.2 Hz); ¹³C-NMR (CDCl₃, 100 MHz) δ 21.8, 23.6, 28.2, 28.9, 30.1, 33.6, 34.8, 38.8, 41.5, 42.2, 45.2, 55.8, 68.7, 72.8, 127.4, 127.6, 127.9, 128.5, 129.7, 137.4, 138.9, and 143.1; HRMS (direct probe) 441.2354 observed (441.2338 calculated).

6.7. [2α,3β]-N-[2-(2-Benzylloxyethyl)-6,6-dimethylbicyclo[3.1.1]hept-3-yl]-N-ethyl-p-toluene sulphonamide (**10**)

Substitution of KH (10.0 mg, 0.28 mmol), in 1.5 mL THF, 0.10 g (0.34 mmol) of sulphonamide **8** dissolved in

0.5 THF and 54.0 mg (0.34 mmol) of iodoethane for the reaction procedure outlined for compound **9**, afforded 79.0 mg (92%) of the *N*-ethyl sulphonamide **10** as a white solid: mp = 75–77 °C; R_f 0.57, (20% ethyl acetate/hexanes); IR (neat) 2988, 2919, 2871, 1722, 1597, 1460, 1336, 1157, 1102, 1005 and 950 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.81 (m, 1H), 0.93 (s, 3H), 1.17 (s, 3H), 1.46 (t, 3H, J = 7.4 Hz), 1.52–1.63 (m, 3H), 1.77–2.11 (m, 4H), 2.28–2.36 (m, 1H), 2.41 (s, 3H), 3.08 (dq, 1H, J = 15.1, 7.4 Hz), 3.37 (dq, 1H, J = 15.1, 7.4 Hz), 3.53 (t, 2H, J = 6.2 Hz), 4.34 (dt, 1H, J = 9.5, 8.1 Hz), 4.43 (d, 1H, J_{AB} = 12.0 Hz), 4.48 (d, 1H, J_{AB} = 12.0 Hz), 7.27 (d, 2H, J = 8.0 Hz), 7.30–7.35 (m, 5H) and 7.76 (d, 2H, J = 8.0 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 17.9, 21.5, 23.1, 28.1, 29.8, 32.3, 33.8, 33.9, 39.3, 41.4, 43.2, 44.5, 57.1, 68.3, 72.6, 127.38, 127.40, 127.6, 128.3, 129.5, 138.2, 138.7, and 142.8; HRMS (direct probe) 455.2483 observed (455.2494 calculated).

6.8. $[2\alpha,3\beta]$ -(6,6-Dimethyl-3-*p*-tolylamino-bicyclo[3.1.1]hept-2-yl)-methanol (**11**)

To a slurry of 50.0 mg of palladium on carbon (10% wt on activated carbon) in 9.0 mL of ethanol was added a solution of 0.44 g (1.0 mmol) of sulphonamide **8** dissolved in 1.0 mL of ethanol via cannula under an atmosphere of N_2 . The reaction was allowed to stir under an atmosphere of H_2 at rt for 24 h. The H_2 gas was removed and the mixture was purged with an atmosphere of N_2 before it was passed through a layer of celite. The ethanol was removed under reduced pressure and the crude mixture was purified by recrystallisation from 5% ethyl acetate/hexanes (TLC 20% ethyl acetate/hexanes, R_f = 0.11) to afford 34.0 mg (95%) of the corresponding alcohol **11** as a white solid: mp = 141–143 °C; IR (neat) 3280–3497, 2928, 1714, 1610, 1451, 1325, 1156, 1092, 814 and 668 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.82 (s, 3H), 0.86 (d, 1H, J = 9.6 Hz), 1.09 (s, 3H), 1.45–1.74 (m, 4H), 1.81–1.85 (m, 1H), 1.93–1.99 (m, 1H), 2.06 (dt, 1H, J = 12.0, 10.8 Hz), 2.24 (dt, 1H, J = 7.2, 6.4 Hz), 2.36 (s, 3H), 3.16 (s, 1H), 3.48–3.60 (m, 3H), 6.02 (d, 1H, J = 7.6 Hz), 7.25 (d, 2H, J = 8.0 Hz), and 7.78 (d, 2H, J = 8.0 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 21.7, 23.6, 27.9, 34.5, 37.5, 37.6, 38.3, 41.6, 45.2, 46.4, 51.8, 60.2, 127.4, 129.8, 138.4 and 143.4.

6.9. $[2\alpha,3\beta]$ -*N*-[6,6-Dimethyl-2-(2-oxoethyl)bicyclo[3.1.1]hept-3-yl]-*p*-toluene sulphonamide (**14**)

To a stirred solution of 0.20 g (0.6 mmol) of alcohol **11** and 0.2 g (4 Å) powdered molecular sieves in 10.0 mL of CH_2Cl_2 was added 0.11 g (0.92 mmol) of NMO. The reaction was allowed to stir for 10 min at rt, after this time, 11.0 mg (0.031 mmol) TPAP was added. The

reaction mixture was stirred for 1 h and the CH_2Cl_2 was removed under reduced pressure. The crude mixture was purified by flash chromatography (20% ethyl acetate/hexanes, R_f = 0.51) and recrystallised from 5% ethyl acetate/hexanes to afford 0.18 (92%) of aldehyde **14** as a white solid: mp = 131–133 °C; IR (neat) 3281, 2927, 1718, 1598, 1448, 1330, 1156, 1091, 1061, 814, and 668 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.90 (s, 3H), 0.91 (d, 1H, J = 10.8 Hz), 1.15 (s, 3H), 1.20–1.24 (m, 1H), 1.59 (ddd, 2H, J = 14.0, 6.0, 2.4 Hz), 1.78–1.87 (m, 1H), 2.18 (tm, 2H, J = 11.8 Hz), 2.42 (s, 3H), 2.48 (ddd, 1H, J = 17.6, 8.8, 2.4 Hz), 3.51 (ddt, 1H, J = 12.0, 8.0, 4.8 Hz), 5.04 (d, 1H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.2 Hz), 7.76 (d, 2H, J = 8.2 Hz), and 9.59 (d, 1H, J = 2.0 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 21.8, 23.6, 27.8, 34.9, 37.7, 38.3, 41.4, 44.7, 45.6, 49.4, 51.4, 127.3, 129.9, 138.0, 143.4 and 202.1; $\text{M}+\text{H}$ HRMS (electrospray) 336.1622 observed (336.1633 calculated).

6.10. $[2\alpha,3\beta]$ -[6,6-Dimethyl-3-(methyl-*p*-tolyl-amino)-bicyclo[3.1.1]hept-2-yl]-methanol (**12**)

The reaction procedure described for alcohol **11** was used on sulphonamide **9** (of 0.26 g, 0.59 mmol) to yield alcohol **12** (0.20, 98%) as a colourless oil: TLC R_f = 0.07 (20% ethyl acetate/hexanes), IR (neat) 3406 (br), 2918, 1686, 1620, 1457, 1338, 1151, 1088 and 912 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) 80.81 (d, 1H, J = 10.0 Hz), 1.02 (s, 3H), 1.18 (s, 3H), 1.37–1.43 (m, 2H), 1.65–1.75 (m, 1H), 1.78–2.0 (m, 3H), 2.05–2.13 (m, 2H), 2.27–2.35 (m, 1H), 2.42 (s, 3H), 2.80 (s, 3H), 3.62–3.70 (m, 1H), 4.49–4.56 (m, 1H), 7.29 (d, 2H, J = 8.4 Hz), and 7.74 (d, 2H, J = 8.4 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 21.6, 23.4, 27.9, 28.6, 29.6, 33.3, 37.8, 38.6, 41.3, 41.7, 45.0, 55.4, 60.9, 127.2, 129.5, 136.8 and 143.3.

6.11. $[2\alpha,3\beta]$ -*N*-[6,6-Dimethyl-2-(2-oxoethyl)bicyclo[3.1.1]hept-3-yl]-*N*-methyl-*p*-toluene sulphonamide (**15**)

Following the procedure for the aldehyde **14**, oxidation of alcohol **12** (0.10 g, 0.28 mmol) gave 77.0 mg (77%) of aldehyde **15** as a white foam: R_f = 0.30 (20% ethyl acetate/hexanes); IR (neat) 2985, 1720, 1598, 1454, 1335, 1110, 970, 815 and 753 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.81 (d, 1H, J = 10.0 Hz), 1.04 (s, 3H), 1.17 (s, 3H), 1.46 (ddd, 1H, J = 13.6, 7.2, 2.0 Hz) 1.70–1.79 (m, 1H), 1.80–1.85 (m, 1H), 1.90 (td, 1H, J = 5.8, 1.6 Hz), 2.31–2.38 (m, 1H), 2.42 (s, 3H), 2.51–2.58 (m, 1H), 2.63 (ddd, 1H, J = 17.2, 10.0, 2.0 Hz), 2.87 (s, 3H), 2.89 (dd, 1H, J = 17.6, 3.0 Hz), 4.46 (dd, 1H, J = 18.0, 7.7 Hz), 7.30 (d, 2H, J = 8.4 Hz), 7.70 (d, 2H, J = 8.4 Hz), and 9.78 (d, 1H, J = 2.0 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 21.9, 23.5, 28.1, 28.9, 29.2, 34.1, 38.8, 40.0, 41.4, 45.4, 48.8, 54.6, 127.3, 129.9, 136.9, 143.4 and 202.0;

M+H HRMS (electrospray) 350.1795 observed (350.1790 calculated).

6.12. *[2 α ,3 β]-[3-(Ethyl-*p*-tolyl-amino)-6,6-dimethyl-bicyclo[3.1.1]hept-2-yl]-methanol (13)*

The reaction procedure described for alcohol **11** was used on sulphonamide **10** (91.0 mg, 0.20 mmol) to yield alcohol **13** (64.0 mg, 88%) as a colourless oil: R_f = 0.11 (20% ethyl acetate/hexanes); IR (neat) 3439 (br), 2929, 1598, 1466, 1332, 1151, 1088 and 1000 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.83 (d, 1H, J = 10.4 Hz), 0.98 (s, 3H), 1.16 (s, 3H), 1.22 (t, 3H, J = 7.3 Hz), 1.30–1.37 (m, 3H), 1.58–1.64 (m, 1H), 1.74–2.10 (m, 3H), 2.29–2.35 (m, 1H), 2.40 (s, 3H), 3.11 (dq, 1H, J = 14.9, 7.3 Hz), 3.33 (dq, 1H, J = 14.9, 7.3 Hz), 3.59–3.74 (m, 3H), 4.33 (q, 1H, J = 8.5 Hz), 7.26 (d, 2H, J = 7.6 Hz), and 7.73 (d, 2H, J = 7.6 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 18.2, 21.7, 23.3, 28.3, 32.2, 34.0, 37.3, 39.3, 39.5, 41.5, 43.0, 44.8, 57.3, 61.0, 127.6, 129.8, 138.2 and 143.3.

6.13. *[2 α ,3 β]-N-[6,6-dimethyl-2-(2-oxoethyl)bicyclo[3.1.1]hept-3-yl]-4-methylbenzene sulphonamide (16)*

Following the procedure for aldehyde **14**, the oxidation of alcohol **13** (60.0 mg, 0.16 mmol) gave 56.0 mg (97%) of aldehyde **16** as a white foam: R_f = 0.35 (20% ethyl acetate/hexanes); IR (neat) 2929, 1724, 1608, 1474, 1330, 1169, 1087, 814, 732, and 662 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.89 (d, 1H, J = 10.8 Hz), 0.99 (s, 3H), 1.15 (s, 3H), 1.32 (t, 2H, J = 7.2 Hz), 1.35–1.41 (m, 2H), 1.72–1.87 (m, 3H), 2.31–2.38 (m, 1H), 2.41 (s, 3H), 2.52–2.62 (m, 2H), 3.11 (dq, 1H, J = 14.6, 7.2 Hz), 3.36 (dq, 1H, J = 14.6, 7.2 Hz), 4.31 (q, 1H, J = 8.3 Hz), 7.27 (d, 2H, J = 8.2 Hz), 7.72 (d, 2H, J = 8.2 Hz), and 9.72 (d, 1H, J = 1.2 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 18.3, 21.7, 23.2, 28.2, 31.7, 34.5, 39.2, 39.4, 40.6, 41.4, 45.4, 48.3, 55.9, 127.4, 129.9, 138.2, 143.4 and 202.1; M+H HRMS (electrospray) 364.1936 observed (364.1946 calculated).

6.14. *[2 α ,3 β ,5 α]-7-[6,6-Dimethyl-3-(toluene-4-sulphonylamino)bicyclo[3.1.1]hept-5-enoic acid (1)*

To a stirred solution of 0.28 g (0.63 mmol) of 4-carboxybutyl triphenyl phosphonium bromide in 8.0 mL THF was added 1.0 mL of 1.0 M (1.0 mmol) of *tert*-BuOK in pentane. The mixture was allowed to stir under an atmosphere of Ar for 1 h at rt. A solution of 70 mg (0.21 mmol) of aldehyde **14** dissolved in 1.0 mL THF was added via cannula. The reaction mixture was allowed to stir for 1 h and was quenched by the addition of 1 N HCl (3 \times 10 mL). The crude mixture was extracted with ether and the combined organics were washed with 1 N HCl (3 \times 10 mL) and a saturated NaCl

solution (3 \times 10 mL), dried over MgSO_4 and the solvent was removed under reduced pressure. The mixture was purified by preparative TLC (20% ethyl acetate/1% acetic acid/hexanes, R_f = 0.28) to afford 68.0 mg (77%) of olefin **1** as a yellow oil: IR (neat) 3267, 2922, 1707, 1618, 1441, 1327, 1304, 1245, 1154, 1099, 1020, 999, 956 and 913 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.78 (d, 1H, J = 13.2 Hz), 0.92 (s, 3H), 1.15 (s, 3H), 1.19–1.25 (m, 1H), 1.43–2.30 (m, 12H), 2.34 (t, 2H, J = 9.6 Hz), 2.42 (s, 3H), 3.57 (quintet, 1H, J = 10.6 Hz), 5.11 (d, 1H, J = 11.2 Hz), 5.25–5.37 (m, 2H), 7.30 (d, 2H, J = 10.8 Hz), and 7.79 (d, 2H, J = 10.8 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) 821.8, 23.6, 24.9, 26.6, 28.0, 31.4, 33.6, 35.1, 38.2, 38.4, 41.8, 41.8, 43.7, 51.8, 52.0, 127.3, 129.2, 129.9, 130.0, 138.6, 143.5 and 179.1; HRMS (direct probe) 419.2137 observed (419.2130 calculated).

6.15. *[2 α ,3 β ,5 α]-7-(6,6-Dimethyl-3-[methyl(toluene-4-sulphonylamino)bicyclo[3.1.1]hept-2-yl]hept-5-enoic acid (2)*

The reaction procedure described for olefin **1** was used on aldehyde **15** (0.64 g, 0.18 mmol) to give olefin **2** (53.0 mg, 68%) as a yellow oil: R_f = 0.34 (40% ethyl acetate/1% acetic acid/hexanes); IR (neat) 3385 br, 2921, 2367, 1707, 1606, 1458, 1401, 1333, 1152, 1025, 968, 814, 767, and 662 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.75 (d, 1H, J = 10.0 Hz), 1.04 (s, 3H), 1.16 (s, 3H), 1.39–1.46 (m, 1H), 1.64–1.72 (m, 2H), 1.78–1.85 (m, 2H), 1.92–2.21 (m, 2H), 2.35 (t, 2H, J = 7.6 Hz), 2.41 (s, 3H), 2.79 (s, 3H), 4.39–4.50 (m, 1H), 5.31–5.41 (m, 2H), 7.28 (d, 2H, J = 8.2 Hz), and 7.71 (d, 2H, J = 8.2 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 21.5, 23.3, 24.6, 26.5, 27.8, 28.5, 29.4, 31.3, 33.47, 33.51, 38.6, 41.4, 43.3, 46.4, 55.2, 127.2, 129.47, 129.50, 137.0, 143.0 and 179.5; HRMS (direct probe) 433.2296 observed (433.2287 calculated).

6.16. *[2 α ,3 β ,5 α]-7-(3-Ethyl-(toluene-4-sulphonyl)amino-6,6-dimethylbicyclo[3.1.1]hept-2-yl]hept-5-enoic acid (3)*

The reaction procedure described for olefin **1** was used on aldehyde **16** (20.0 mg, 0.055 mmol) to give olefin **3** (17.0 mg, 70% yield) as a yellow oil: R_f = 0.28 (20% ethyl acetate/5% acetic acid/hexanes); IR (neat) 3452 br, 2923, 1707, 1608, 1463, 1334, 1152, 1088, 951, 814, 735 and 665 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 0.75 (d, 1H, J = 10.0 Hz), 1.04 (s, 3H), 1.16 (s, 3H), 1.39–1.46 (m, 1H), 1.64–1.72 (m, 2H), 1.78–1.85 (m, 2H), 1.92–2.21 (m, 2H), 2.35 (t, 2H, J = 7.6 Hz), 2.41 (s, 3H), 2.79 (s, 3H), 4.39–4.50 (m, 1H), 5.31–5.41 (m, 2H), 7.28 (d, 2H, J = 8.2 Hz), and 7.71 (d, 2H, J = 8.2 Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ 18.2, 21.1, 23.1, 24.8, 26.8, 28.2, 31.3, 32.5, 33.5, 34.4, 39.2, 39.3, 41.8, 43.7, 47.6, 56.9, 129.6, 129.9, 130.2, 131.0, 139.5, 139.6 and 179.9;

HRMS (direct probe) 447.2446 observed (447.2443 calculated).

Acknowledgements

This work was supported in part through grants HL36838 and RR1070 to P.V.H., M.D., Ph.D.

References

- [1] L.M. Lima, C.B. Ormelli, F.F. Brito, A.L.P. Miranda, C.A.M. Fraga, E.J. Barreiro, Synthesis and antiplatelet evaluation of novel aryl-sulfonamide derivatives, from natural safrole, *Pharm. Acta Hel.* 73 (6) (1999) 281–292.
- [2] M. Hamberg, J. Svensson, B. Samuelsson, Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides, *Proc. Natl. Acad. Sci. USA* 72 (8) (1975) 2994–2998.
- [3] R.-F. Shen, H.-H. Tai, Thromboxanes: synthase and receptors, *J. Biomed. Sci.* 5 (1998) 153–172.
- [4] P.V. Halushka, Thromboxane A₂ receptors: Where have you gone?, *Prostaglandins Other Lipid Mediators* 60 (2000) 175–189.
- [5] Y. Djellas, K. Antonakis, G.C. Le Breton, Shifts in the affinity distribution of one class of seven-transmembrane receptors by activation of a separate class of seven-transmembrane receptors, *Biochem. Pharm.* 59 (2000) 1521–1530.
- [6] Y. Yamamoto, K. Kamiya, S. Terao, Modeling of human thromboxane A₂ receptor and analysis of the receptor–ligand interaction, *J. Med. Chem.* 36 (1993) 820–825.
- [7] B. Jin, A.J. Hopfinger, A proposed common spatial pharmacophore and the corresponding active conformations of some TXA₂ receptor antagonists, *J. Chem. Inf. Comput. Sci.* 34 (1994) 1014–1021.
- [8] M.G. Albuquerque, A.J. Hopfinger, E.J. Barreiro, R.B. de Alencastro, Four-dimensional quantitative structure–activity relationship analysis of a series of interphenylene 7-oxabicycloheptane oxazole thromboxane A₂ receptor antagonists, *J. Chem. Inf. Comput. Sci.* 38 (1998) 925–938.
- [9] N. Chiang, W.M. Kan, H.-H. Tai, Site-directed mutagenesis of cysteinyl and serine residues of human thromboxane A₂ receptor in insect cells, *Arch. Biochem. Biophys.* 334 (1) (1996) 9–17.
- [10] C.D. Funk, L. Furci, N. Moran, G.A. Fitzgerald, Point mutation in the seventh hydrophobic domain of the human thromboxane A₂ receptor allows discrimination between agonist and antagonist binding sites, *Molec. Pharmacol.* 44 (1993) 934–939.
- [11] M.-T. Walsh, J.F. Foley, T. Kinsella, Characterization of the role of N-linked glycosylation on the cell signaling and expression of the human thromboxane A₂ receptor alpha and beta isoforms, *J. Pharmacol. Exp. Ther.* 286 (1998) 1026–1036.
- [12] H. Zhou, F. Yan, S. Yamamoto, H.-H. Tai, Phenylalanine 138 in the second intracellular loop of human thromboxane receptor is critical for receptor-G-protein coupling, *Biochem. Biophys. Res. Commun.* 264 (1999) 171–175.
- [13] M. Hirata, A. Kakizuka, F. Ushikubi, I. Fuse, M. Okuma, S. Narumiya, Arg⁶⁰ to Leu mutation of the human thromboxane A₂ receptor in a dominantly inherited bleeding disorder, *J. Clin. Invest.* 94 (1994) 1662–1667.
- [14] M. Unoki, S. Furuta, Y. Onouki, O. Watanabe, S. Doi, H. Fujiwara, A. Miyatake, K. Fujita, M. Tamari, Y. Nakamura, Association studies of 33 single nucleotide polymorphisms (SNPs) in 29 candidate genes for bronchial asthma: positive association a T924C polymorphism in the thromboxane A₂ receptor gene, *Hum. Gen.* 106 (2000) 440–446.
- [15] M. Narisada, M. Ohtani, F. Watanabe, K. Uchida, H. Arita, M. Doteuchi, K. Hanasaki, H. Kakushi, K. Otani, S. Hara, Synthesis and in vitro activity of various derivatives of a novel thromboxane receptor antagonist, (+/–)-(5*Z*)-7-[3-endo-[(phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-exo-yl]heptenoic acid, *J. Med. Chem.* 31 (1988) 1847–1854.
- [16] N. Hamanaka, T. Seko, T. Miyazaki, M. Naka, The synthesis of potent thromboxane A₂/prostaglandin H₂ endoperoxide receptor antagonist, *Tetrahedron Lett.* 30 (18) (1989) 2399–2402.
- [17] S.E. Hall, W.-C. Han, D.N. Harris, A. Hedberg, M.L. Ogletree, *J. Med. Chem.* 32 (1989) 974.
- [18] R.N. Misra, B.R. Brown, P.M. Sher, M.M. Patel, S.E. Hall, W.-C. Han, J.C. Barrish, O. Kocy, D.N. Harris, H.J. Goldenberg, I.M. Michel, W.A. Schumacher, H. Monshizadegan, M.E. Ogletree, Interphenylene-7-oxabicyclo[2.2.1]heptane oxazoles. Highly potent, selective, and long-acting thromboxane A₂ receptor antagonists, *J. Med. Chem.* 36 (10) (1993) 1401–1417.
- [19] M. Sato, Y. Kawashima, J. Goto, Y. Yamane, Y. Chiba, S. Jinno, M. Satake, C. Iwata, Synthesis and evaluation of novel fluorinated sulotroban-related sulfonamide derivatives as thromboxane A₂ receptor antagonists, *Eur. J. Med. Chem.* 30 (1995) 403–413.
- [20] S.E. Hall, Thromboxane A₂ receptor antagonists, *Med. Res. Rev.* 11 (5) (1991) 503–579.
- [21] D.E. Mais, C. Dunlap, N. Hamanaka, P.V. Halushka, Further studies on the effects of epimers of thromboxane A₂ antagonists on platelets and veins, *Eur. J. Pharmacol.* 111 (1985) 125–128.
- [22] M. Takasuka, M. Yamakawa, M. Ohtani, FTIR spectral study of intramolecular hydrogen bonding in a thromboxane A₂ receptor antagonist S-145 and related compounds, *J. Chem. Soc. Perkin Trans. 2* (1989) 1173–1179.
- [23] M. Takasuka, M. Yamakawa, M. Ohtani, FTIR spectral study of intramolecular hydrogen bonding in thromboxane A₂ receptor antagonist S-145 and related compounds. Part 2, *J. Chem. Soc. Perkin Trans. 2* (1990) 1467–1479.
- [24] M. Takasuka, M. Yamakawa, M. Ohtani, FTIR spectral study of intramolecular hydrogen bonding in thromboxane A₂ receptor antagonist S-145 and related compounds. 3. Conformation and activity of S-145 analogs, *J. Med. Chem.* 34 (1991) 1885–1891.
- [25] J.M. Langenhan, J.D. Fisk, S.H. Gellman, Evaluation of hydrogen bonding complementarity between a secondary sulfonamide and an α -amino acid, *Org. Lett.* 3 (16) (2001) 2559–2562.
- [26] S.E. Hall, W.-C. Han, D.N. Harris, A. Hedberg, M.L. Ogletree, 9,11-Epoxy-9-homo-14-thiaprost-5-enoic acid derivatives: potent thromboxane A₂ antagonists, *J. Med. Chem.* 32 (1989) 974–984.
- [27] K. Seno, S. Hagishita, Thromboxane A₂ receptor antagonists. III. Synthesis and pharmacological activity of 6,6-dimethylbicyclo[3.1.1]heptane derivatives with a substituted sulfonyl amino group at C-2, *Chem. Pharm. Bull.* 37 (6) (1989) 1524–1533.
- [28] W.P. Griffith, S.V. Ley, TPAP: tetra-*n*-propylammonium per-ruthenate, a mild and convenient oxidant for alcohols, *Aldrichim. Acta* 23 (1990) 13–19.
- [29] D. Komiotis, S. Pananookooln, K. Zaw, J.P. Dieter, G.C. Le Breton, D.L. Venton, Synthesis of DL-*cis*- and (4*R*,5*R*)-*trans*-7-2,2-dimethyl-4-(phenylsulfonyl)-aminomethyl-1,3-dioxolan-5-yl]-5(*Z*)-heptenoic acid analogues as platelet thromboxane A₂ receptor antagonists, *Eur. J. Med. Chem.* 30 (1995) 321–326.
- [30] P.R. Mayeux, T.A. Morinelli, T.C. Williams, E.S. Hazard, D.E. Mais, J.E.J. Oatis, D.A. Baron, P.V. Halushka, Differential

- effects of pH on thromboxane A_2 /prostaglandin H_2 receptor agonist and antagonist binding in human platelets, *J. Biol. Chem.* 299 (21) (1973) 13752–13758.
- [31] D.E. Mais, D. DeHoll, H. Sightler, P.V. Halushka, Different pharmacological activities for 13-azapinane thromboxane A_2 analogs in platelets and blood vessels, *Eur. J. Pharmacol.* 148 (1988) 309–315.
- [32] Y.-C. Cheng, W.H. Prusoff, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction, *Biochem. Pharm.* 22 (1973) 3099–3108.
- [33] J. Mitchel, P. Seeman, in: H. Kalant, W.H.E. Roschlau (Eds.), *Drug receptors. Principles of medicinal pharmacology*, 6th ed, Oxford, New York, 1998, p. 97.