Synthesis, NMR studies and conformational analysis of oxazolidine derivatives of the β-adrenoreceptor antagonists metoprolol, atenolol and timolol

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Formaldehyde-derived oxazolidine derivatives 4–7 of the β-adrenoreceptor antagonists metoprolol 1, atenolol 2 and timolol 3 have been synthesised. Conformational analysis of 1–3 and the oxazolidine derivatives 4–7 has been performed using ^{1}H NMR spectroscopy and computational methods. The ^{1}H NMR studies show that for the aryloxypropanolamine β-adrenoreceptor antagonists there is a predominance of the conformer in which the amine group is approximately antiperiplanar or *trans* to the aryloxymethylene group. Both ^{1}H NMR data and theoretical studies indicate that the oxazolidine derivatives 4–7 and the aryloxypropanolamine β-adrenoreceptor antagonists 1–3 adopt similar conformations around the β-amino alcohol moiety. Thus, oxazolidine ring formation does not dramatically alter the preferred conformation adopted by the β-amino alcohol moiety of 1–3. Oxazolidine derivatives of aryloxypropanolamine β-adrenoreceptor antagonists may therefore be appropriate as prodrugs, or semi-rigid analogues, when greater lipophilicity is required for drug delivery.

Introduction

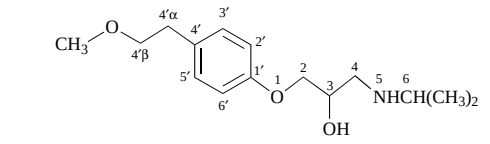
β-Adrenoreceptor antagonists are a group of compounds that competitively inhibit the effects of catecholamines at β-adrenergic receptors.[1] These agents are used widely in clinical medicine for the treatment of various conditions including hypertension,[2,3] angina pectoris,[4] cardiac arrhythmias,[5,6] hypothyroidism[7] and glaucoma.[8] As the β-adrenoreceptor antagonists (β-blockers) have such a diverse range of clinical applications, the mode of delivery of these drugs becomes crucial. In particular there has been great interest in the percutaneous transport of β-blockers for hypertension and ocular delivery for glaucoma.[8–10]

Ocular β-blockers are primary agents currently used in the treatment of glaucoma. They reduce aqueous humour production, thereby decreasing intraocular pressure and thus preventing the loss of visual fields.[10] Direct ocular application of many of these drugs causes severe irritation, presumably as a result of alkalinity produced by the strongly basic amines in an aqueous environment (*e.g.* pK_a of propranolol is 9.5).[11,12] The therapeutic usefulness of these drugs in treating glaucoma is also often limited by a relatively high incidence of cardiovascular and respiratory side effects.[10,13] These side effects arise as a result of the highly polar nature of the β-blockers and their consequent low lipophilicity which, in turn, results in poor absorption of the drugs into the eye upon topical administration. As such, large concentrations of the drugs have to be used, which ultimately results in the topically applied drug being absorbed into the systemic circulation *via* the nasolacrimal duct.[10,14,15] A potentially useful approach to decrease the systemic absorption of topically applied β-blockers, thereby diminishing adverse effects, may be the development of transient derivatives or prodrugs[16] with appropriate moieties included in their structures designed to improve corneal absorption through increased lipophilicity. This approach has been successfully applied in the past to a variety of drug classes, including β-blockers.[8,9,17–23]

In the aryloxypropanolamine class of β-blockers there are two functional groups which are obvious candidates for manipulation to produce prodrugs. They are the β-hydroxy and the amino groups. A range of β-blocker prodrugs have previously been synthesised by converting the β-hydroxy group into bioreversible derivatives such as esters.[8,9,17–21] There has also been considerable research on the conversion of the amino functional group into prodrug forms, for example carbamates.[12] As well as exhibiting chemical stability, these derivatives have been shown to have favourable lipophilic properties.

Another approach involves combining both β-hydroxy and amino functional groups into a cyclic group such as an oxazolidine ring. Oxazolidine derivatives of arylethanolamines have been synthesised as potential β_3-adrenoreceptor agonists.[24] Previous studies have been carried out on oxazolidine derivatives of (−)-ephedrine and a range of aldehydes and ketones.[25,26] These studies proposed that oxazolidines may have potential as prodrug forms for β-amino alcohols or carbonyl containing compounds. The oxazolidines are much weaker bases than the parent β-amino alcohols and this results in higher lipophilicity at physiological pH. Such increased lipophilicity may become advantageous in situations where delivery problems for the β-amino alcohol-type drugs are due to low lipophilicity, for example glaucoma.

Oxazolidines and their properties have been examined in detail in our laboratories.[27] They appear to be useful as prodrugs for β-blockers because the resulting 'masked' amines do not ionise and hence are more compatible with organic and lipophilic media. It has been demonstrated that an oxazolidine can penetrate a biological membrane from water faster than a β-amino alcohol at pH values around 7.[28] Control over the chemical stability of the oxazolidine systems can be enforced by the choice of different aldehyde moieties. In addition,



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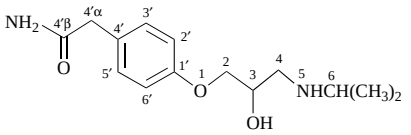

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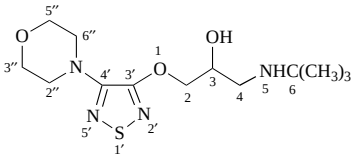

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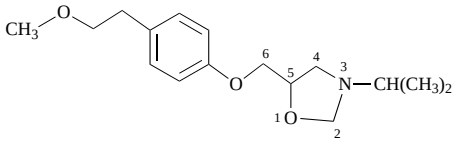

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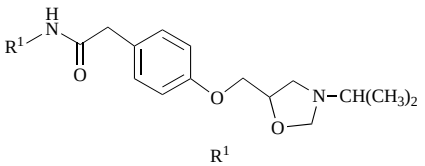

	R^1
5	H
6	CH_2OH

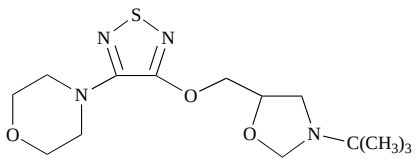

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Fig. 1 Structures for the β-blockers **1**–**3** and the oxazolidine derivatives **4**–**7**. The numbering system for the compounds is indicated.

oxazolidines can be regenerated easily to the parent drugs *via* hydrolysis.[25,27–30]

Structural properties of arylethanolamines and aryloxypropanolamines possessing β-adrenergic agonistic and/or antagonistic activity have been extensively studied using a variety of methods.[31–54] Data obtained from X-ray crystallography and quantum mechanical calculations on several β-blockers show, without exception, that the preferred conformation around the β-amino alcohol group has the hydroxy and the amino group located *gauche* to each other, irrespective of whether the amino group is charged or uncharged.[31,32,42–45,54]

^{1}H NMR spectroscopic studies on several arylethanolamines support this conclusion, indicating that the main conformation adopted in solution has a *gauche* arrangement for the β-hydroxy and amino groups.[46–48,54] The major interaction leading to the predominance of this rotamer has been postulated to be hydrogen bonding between the two groups.[48,49] These NMR studies agreed well with X-ray diffraction analysis.[50,51] ^{1}H NMR studies performed on a range of aryloxypropanolamine compounds[52–57] including propranolol,[53] metoprolol,[56] atenolol[56] and timolol[57] also supported the proposed conformation in which the β-hydroxy and the amino group are *gauche* and the amino group and the *O*-aryl substituent are antiperiplanar to each other.

In this work we describe the results of conformational analysis of the β-blockers **1**–**3** and the formaldehyde-derived oxazolidine derivatives **4**–**7** (Fig. 1), using ^{1}H NMR spectroscopy and computational methods.

Experimental

NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded in 5 mm tubes at 300 K on a Bruker AM 300 WB spectrometer. All solutions of the β-blockers were 0.06–0.07 M in D_2O and $CDCl_3$ while the oxazolidine derivatives were 0.06–0.07 M in $CDCl_3$ unless otherwise stated. The deuterium signal of the solvent was used as the lock and tetramethylsilane (TMS) was the internal standard. *J* values are in Hz. One-dimensional NMR experiments were carried out with a spectral width of 3 kHz, a 45° pulse angle, 16 384 data points and a repetition delay of 2.0 s. 32 scans were accumulated prior to Fourier transformation. DQF–COSY spectra were recorded using a spectral width of 3 kHz, a 90° pulse of 7.8 μs and a repetition delay of 2.0 s. For each FID, 32 scans were accumulated. The two-dimensional data were collected as a 512 × 1024-word matrix and zero-filled to 1024 × 2048 prior to Fourier transformation. A sine-bell window function was applied in both dimensions.

Chemistry

Mass spectra and high resolution mass spectra (HRMS) were obtained on a JEOL JMS DX-300 double focussing instrument. Infra-red spectra were run in KBr disks on a Bruker IFS66 FTIR spectrometer. Melting points were determined on a Gallencamp melting point apparatus and are uncorrected. Methanol was distilled from iodine and magnesium and stored over 3 Å molecular sieves. Solutions were concentrated on a Buchi rotary evaporator.

Synthesis

All compounds were prepared as racemic mixtures and no attempts were made to maximise yields. The oxazolidine derivatives **4**–**7** were synthesised in a one step reaction by condensation of the β-amino alcohol derivatives **1**–**3** with formaldehyde. Experimental details for the synthesis of **4** are outlined below. For the oxazolidine derivatives **5** and **7** any variations on this procedure are described. The cyclisation of **2** was initially performed in methanol using 38% formalin solution resulting in hydroxymethylation of the primary amide group in addition to oxazolidine ring formation to form **6**. The desired oxazolidine **5** was eventually synthesised employing the conditions described for **4**.

3-Isopropyl-5-[4-(2-methoxyethyl)phenoxymethyl]oxazolidine 4. A solution of the free base of metoprolol **1** (0.79 g, 3.0 mmol) in super-dry methanol (20 ml) was added to a solution of paraformaldehyde (0.45 g, 15.0 mmol) and potassium hydroxide (50 mg) in super-dry methanol (50 ml). Sodium sulfate (0.5 g) was added to the mixture and the resulting suspension was refluxed for 8 h. The solution was filtered hot and the methanol removed under reduced pressure. Radial chromatography (chloroform–methanol 8:2, R_f 0.65) afforded the desired oxazolidine **4** (0.76 g, 91%) as a clear viscous oil (Found: C, 67.1; H, 8.7; N, 4.6. $C_{16}H_{25}NO_3{\cdot}0.5H_2O$ requires C, 66.7; H, 9.1; N, 4.8%) (Found: M^+, 279.186. $C_{16}H_{25}NO_3$ requires M^+, 279.184). MS *m*/*z* 279 (M^+, 60%). δ_H 1.09 [d, 6H, $CH(CH_3)_2$, *J* 6.2], 2.57 [sept, 1H, $CH(CH_3)_2$, *J* 6.3], 2.74 (dd, 1H, $H4_B$, *J* 6.6, 10.1), 2.80 (t, 2H, $CH_2$4′α, *J* 7.1), 3.10 (dd, 1H, $H4_A$, *J* 6.9, 10.1), 3.33 (s, 3H, OCH_3), 3.54 (t, 2H, $CH_2$4′β, *J* 7.1), 3.93 (dd, 1H, $H6_B$, *J* 5.4, 9.7), 4.00 (dd, 1H, $H6_A$, *J* 5.7, 9.5), 4.34 (d, 1H, $H2_B$, *J* 3.6), 4.36 (m, 1H, H5), 4.39 (d, 1H, $H2_A$, *J* 4.0), 6.83 (d, 2H, H2′, H6′, *J* 8.5), 7.11 (d, 2H, H3′, H5′, *J* 8.5). δ_C(CD_3OD) 21.9, $CH(CH_3)_2$; 36.1, C4′α; 53.0, C4; 53.9, $CH(CH_3)_2$; 58.7, OCH_3; 70.3, C4′β; 74.7, C6; 76.9, C5; 85.9, C2; 115.6, C2′, C6′; 130.8, C3′, C5′; 132.8, C4′; 158.7, C1′.

3-Isopropyl-5-[(4-acetamido)phenoxymethyl]oxazolidine 5. White crystals from methanol (70.4%) mp 156–157 °C (Found: C, 63.0; H, 7.8; N, 9.0. $C_{15}H_{22}N_2O_3 \cdot 0.5H_2O$ requires C, 62.8; H, 8.1; N, 9.7%) (Found: M^+, 278.164. $C_{15}H_{22}N_2O_3$ requires M^+, 278.187). MS *m*/*z* 278 (M^+, 47%). δ_H 1.10 [d, 6H, $CH(CH_3)_2$, *J* 6.6], 2.59 [sept, 1H, $CH(CH_3)_2$, *J* 6.36], 2.76 (dd, 1H, $H4_B$, *J* 6.6, 10.2), 3.11 (dd, 1H, $H4_A$, *J* 6.9, 10.1), 3.52 (s, 2H, CH_2CO), 3.95 (dd, 1H, $H6_B$, *J* 5.2, 9.6), 4.02 (dd, 1H, $H6_A$, *J* 5.7, 9.6), 4.35 (d, 1H, $H2_B$, *J* 3.7), 4.41 (d, 1H, $H2_A$, *J* 3.6), 4.41 (m, 1H, H5), 5.39 (s, 2H, NH_2), 6.90 (d, 2H, H2′, H6′, *J* 8.5), 7.17 (d, 2H, H3′, H5′, *J* 8.6). $\delta_C(CD_3OD)$ 21.9, $CH(CH_3)_2$; 42.6, CH_2CO; 52.9, $CH(CH_3)_2$; 53.9, C4; 70.5, C6; 76.8, C5; 85.9, C2; 115.9, C2′, C6′; 129.4, C4′; 131.2, C3′, C5′; 159.3, C1′; 177.3, CO.

3-Isopropyl-5-[4-(*N*-hydroxymethylacetamido)phenoxymethyl]oxazolidine 6. Atenolol **2** (0.24 g, 0.9 mmol) in superdry methanol was added to a solution of formalin (38%) (600 μl, 7.27 mmol) and the resulting mixture was refluxed for 8 h. The methanol was removed under reduced pressure to a volume of approximately 1 ml. Radial chromatography (chloroform–methanol 8:2, R_f 0.44) gave the oxazolidine **6** (0.17 g, 61%) as white crystals. Mp 88–89 °C (Found: C, 62.1; H, 8.0; N, 9.0. $C_{16}H_{24}N_2O_4$ requires C, 62.3; H, 7.8; N, 9.1%) (Found: M^+, 308.174. $C_{16}H_{24}N_2O_4$ requires M^+, 308.175). MS *m*/*z* 308 (M^+, 3%). δ_H 1.05 [d, 6H, $CH(CH_3)_2$, *J* 6.5], 2.54 [sept, 1H, $CH(CH_3)_2$, *J* 6.4], 2.70 (dd, 1H, $H4_B$, *J* 6.6, 10.2), 3.05 (dd, 1H, $H4_A$, *J* 6.9, 10.1), 3.46 (s, 2H, CH_2CO), 3.88 (dd, 1H, $H6_B$, *J* 5.3, 9.6), 3.96 (dd, 1H, $H6_A$, *J* 5.6, 9.6), 4.30 (d, 1H, $H2_B$, *J* 3.7), 4.32 (m, 1H, H5), 4.35 (d, 1H, $H2_A$, *J* 3.6), 4.60 (d, 2H, CH_2OH, *J* 6.5), 6.40 (t, 1H, NH, *J* 6.5), 6.83 (d, 2H, H2′, H6′, *J* 8.5), 7.09 (d, 2H, H3′, H5′, *J* 8.6). $\delta_C(CDCl_3)$ 21.7, $CH(CH_3)_2$; 42.6, CH_2CO; 52.4, $CH(CH_3)_2$; 52.5, C4; 64.3, C6; 69.4, CH_2OH; 74.9, C5; 85.1, C2; 114.9, C2′, C6′; 126.6, C4′; 130.5, C3′, C5′; 157.9, C1′, 172.9, CO.

3-*tert*-Butyl-5-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxymethyl]oxazolidine 7. Purification by radial chromatography (chloroform–methanol, 8:2, R_f 0.46) gave the desired oxazolidine **7** (84.0%) (Found: C, 50.2; H, 7.4; N, 16.5. $C_{14}H_{24}N_4O_3S \cdot 0.5H_2O$ requires C, 49.8; H, 7.4; N, 16.6%) (Found: M^+, 328.156. $C_{14}H_{24}N_4O_3S \cdot 0.5H_2O$ requires M^+, 328.280). MS *m*/*z* 328 (M^+, 17). δ_H 1.06 [s, 9H, $C(CH_3)_3$], 2.66 (dd, 1H, $H4_B$, *J* 6.70, 9.89), 3.10 (dd, 1H, $H4_A$, *J* 6.61, 9.87), 3.47 [t, 4H, CH_2(3″, 5″), *J* 4.79], 3.74 [t, 4H, CH_2(2″, 6″), *J* 4.74], 4.35 (d, 1H, $H2_B$, *J* 3.37), 4.46 (d, 1H, $H2_A$, *J* 3.65), 4.37 (m, 1H, H5), 4.40 [m, 2H, CH_2(6)]. $\delta_C(CDCl_3)$ 26.6, $C(CH_3)_3$; 47.1, C4; 47.7, C3″, C5″; 52.3, $C(CH_3)_3$; 66.3, C2″, C6″; 71.2, C6; 74.8, C5; 81.1, C2; 149.7, C4′; 153.9, C3′.

Computational conformational analysis

Computer-aided conformational analyses were carried out to further define the conformations of **1**–**7**. The primary aim of these conformational studies was to examine closely the orientations of the β-amino alcohol group. The (*S*)-aryloxypropanolamine isomers were examined in the conformational analysis since it is known that most of the pharmacological activity resides with this isomer.[58] As a consequence, only the (*S*)-oxazolidine isomers were considered here. All molecules were constructed using standard bond angles and bond lengths within the sketch functionality of the program SYBYL.[59] The molecules were then minimised using the TRIPOS force field,[60] Gasteiger–Hückel atom charges (an algorithm which incorporates Gasteiger–Marsili[61] and Hückel[62] charge calculations) and the Powell optimisation method.[63] Minimisation was terminated for each structure when the gradient fell below 0.05 kcal mol^{-1} $Å^{-1}$ (1 cal = 4.184 J). The default values were used for all other parameters.

Conformer generation

The conformers for **3** and **8** were generated using the systematic search algorithm implemented within SYBYL. The acyclic torsion angles (defined in Fig. 2) were varied in 30° increments over 360°. The default values were used for all other parameters. The resulting structures were then minimised using the protocol described above. All unique conformers within 10 kcal mol^{-1} of the lowest energy conformer found were reported.

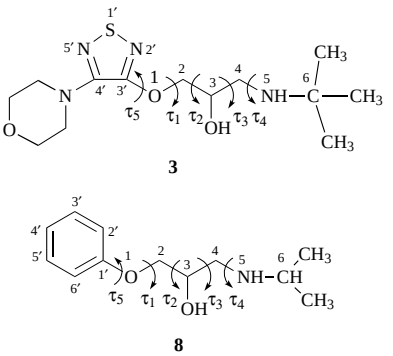

Fig. 2 Fragments **3** and **8** used for theoretical calculations. The torsion angles τ_1–τ_5 are defined in the text.

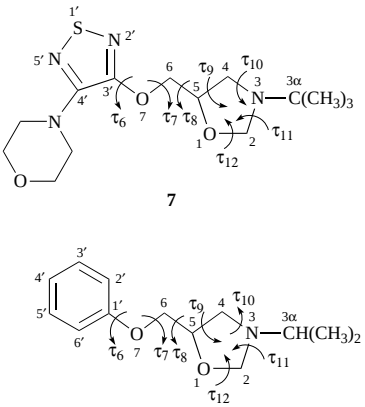

Fig. 3 Oxazolidine fragments **7** and **9** used in the theoretical calculations. Torsion angles τ_6–τ_{12} are defined in the text.

The conformers of **7** and **9** were generated in a similar manner to that of **3** and **8**, with the addition of conformational analysis of the oxazolidine ring. The acyclic torsion angles (defined in Fig. 3) were varied in 30° increments over 360°.

The bond between atoms C5 and O1 was chosen as the ring closure bond and torsion angles τ_9–τ_{12} were varied in 5° steps over the full 360° circle. A detailed description of ring conformational analysis has been given by Lipton and Still[64] and therefore will not be included here. The default values were used for all other parameters. The resulting structures were then minimised as previously described for **3** and **8**. The torsion angle to the morpholine group in **3** and **7** was not considered because ^{1}H NMR data indicated that the oxypropanolamine sidechain of timolol **3** adopted a similar conformation to metoprolol **1** and atenolol **2** indicating that the morpholine group has no effect upon the conformation adopted by the oxypropanolamine sidechain in solution.

Superimposition of the selected minimised structures was performed using the linear least squares fitting algorithm within SYBYL. All calculations were performed on a Silicon Graphics Indigo 2 XZ Unix workstation.

Results and discussion

NMR studies

Investigation of the low energy conformations of the β-amino alcohol moiety of **1**–**3** was undertaken to determine whether constraining the β-blocker backbone into an oxazolidine ring affected the orientation of the β-hydroxyl oxygen and the amino group. If the oxazolidine ring constrains the molecule

Table 1 ^{1}H NMR spectral data for the free base of metoprolol **1** in $CDCl_3$ and metroprolol tartrate in D_2O

	δ_H			J/Hz	
Proton	$CDCl_3$	D_2O	Multiplicity	$CDCl_3$	D_2O
$CH(CH_3)_2$	1.05	1.32	d	6.5	6.6
$CH(CH_3)_2$	2.83	3.47	sept	6.5	6.6
$H4_A$	2.83	3.35	dd	3.2, 11.9	3.3, 13.0
$H4_B$	2.65	3.20	dd	8.9, 11.9	9.3, 13.1
H3	4.01	4.29	m	—	—
$H2_A$	3.88	4.10	dd	4.4, 9.6	4.2, 10.4
$H2_B$	3.86	4.05	dd	5.4, 10.4	5.1, 10.5
H2′, H6′	6.80	6.95	d	8.5	8.6
H3′, H5′	7.07	7.22	d	8.6	8.6
H4′α	2.79	2.82	t	7.5	6.5
H4′β	3.52	3.67	t	7.1	6.6
OCH_3	3.31	3.29	s	—	—

Table 2 ^{1}H NMR spectral data for the free base of atenolol **2** in $CDCl_3$ and in D_2O

	δ_H			J/Hz	
Proton	$CDCl_3$	D_2O	Multiplicity	$CDCl_3$	D_2O
$CH(CH_3)_2$	1.09	1.05	d	6.2	6.3
$CH(CH_3)_2$	2.86	2.86	sept	6.3	6.3
$H4_A$	2.85	2.82	dd	3.8, 12.0	4.0, 13.2
$H4_B$	2.66	2.72	dd	8.3, 12.0	7.9, 12.8
H3	4.04	4.08	m	—	—
CH_2-2[a]	3.94	—	d	4.4	—
$H2_A$[b]	—	4.06	dd	—	3.4, 11.0
$H2_B$[b]	—	3.97	dd	—	7.0, 11.0
H2′, H6′	6.90	6.96	d	8.6	8.6
H3′, H5′	7.22	7.22	d	8.6	8.6
H4′α	3.44	3.51	s	—	—

[a] CH_2-2 protons of **2** in $CDCl_3$ are equivalent. [b] H2 protons are inequivalent in D_2O appearing as doublets of doublets.

Table 3 ^{1}H NMR spectral data for the free base of timolol **3** in $CDCl_3$ and timolol maleate in D_2O

	δ_H			J/Hz	
Proton	$CDCl_3$	D_2O	Multiplicity	$CDCl_3$	D_2O
$CH(CH_3)_3$	1.09	1.36	s	—	—
$H4_A$	2.71	3.28	dd	3.9, 11.9	2.7, 12.8
$H4_B$	2.52	3.08	dd	8.2, 11.9	10.0, 12.7
H3[a]	3.88	—	dddd	3.7, 4.3	—
				5.8, 8.2	—
H3[b]	—	4.31	m	—	—
$H2_A$	4.38	4.51	dd	4.4, 11.0	4.1, 10.9
$H2_B$	4.30	4.43	dd	5.7, 11.0	5.5, 11.1
CH_2-2″, CH_2-6″	3.71	3.82	t	4.6	4.5
CH_2-3″, CH_2-5″	3.44	3.46	t	4.6	4.5

[a] H3 protons of **3** in $CDCl_3$ can be resolved as eight peaks coupled to H2 and H4. [b] H3 in D_2O is not resolved.

close to one of the β-blocker's preferred conformations, then the drug may have potential as a rigid analogue. On the other hand, controlled oxazolidine ring hydrolysis could provide an effective prodrug agent for the delivery of the β-blocker by improving the lipophilic characteristics of the molecule.

Compounds **1** and **2** have the same atomic constitutions around the oxypropanolamine moiety while **3** possesses an *N*-*tert*-butyl group instead of an *N*-isopropyl group. The spectral characteristics of these compounds were very similar and functional groups within the structures were easily identified in the ^{1}H NMR spectra. Bridging of the hydroxy and amino functions of **1**–**3** with formaldehyde to form the oxazolidine derivatives **4**–**7** did not appreciably change the chemical shifts of the various protons although changes in the coupling constants were observed.

^{1}H NMR analysis of β-blockers 1–3

A combination of 2D homonuclear double quantum filtered phase sensitive ^{1}H–^{1}H correlated spectroscopy (DQF–COSY) and selective homonuclear decoupling experiments was utilised in the ^{1}H NMR assignment of the β-blockers and their corresponding oxazolidine derivatives. The ^{1}H NMR spectral data for **1**–**3** are given in Tables 1–3. The three bond vicinal coupling constants of the β-amino alcohol moiety in **3** and **8** (Fig. 2) provide valuable information about the torsion angles τ_1–τ_5.

The non-equivalence of the protons in the methylene groups CH_2-2 and CH_2-4 is easily observed. For compounds **1**–**3**, $H4_A$ exhibits couplings of $J_{4A,3}$ 2.7–4.0 with H3 and $J_{4A,4B}$ 11.9–13.2 with $H4_B$. Proton $H4_B$ couples to H3 with a coupling constant in the range $J_{4B,3}$ 7.9–10.0. The CH_2-2 protons appear further downfield, proton $H2_A$ displaying couplings of $J_{2A,3}$ 3.4–4.4 with H3 and $J_{2A,2B}$ 9.6–11.0 with $H2_B$. Proton $H2_B$ shows couplings of $J_{2B,3}$ 5.1–7.0 with H3. An exception to this was noted in the ^{1}H NMR spectral data of **2** in $CDCl_3$, Table 2. The CH_2-2 protons were found to be equivalent and appeared as a doublet at δ 3.94 (J 4.4).

Conformation around the C2–C3 bond (τ_2)

The three classical conformers about the C2–C3 bond are shown in Fig. 4. The observed coupling constants of $J_{2B,3}$ 5.1–7.0 and $J_{2A,3}$ 3.4–4.4 do not correspond to any of the conformers $g+$, t or $g-$. This suggests a rapid equilibrium involving more than one rotational isomer. Kulkarni observed the coexistence of the three staggered rotamers for atenolol, metoprolol and timolol with the equilibrium dominated by the $g+$ rotamer.[54,56,57]

Conformation around the C3–C4 bond (τ_3)

An examination was made of the coupling constants observed between H3 and the two H4 protons for **1**–**3** recorded in both $CDCl_3$ and D_2O and from these values approximate torsion

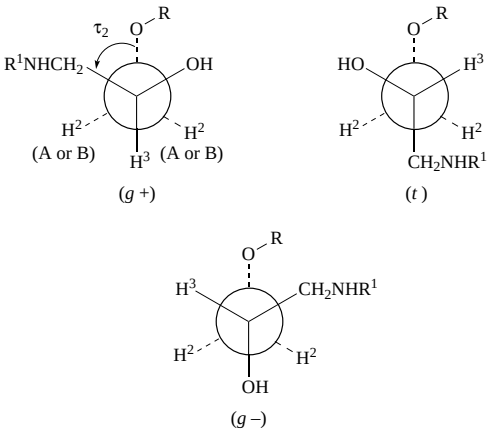

Fig. 4 Newman projections depicting the conformations around the C2–C3 bond (τ_2) of the aryloxypropanolamine sidechain of the β-blockers

angles were predicted using the Karplus equation.[65] These results indicate a predominance of conformation t, Fig. 5.

These findings are consistent with those of Kulkarni and coworkers[53,56,57] who examined their molecules in D_2O and indicate that the most likely conformer is an extended form with the possibility of H-bonding between the NH and the OH groups and that an interaction involving H-bonding between the ether oxygen and the amino group is unlikely.[53–57]

^{1}H NMR analysis of the oxazolidine derivatives 4–7

NMR data for the oxazolidine derivatives **4**–**7** are given in the Experimental section. The DQF–COSY spectrum of the oxazolidine derivative **4** in $CDCl_3$ at 300 K is shown in Fig. 6 with

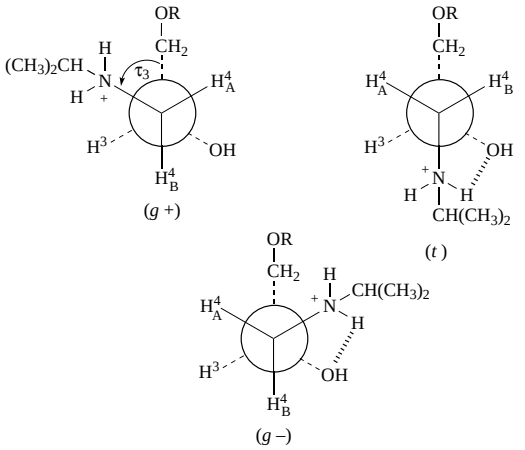

Fig. 5 Newman projections depicting the likely conformers around the C3–C4 bond (τ_3)

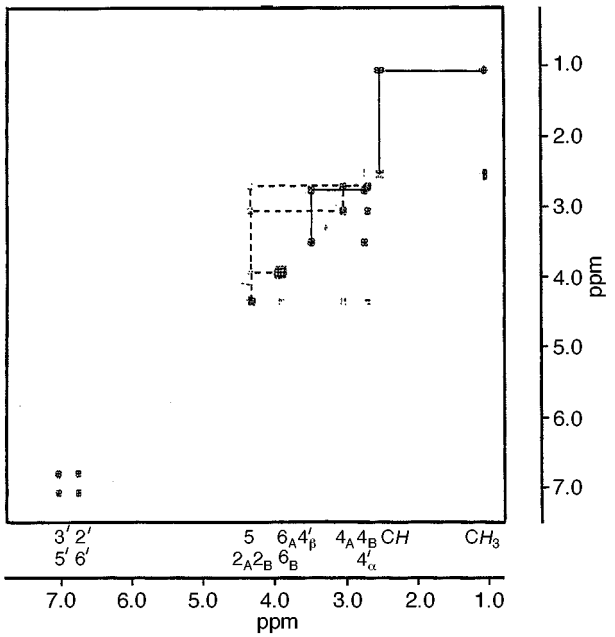

Fig. 6 Contour plot of the 2D DQF–COSY spectrum of **4** in $CDCl_3$

assignments and connections between cross peaks indicated. As the assignments of **4**–**7** have not been reported previously, the assignment procedure for **4** is described now in some detail. The torsion angles of interest within the oxazolidine derivatives (τ_6–τ_{12}) are shown in Fig. 3.

The high field doublet in the 1D spectrum of **4** (see Experimental section for ^{1}H NMR data) at δ 1.09 (J 6.2) is readily assigned to the isopropyl methyl protons. Examination of the DQF–COSY spectrum reveals a connection to the septet for the isopropyl methine proton at δ 2.57. The protons of the methoxy group were easily assigned as the singlet at δ 3.33. The methylene protons of the methoxyethyl side chain were also assigned as the two triplets at δ 2.80 (CH_2-4′α, J 7.1) and 3.54 (CH_2-4′β, J 7.1). As expected, the two H4 protons of the oxazolidine ring are non-equivalent and appear as doublets of doublets at δ 2.74 ($H4_B$: $J_{4B,5}$ 6.6; $J_{4A,4B}$ 10.1) and 3.10 ($H4_A$: $J_{4A,5}$ 6.9; $J_{4A,4B}$ 10.1). Connections from these two protons can be traced to the multiplet centred at δ 4.36 which can be assigned as proton H5. The H5 multiplet overlaps with the doublets for $H2_B$ ($J_{2B,2A}$ 3.6) at δ 4.34 and $H2_A$ ($J_{2A,2B}$ 4.0) at δ 4.39. A connection can be traced from H5 to the doublet of doublets at δ 3.93 ($J_{5,6B}$ 5.4, 9.7) and 4.00 ($J_{5,6A}$ 5.7, 9.5) assigned to $H6_B$ and $H6_A$, respectively. The four aromatic protons H2′, H6′ at δ 6.83 (J 8.5) and H3′, H5′ at δ 7.11 (J 8.5) are clearly defined.

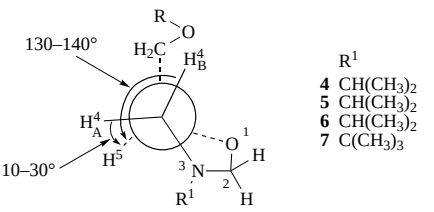

Fig. 7 Newman projection showing the proposed conformation of the oxazolidine ring system of **4**–**7** about the C4–C5 bond (τ_9)

Conformation around the C4–C5 bond (τ_9)

The coupling between $H4_A$ and H5 of $J_{4A,5}$ 6.6–7.0 is considerably larger than the values of 2.7–4.0 recorded for the corresponding protons ($H4_A$ and H3) of **1**–**3**. The bridging of the nitrogen and the oxygen atom changes the conformation, leading to a decreased torsion angle between these vicinal hydrogens which results in the observed larger coupling constant. An approximate value of 10–30° for τ_9 (depicted in Fig. 7) was estimated from the Karplus equation. Conversely the coupling between $H4_B$ and H5 ($J_{4B,5}$ 6.6–6.7) is smaller than that between the corresponding protons ($H4_B$ and H3) in the linear β-blocker ($J_{3,4B}$ 7.9–10.0). Thus in **4**–**7** the $H4_B$ and H5 protons are orientated at approximately 130–140° to each other, compared to a torsion angle of 140–165° between the corresponding protons $H4_B$ and H3 of **1**–**3**. Oxazolidine formation changes the conformation of the protons H3 and $H4_B$ of the acyclic β-blocker from an essentially *trans* configuration to one shown in Fig. 7, with the corresponding oxazolidine protons $H4_B$ and H5 in a less *gauche* configuration.

Conformation around the C5–C6 bond (τ_8)

Cyclisation to form the oxazolidine ring does not affect the coupling constants between the H6 and H5 protons as dramatically as it does the couplings between the H4 and H5 protons. The couplings between $H6_A$ and H5 ($J_{6A,5}$ 5.6–5.7) for the oxazolidine derivatives **4**–**7** are slightly larger than the corresponding $H2_A$–H3 coupling (of **1**–**3**) of $J_{2A,3}$ 3.4–4.4. The H5–$H6_B$ coupling of $J_{5,6B}$ 5.3–5.4 is slightly smaller than $J_{2B,3}$ 5.1–7.0 observed for the coupling between $H2_B$–H3 of the linear parent β-blockers **1**–**3**. Thus, the conformation about this part of the molecule is only slightly affected by oxazolidine ring formation. Considering that the most likely conformation of the acyclic β-blockers around τ_2 corresponds to $g+$ in Fig. 4, the most likely cause of conformational change as a result of oxazolidine formation appears to be due to the loss of hydrogen bonding between the ether oxygen and the 3-hydroxy group which was shown to be likely given the proposed conformer $g+$ in Fig. 5. In the oxazolidine derivatives, the 3-hydroxy group becomes involved in the five-membered ring and can no longer contribute to hydrogen bonding.

Conclusion

The ^{1}H NMR data indicate that the cyclic oxazolidine derivatives **4**–**7** and the linear β-blockers **1**–**3** have similar conformations around the β-amino alcohol moiety.

Computational analysis

The ^{1}H NMR data are valuable for the determination of the lowest energy conformer in solution or for establishing the presence of several conformers in rapid equilibrium. To check for the presence of other conformers which may be of slightly higher energy, yet which might become populated at the receptor site it was of interest to perform an extensive computational analysis of the energy surface of these molecules.

Computational analysis of the linear β-blocker fragments 3 and 8. The inherent flexibility of β-blockers results in an enormous range of possible conformations that these compounds can adopt. Since our interest lay only in the conformation of the oxypropanolamine backbone, the parent molecules **1** and

2 were pruned to the 4′-unsubstituted aryloxypropanolamine derivative **8**, where conformational preferences are unlikely to be significantly different from the whole structure. Analysis of both **1** and **2** showed that the methoxyethyl group of **1** and the amidomethyl substituent of **2** were unlikely to fold in a way that would affect the conformation of the β-amino alcohol functionality. The entire structure of timolol (**3**) was built up to examine the conformation around the β-amino alcohol moiety since it contains a different aromatic moiety than either metoprolol (**1**) or atenolol (**2**).

The torsion angles of the two β-amino alcohol fragments **3** and **8** (Fig. 2) considered were τ_1–τ_5 where τ_1 is defined by the atoms C3′, O1, C2, C3 for fragment **3** and C1′, O1, C2, C3 for the fragment **8**; τ_2 by atoms O1, C2, C3, C4; τ_3 by the atoms C2, C3, C4, N5; τ_4 by the atoms C3, C4, N5, C6 and τ_5 by the atoms N2′, C3′, O1, C2 for fragment **3** and C2′, C1′, O1, C2 for fragment **8**. The torsion angles were varied in 30° increments over 360° and the resulting structures were minimised using molecular mechanics. Application of this protocol to fragment **8** resulted in the generation of 8857 conformers which were within 10 kcal mol^{-1} of the lowest energy conformer found (E = 5.9 kcal mol^{-1}), while **3** afforded 596 conformers within 10 kcal mol^{-1} of the lowest energy conformer (E = 15.0 kcal mol^{-1}).

Computational analysis of the oxazolidine derivatives 4–7. As for the β-blockers **1** and **2**, the oxazolidine derivatives **4**–**6** were pruned back to the 4′-unsubstituted analogue **9**. The entire structure of **7** was used in the conformational analysis. The torsion angles τ_6 (defined by atoms N2′, C3′, O7, C6 for **7** and C2′, C1′, O7, C6 for fragment **9**), τ_7 (defined by atoms C3′, O7, C6, C5 for **7** and C1′, O7, C6, C5 for **9**) and τ_8 (defined by atoms O7, C6, C5, C4) were varied in 30° increments. The oxazolidine ring torsion angles τ_9 (defined by atoms C6, C5, C4, N3), τ_{10} (defined by atoms C5, C4, N3, C2), τ_{11} (defined by atoms C4, N3, C2, O1) and τ_{12} (defined by atoms N3, C2, O1, C5) were varied in 5° steps over 360°. The resulting structures were then minimised using molecular mechanics. Using this protocol there were 8195 conformers of **9** all within 10 kcal mol^{-1} of the lowest energy conformer (E = 10.8 kcal mol^{-1}) while for **7**, 1058 conformers were within 10 kcal mol^{-1} of the lowest energy conformer (E = 8.8 kcal mol^{-1}).

Superimposition of low energy conformers of the β-blocker fragments 3 and 8 and the oxazolidine analogues 7 and 9

The purpose of this study was to see whether the incorporation of the oxazolidine ring significantly altered the conformation adopted by the β-amino alcohol moiety of **1**–**3**. Therefore, a comparison was carried out between the theoretically determined conformers and the conformers predicted by ^{1}H NMR analysis.

The torsion angles between the vicinal protons $H2_A$–H3, $H2_B$–H3, H3–$H4_A$ and H3–$H4_B$ within each of the conformers generated from fragment **8** (representative of **1** and **2**), and timolol (**3**) were examined. Torsion angles between the protons $H4_A$–H5, $H4_B$–H5, H5–$H6_A$ and H5–$H6_B$ of all the conformers generated from the two oxazolidine fragments **7** and **9** were also determined, Fig. 8. A comparison was then carried out between the torsion angles of all the theoretically determined conformers and those experimentally determined using ^{1}H NMR spectroscopy.

The chemical shifts and coupling constants obtained from the ^{1}H NMR spectra of a species which consists of a mixture of rapidly interconverting forms are average values and this is certainly the case with the flexible β-blocker molecules **1**–**3**. As such, it is difficult to derive torsion angles for these molecules from the Karplus rule.[65] However, the torsion angles predicted from the ^{1}H NMR data of the semi-rigid oxazolidine derivatives are more tractable. The torsion angles estimated from ^{1}H NMR studies were used to discount unlikely conformers generated by theoretical studies. This allowed a large

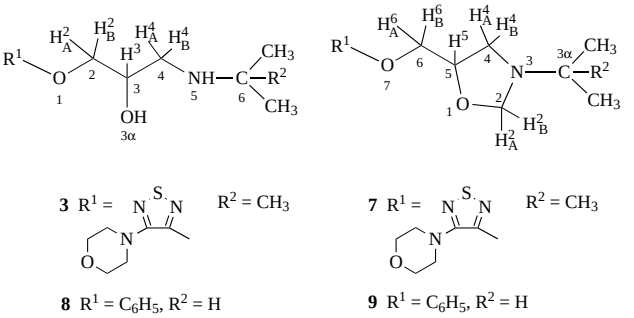

Fig. 8 Fragments indicating the protons between which torsion angles were measured in the β-amino alcohol and oxazolidine low energy conformers

number of theoretically determined conformers to be discarded as not all conformations around the bonds of interest were compatible with the coupling constants observed.

For the β-blockers **1** and **2** the coupling constants recorded between the protons $H2_A$–H3 (τ_{13} defined by $H2_A$, C2, C3, H3) ranges from $J_{2A,3}$ 3.4–4.4 while the magnitude of coupling between protons $H2_B$–H3 (τ_{14} defined by $H2_B$, C2, C3, H3) is $J_{2B,3}$ 5.1–7.0, Tables 1–3. As such, theoretically determined conformers containing torsion angles about τ_{13} and τ_{14} of a very large (160–180°) or small magnitude (0–20°) were not considered. The magnitude of coupling between $H4_A$–H3 (τ_{15} defined by $H4_A$, C4, C3, H3) and $H4_B$–H3 (τ_{16} defined by $H4_B$, C4, C3, H3) was $J_{4A,3}$ 3.2–4.0 and $J_{4B,3}$ 7.9–9.3, respectively. As such, torsion angles in the approximate range τ_{15} = 40–60° and 110–140° and τ_{16} = 0–20° and 135–180° were considered. A similar process was used when selecting conformers generated from timolol (**3**) and the oxazolidine fragments **7** and **9**.

Comparison of timolol 3 with the oxazolidine 7

Conformational analysis of **3** generated 15 conformers consistent with those expected from the ^{1}H NMR data. All 15 conformers were subjected to a rigid superimposition using the atoms C2, C3, C4 and N5 in order to check that all the conformers were unique. There were three distinct conformational families evident. The lowest energy conformer from each family was then used in the superimposition with the oxazolidine **7**.

Examination of the torsion angles around the protons $H4_A$–H5 (τ_{17} defined by the atoms $H4_A$, C4, C5, H5), $H4_B$–H5 (τ_{18} defined by the atoms $H4_B$, C4, C5, H5), H5–$H6_A$ (τ_{19} defined by the atoms H5, C5, C6, $H6_A$) and H5–$H6_B$ (τ_{20} defined by the atoms H5, C5, C6, $H6_B$) (Fig. 8) showed that of the 1058 low energy conformers generated from **7**, 20 were found to resemble the conformations predicted from ^{1}H NMR measurements.

Superimposition of all 20 conformers *via* atoms C6, C5, C4 and N3 revealed three distinct conformational families; the lowest energy conformer from each family was then used in the superimpositions with **3**.

Fig. 9 shows the result of rigid superimposition (RMS = 0.15) of the three representative conformers generated from **3** (*via* atoms C2, C3, C4, N5) and the three oxazolidine conformers of **7** (*via* atoms C6, C5, C4, N3). The distance between the β-hydroxy oxygen atom and the oxazolidine oxygen atom is 0.45 Å and the distance between C6 of **3** and C3α of **7** is 0.34 Å.

Comparison of fragment 8 (representative of 1 and 2) with the oxazolidine 9

From the 8857 low energy conformers resulting from conformational analysis of fragment **8**, 205 different conformers were shown to closely match the conformations predicted from the ^{1}H NMR measurements of **1** and **2**. The conformers were superimposed using the atoms C2, C3, C4 and N5 and six dis-

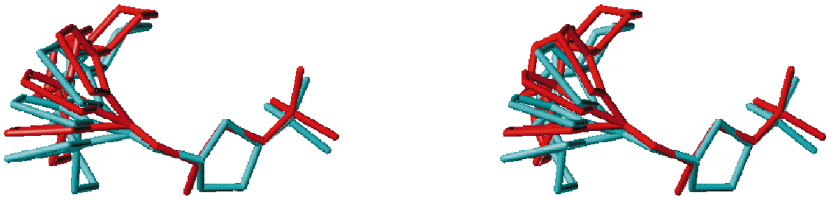

Fig. 9 Stereoview of the superimposition of three low energy conformers of **3** (red) and three from **7** (blue) consistent with the ^{1}H NMR data. For clarity hydrogen atoms have not been displayed.

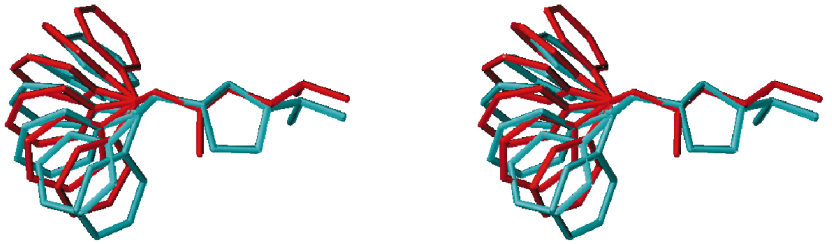

Fig. 10 Stereoview of the superimposition of six low energy conformers of fragment **8** (red) and six of fragment **9** (blue) consistent with the ^{1}H NMR data. For clarity hydrogen atoms have not been displayed.

tinct conformational families of fragment **8** were evident, the variations within each family being due to different τ_4 and τ_5 values. The lowest energy conformer within each distinct conformational family was used in the superimposition with the oxazolidine **9**.

Using the same procedure used to examine the oxazolidine **7**, 150 low energy conformers of **9** compared favourably with the conformations predicted from the ^{1}H NMR parameters for the oxazolidine derivatives **4**–**6**. Superimposition of the 150 conformers (*via* atoms C6, C5, C4, N3) showed that six distinct conformational families existed. Again, the lowest energy conformer within each family was used in the superimposition with the conformers of fragment **8**.

Thus six conformers generated from the β-amino alcohol fragment **8** and six conformers generated from the oxazolidine fragment **9** were subjected to a rigid superimposition using the atoms C2, C3, C4 and N5 of the β-amino alcohol fragment and the corresponding atoms of the oxazolidine fragment, C6, C5, C4 and N3 (RMS = 0.16). The superimpositions are shown in Fig. 10 and illustrate the close proximity of comparable atoms in the two fragments. For example, the distance between the β-hydroxy oxygen atom and the oxazolidine oxygen atom is 0.48 Å, while the distance between C6 of **8** and C3α of **9** is 0.58 Å.

The superimpositions shown in Figs. 9 and 10 illustrate that many of the low energy oxazolidine conformers of **7** and **9** compare well with the low energy β-amino alcohol conformers of **3** and **8**.

Conclusions

Conformational analysis of the β-blockers **1**–**3**, and the corresponding oxazolidine derivatives **4**–**7**, indicate that oxazolidine formation results in the general preservation of the solution conformation adopted by the β-amino alcohol moiety of the β-blockers. The oxazolidines could therefore potentially serve one of two purposes. If the cyclic systems prove to be labile, they could become useful delivery systems where an increase in lipophilicity is required, for example ocular delivery. Alternatively, if stable oxazolidine derivatives can be developed, they may be useful as semi-rigid β-blockers.

Acknowledgements

D. J. C. is grateful for the support of an Australian Research Council Senior Fellowship.

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Paper 7/06442J
Received 3rd *September* 1997
Accepted 14*th October* 1997