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# Synthesis and Biological Activity of New Melatonin Receptor Ligands

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**Abstract**

To discover analogues of melatonin with a longer half-life, novel non-indole analogues of the compound, in which the amide group of the side-chain has been reversed, have been prepared and evaluated in binding assays to determine their activity on melatonin receptors.

The two most active compounds were those with the *N*-methylbutyramide side-chain. Butyramide and pentanoylamide side-chains resulted in similar affinities, irrespective of the skeleton tested whereas a propionamide side-chain led to loss of affinity. The biological activity of the molecules was more influenced by the length of the side-chain than by the nature of the skeleton, which had little effect.

The results obtained show the relative importance of the length of the side-chain and of the nature of the skeleton in both the binding to and the activity on the melatonin receptor of the retroamide series.

Since the hormone melatonin was isolated and identified as *N*-acetyl-5-methoxytryptamine (Lerner et al 1958) interest in it has been steadily growing. In vertebrates this hormone has its primary sites of production in the pineal gland and in the photoreceptor cells of the retina, where it is synthesized from 5-hydroxytryptamine (5-HT) via a two-step biochemical pathway (Weichmann 1986; Reiter 1991). Its activity, mediated through high-affinity G protein-coupled receptors, is principally related to regulation of photoperiodic responses (Hagan & Oakley 1995), entrainment of mammalian circadian rhythms (Armstrong 1989), induction of sleep in man (Dollins et al 1994), and retinal physiology (Cahill & Besharse 1995). Recently, the antitumour properties of melatonin, its implication in the responsiveness of the immune system (Maestroni 1993), and its free-radical scavenger properties have also been described (Reiter et al 1995).

Despite its potential involvement in the regulation of many physiological processes, the very short biological half-life (15–30 min) of melatonin, because of its rapid catabolism to 6-hydroxymelatonin and *N*-acetylkynurenamines, currently limits its use as a therapeutic agent (Claustrat et al 1989; Di et al 1997). For this reason there is considerable interest in discovering new molecules capable of mimicking or antagonizing responses to melatonin. Such compounds constitute important tools in the elucidation of the physiological roles of melatonin. Several indole analogues (Spadoni et al 1993, 1997, 1998; Garratt et al 1994, 1995; Henin et al 1997; Tarzia et al 1997; Davies et al 1998) of melatonin have been found to act as ligands and many papers have reported the synthesis of several non-indole bioisosteres (Yous et al 1992; Copinga et al 1993; Depreux et al 1994; Sugden 1994; Langlois et al 1995; Garratt et al 1996; Jansen et al 1996; Leclerc et al 1996; Li et al 1997; Fourmaintraux et al 1998; Kloubert et al 1998).

We recently synthesized a series of benzopyran and benzodioxin analogues of melatonin (Viaud et al 1998; Mamai et al 1999) and evaluated their competitive inhibition of 2-[$^{125}$I]melatonin binding in ovine pars tuberalis membrane preparation.

In our continuing study of heterocyclic compounds with potential biological activity (Savelon

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et al 1998; Boyé et al 1999), we present here initial results on the design of new non-indole melatonin-like compounds in which the amide group has a carbon-nitrogen sequence (Leclerc et al 1998), in contrast with that of melatonin in which the order is opposite (nitrogen-carbon). The aim of this study was to evaluate for three fixed skeletons—1,4-benzodioxin, 2,3-dihydro-1,4-benzodioxin and 2,3-dihydro-1,4-benzoxathiin—the importance of the length of the retroamide side-chain in the interaction of the molecule when binding to, and acting on, the melatonin receptor.

Compounds **1**–**8** (Figure 1) were synthesized and evaluated in binding assays; the results obtained were compared with those from *N*-[3-(2,3-dihydro-1,4-benzodioxin-5-yl)propyl]acetamide (**A**; Guillaumet et al 1998; Charton et al 1999).

## Materials and Methods

*Chemistry*

Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. Proton and carbon NMR were recorded on a Bruker AM 300 W. B. or DPX 250 spectrometer. Coupling constants are in Hz and chemical shifts are $\delta$ ppm downfield from tetramethylsilane, which was used as an internal standard. IR spectra were obtained by use of a Perkin-Elmer 297 spectrophotometer. Mass spectra were recorded on a Nermag R 10-10 C (70 eV) apparatus. Organic solvents were purified as necessary as described by Perrin et al (1986) or purchased from Aldrich. Analytical thin-layer chromatography was performed on precoated plates (silica gel, $60F_{254}$) and spots were visualized with UV light or with a solution of ammonium cerium nitrate in ethanol. Column chromatography was performed on silica gel 60 (Merck) j 70–400 mesh for gravity columns and 230–400 mesh for flash columns. The solvents used for column chromatography were distilled before use; solvent mixtures are reported as v/v ratios.

*3-(2,3-Dihydro-1,4-benzodioxin-5-yl)propionic acid (**10a**).* Sodium hydroxide solution (10%, 3·4 mL, 8·46 mmol) was added to a solution of ethyl 3-(2,3-dihydro-1,4-benzodioxin-5-yl)propionate **9a** (1·00 g, 4·23 mmol) in ethanol (15 mL) under argon. The reaction mixture was heated under reflux for 2 h, then extracted with ethyl acetate. The aqueous layer was acidified with 3 M HCl which caused precipitation of acid **10a**. The white solid was collected by filtration and dried under high vacuum over $P_2O_5$ to give a white solid (91%), mp 73°C. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 2·44 (t, 2H, J = 7·8, $CH_2$), 2·73 (t, 2H, J = 7·8, $CH_2$), 4·19–4·24 (m, 4H, $H_2$ and $H_3$), 6·65–6·73 (m, 3H, $H_6$, $H_7$ and $H_8$), 12·1 (br s,1H, $CO_2H$). IR (KBr) $\nu$ 3300–2700, 1712, 1282, 1101 cm$^{-1}$. Calculated for $C_{11}H_{12}O_4$: C, 63·44; H, 5·82; found: C, 63·29; H, 5·80.

*5-(2,3-Dihydro-1,4-benzodioxin-5-yl)pentanoic acid (**10c**).* This compound was prepared as a white solid, mp 56°C, in 79% yield, from ethyl 5-(2,3-dihydro-1,4-benzodioxin-5-yl)pentanoate **9c** according to the procedure described for **10a**. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1·50–1·55 (m, 4H, 2 × $CH_2$), 2·23–2·29 (m, 2H, $CH_2$), 2·62–2·70 (m, 2H, $CH_2$), 4·20–4·25 (m, 4H, $H_2$ and $H_3$), 6·67–6·72 (m, 3H, $H_6$, $H_7$ and $H_8$), 12·00 (br s,1H, $CO_2H$), IR (KBr) $\nu$ 3300–2700, 1705, 1278, 1100 cm$^{-1}$. Calculated for $C_{13}H_{16}O_4$: C, 66·08; H, 6·84; found: C, 65·82; H, 6·78.

N-*Methyl-[3-(2,3-dihydro-1,4-benzodioxin-5-yl)]-propanamide (**1**).* Hydroxybenzotriazole (379 mg, 2·81 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (538 mg, 2·81 mmol) were added at 0°C to a solution of acid **10a** (500 mg, 2·55 mmol) in anhydrous *N*, *N*-dimethyl-formamide (10 mL) under argon. The argon flow was then stopped and methylamine (10% in benzene, 195 $\mu$L) was introduced. The temperature was left to increase slowly to 20°C, and stirring was continued for 18 h. The reaction mixture was concentrated in-vacuo, and diluted with dichloromethane. The organic layer was washed with

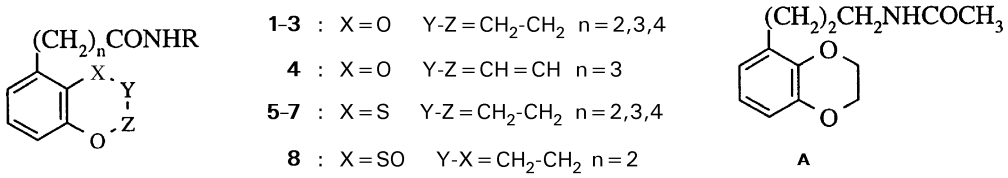


Figure 1. Structures of the retroamides with 1,4-benzodioxin, 2,3-dihydro-1,4-benzodioxin, and 2,3-dihydro-1,4-benzooxathiin skeletons. **A** = *N*-[3-(2,3-dihydro-1,4-benzodioxin-5-yl)propyl]acetamide (Guillaumet et al 1998; Charton et al 1999).

water. The combined organic extracts were dried ($MgSO_4$) and concentrated in-vacuo. Purification by column chromatography (chloroform–ethyl acetate, 7 : 3) gave the retroamide **1** as a solid in 78% yield, mp 99°C. $^1$H NMR (300 MHz, $CDCl_3/D_2O$) $\delta$ 2·35 (t, 2H, J = 8·0, $CH_2$), 2·66 (s, 3H, $CH_3$) 2·81 (t, 2H, J = 8·0, $CH_2$), 4·13–4·18 (m, 4H, $H_2$ and $H_3$), 6·59–6·65 (m, 3H, $H_6$, $H_7$ and $H_8$). IR (KBr) $\nu$ 3326, 1646, 1274, 1192, 1102 cm$^{-1}$. MS (CI/$NH_3$) m/z 222 (M + 1). Calculated for $C_{12}H_{15}NO_3$: C, 65·13; H, 6·85; N, 6·33; found: C, 65·04; H, 6·81; N, 6·28.

N-*Methyl-[5-(2,3-dihydro-1,4-benzodioxin-5-yl)]-pentanamide (**3**)*. This compound (mp 79°C) was prepared in 75% yield from acid **10c** according to the procedure described for **1**. $^1$H NMR (250 MHz, $CDCl_3/D_2O$) $\delta$ 1·56–1·72 (m, 4H, $CH_2$), 2·19 (t, 2H, J = 6·7, $CH_2$), 2·58 (t, 2H, J = 6·7, $CH_2$), 2·78 (s, 3H, $CH_3$), 4·21–4·27 (m, 4H, $H_2$ and $H_3$), 6·61–6·78 (m, 3H, $H_6$, $H_7$ and $H_8$). $^{13}$C NMR (62·9 MHz, $CDCl_3$) $\delta$ 25·6 and 26·3 ($CH_2$ and $CH_3$), 29·3 and 29·5 ($CH_2$), 36·7 ($CH_2$), 64·3 ($C_2$ and $C_3$), 115·1 ($C_7$), 120·6 and 121·9 ($C_6$ and $C_8$), 131·10 ($C_5$), 141·5 ($C_9$ and $C_{10}$), 173·7 (C = O). IR (KBr) $\nu$ 3290, 1636, 1288, 1079 cm$^{-1}$. MS (CI/$NH_3$) m/z 250 (M + 1). Calculated for $C_{14}H_{19}NO_3$: C, 67·45; H, 7·68; N, 5·62; found: C, 67·50; H, 7·70; N, 5·41.

*3-(2,3-Dihydro-1,4-benzodioxin-5-yl)propanol (**12**)*. Lithium aluminium hydride (401 mg, 10·6 mmol) was added in portions to a solution of ethyl 3-(2,3-dihydro-1,4-benzodioxin-5-yl)propionate **9a** (2·5 g, 10·6 mmol) in anhydrous ether (20 mL) at 0°C under argon. The reaction mixture was stirred for 1 h at room temperature. The reaction was quenched by adding water (320 $\mu$L), 15% NaOH (320 $\mu$L) and then water (960 $\mu$L). After stirring for 1 h, the salts were removed by filtration and washed with ether. The organic layer was dried ($MgSO_4$) and concentrated in-vacuo. The crude alcohol **12** was obtained as a colourless oil (97%), and used in the next step without further purification. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·70 (br s,1H, OH), 1·82 (q, 2H, J = 7·3, $CH_2$), 2·66 (t, 2H, J = 7·3, $CH_2$), 3·60 (m, 2H, $CH_2OH$), 4·21–4·27 (m, 4H, $H_2$ and $H_3$), 6·67–6·77 (m, 3H, $H_6$, $H_7$ and $H_8$). IR (neat) $\nu$ 3690–3110, 1282, 1068 cm$^{-1}$.

*[3-(2,3-Dihydro-1,4-benzodioxin-5-yl)propyl]-(4-methylphenyl)sulphonate (**14**)*. Tosyl chloride (1·57 g, 8·24 mmol) was added to a solution of alcohol **12** (800 mg, 4·12 mmol) and triethylamine (1·15 mL, 8·24 mmol) in dichloromethane (20 mL). After stirring at room temperature for 12 h, the product was hydrolysed and extracted with dichloromethane. The combined organic extracts were dried ($MgSO_4$) and concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 3 : 7) gave the tosylate **14** (92%) as a colourless oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·86 (q, 2H, J = 7·0, $CH_2$), 2·38 (s, 3H, $CH_3$), 2·54 (t, 2H, J = 7·0, $CH_2$), 3·98 (t, 2H, J = 7·0, $CH_2O$), 4·14–4·17 (m, 4H, $H_2$ and $H_3$), 6·47 (dd,1H, J = 7·4, 2·9, $H_{arom}$), 6·59–6·66 (m, 2H, $H_{arom}$), 7·27 (m, 2H, $H_{arom}$), 7·72 (m, 2H, $H_{arom}$). IR (neat) $\nu$ 1292, 1184, 1102 cm$^{-1}$. Calculated for $C_{18}H_{20}O_5S$: C, 62·04; H, 5·80; S, 9·20; found: C, 61·82; H, 5·85; S, 9·12.

*4-(2,3-Dihydro-1,4-benzodioxin-5-yl)butyronitrile (**16**)*. Potassium cyanide (448 mg, 6·89 mmol) was added to a solution of tosylate **14** (2·00 g, 5·74 mmol) in *N*, *N*-dimethylformamide. The reaction mixture was heated at 100°C for 5·5 h, and then hydrolysed. The solvent was evaporated and the aqueous layer extracted with dichloromethane. The combined organic extracts were dried ($MgSO_4$) and concentrated in-vacuo. Purification by column chromatography (ethyl acetate-petroleum ether, 3 : 7) gave the cyano compound **16** (77%) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·95 (q, 2H, J = 7·4, $CH_2$), 2·30 (t, 2H, J = 7·4, $CH_2$), 2·71 (t, 2H, J = 7·4, $CH_2$), 4·20–4·26 (m, 4H, $H_2$ and $H_3$), 6·65–6·75 (m, 3H, $H_6$, $H_7$ and $H_8$). IR (neat) $\nu$ 2250, 1292, 1092 cm$^{-1}$. Calculated for $C_{12}H_{13}NO_2$: C, 70·90; H, 6·46; N, 6·89; found: C, 70·75; H, 6·50; N, 6·81.

*4-(2,3-Dihydro-1,4-benzodioxin-5-yl)butanoic acid (**10b**)*. Sodium hydroxide solution (10%, 7·9 mL, 19·65 mmol) was added to a solution of cyano compound **16** (800 mg, 3·93 mmol) in ethanol (10 mL). The reaction mixture was heated at 60°C for 16 h, and the ethanol was then evaporated. Acidification with 2 M HCl led to precipitation of the acid as a white solid which was collected by filtration, and dried under high vacuum over $P_2O_5$ (74%, mp 54°C). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 2·00 (q, 2H, J = 7·8, $CH_2$), 2·46 (t, 2H, J = 7·8, $CH_2$), 2·78 (t, 2H, J = 7·8, $CH_2$), 4·45–4·58 (m, 4H, $H_2$ and $H_3$), 6·90–7·01 (m, 3H, $H_6$, $H_7$ and $H_8$), 12·10 (br s, 1H, $CO_2H$). IR (KBr) $\nu$ 3000–2500, 1730, 1300, 1110 cm$^{-1}$. Calculated for $C_{12}H_{14}O_4$: C, 64·84; H, 6·36; found: C, 64·77; H, 6·34.

N-*Methyl-[4-(2,3-dihydro-1,4-benzodioxin-5-yl)]-butanamide (**2a**)*. This compound (mp 79°C) was prepared in 77% yield from acid **10b** (600 mg, 2·70 mmol) according to the procedure described for **1**. $^1$H NMR (300 MHz, $CDCl_3/D_2O$) $\delta$ 1·86 (q,

2H, J = 7·4, $CH_2$), 2·13 (t, 2H, J = 7·4, $CH_2$), 2·55 (t, 2H, J = 7·4, $CH_2$), 2·73 (s, 3H, $CH_3$), 4·16–4·20 (m, 4H, $H_2$ and $H_3$), 6·60–6·69 (m, 3H, $H_6$, $H_7$ and $H_8$). $^{13}$C NMR (62·9 MHz, $CDCl_3$) $\delta$ 24·4 and 25·0 ($CH_2$ and $CH_3$), 27·8 and 34·8 ($CH_2$), 62·9 and 63·0 ($C_2$ and $C_3$), 114·0 ($C_7$), 119·3 and 120·8 ($C_6$ and $C_8$), 129·0 ($C_5$), 140·2 and 142·1 ($C_9$ and $C_{10}$), 172·2 (C = O). IR (KBr) $\nu$ 3300, 1646, 1284, 1208, 1118 $cm^{-1}$. MS (CI/$NH_3$) m/z 236 (M + 1). Calculated for $C_{13}H_{17}NO_3$: C, 66·36; H, 7·28; N, 5·95; found: C, 66·31; H, 7·21; N, 5·95.

N-*Propyl-4-(2,3-dihydro-1,4-benzodioxin-5-yl)butanamide (**2b**)*. A solution of acid **10b** (260 mg, 1·17 mmol) in *N*, *N*-dimethylformamide (1 mL) was added to a solution of *n*-propylamine hydrochloride (134 mg, 1·75 mmol) in *N*, *N*-dimethylformamide (5 mL) at 0°C. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI; 247 mg, 1·29 mmol) was then added. Stirring was continued for 10 h, and the reaction mixture left to warm to room temperature. After evaporation of *N*, *N*-dimethylformamide, the residue was hydrolysed and then extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), and concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 2 : 8) gave the retroamide **2b** (65%) as a white solid, mp 57–58°C. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 0·92 (t, 3H, J = 7·3, $CH_3$), 1·51 (sext, 2H, J = 7·3, $CH_2$), 1·92 (q, 2H, J = 7·3, $CH_2$), 2·20 (t, 2H, H = 7·3, $CH_2$), 2·62 (t, 2H, J = 7·3, $CH_2$), 3·17–3·25 (m, 2H, $CH_2N$), 4·22–4·27 (m, 4H, $H_2$ and $H_3$), 5·47 (br s, 1H, NH), 6·67–6·76 (m, 3H, $H_6$, $H_7$ and $H_8$). $^{13}$C NMR (62·9 MHz, $CDCl_3$) $\delta$ 11·3 ($CH_3$), 22·9, 25·8, 29·1 and 36·3 ($CH_2$), 41·1 ($CH_2N$), 64·16 and 64·22 ($C_2$ and $C_3$), 115·2 ($C_7$), 122·1 and 120·6 ($C_6$ and $C_8$), 130·2 ($C_5$), 141·5 and 143·4 ($C_9$ and $C_{10}$), 172·8 (C = O). IR (KBr) $\nu$ 3300, 1641. Calculated for $C_{15}H_{21}NO_3$: C, 68·40; H, 8·05; N, 5·32; found: C,68·59; H, 8·14; N, 5·38.

*3-(1,4-Benzodioxin-5-yl)propanol (**13**)*. Lithium aluminium hydride (233 mg, 6·14 mmol) was added to a solution of ethyl 3-(1,4-benzodioxin-5-yl)propanoate **11** (900 mg, 4·09 mmol) in dry ether (30 mL). The reaction mixture was heated under reflux for 30 min. After cooling and hydrolysis, the resulting salts were removed by filtration. The filtrate was dried ($MgSO_4$), and concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 4 : 6) gave alcohol **12** (95%) as an oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·51 (m,1H, OH), 1·76 (m, 2H, $CH_2$), 2·50 (t, 2H, J = 7·2, $CH_2$), 3·60 (m, 2H, $CH_2$), 5·83 (d,1H, J = 3·5, =CH), 5·87 (d,1H, J = 3·5, =CH), 6·43 (dd,1H, J = 7·5, 2·2, $H_{arom}$), 6·84 (dd,1H, J = 7·5, 2·2, $H_{arom}$), 6·70 (m,1H, $H_{arom}$). IR (neat) $\nu$ 3640–3130, 1280 $cm^{-1}$. Calculated for $C_{11}H_{12}O_3$: C, 68·72; H, 6·30; found: C, 68·56; H, 6·25.

*[3-(1,4-Benzodioxin-5-yl)propyl](4-methylphenyl)-sulphonate (**15**)*. This compound was prepared from alcohol **13** in 90% yield according to the procedure described for **14** (24 h). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·86 (m, 2H, $CH_2$), 2·44 (s, 3H, $CH_3$), 2·47 (t, 2H, J = 7·2, $CH_2$), 4·01 (t, 2H, J = 6·2, $CH_2$), 5·83 (d,1H, J = 3·7, =CH), 5·85 (d,1H, J = 3·7, =CH), 6·44 (dd,1H, J = 8·0, 1·7, $H_{arom}$), 6·51 (dd,1H, J = 8·0, 1·7, $H_{arom}$), 6·65 (m,1H, $H_{arom}$), 7·34 (d, 2H, J = 8·5, $H_{arom}$), 7·78 (d, 2H, J = 8·5, $H_{arom}$). IR (neat) $\nu$ 1360, 1250, 1185 $cm^{-1}$. Calculated for $C_{18}H_{18}O_5$: C, 62·40; H, 5·25; S, 9·25; found: C, 62·29; H, 5·26; S, 9·12.

*4-(1,4-Benzodioxin-5-yl)butyronitrile (**17**)*. Potassium cyanide (500 mg, 7·70 mmol) was added to a solution of tosylate **15** (1·07 g, 3·07 mmol) in *N*, *N*-dimethylformamide (15 mL) under argon and the reaction mixture was heated under reflux for 4 h. The solvent was then evaporated and the residue diluted with water. The aqueous layer was extracted with dichloromethane. The combined organic extracts were dried ($MgSO_4$) and concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 1 : 9) gave the cyano compound **17** (85%) as an oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·89 (m, 2H, $CH_2$), 2·32 (t, 2H, J = 7·0, $CH_2$), 2·60 (t, 2H, J = 7·5, $CH_2$), 5·86 (d,1H, J = 3·5, =CH), 5·90 (d,1H, J = 3·5, =CH), 6·49 (dd,1H, J = 7·7, 2·0, $H_{arom}$), 6·66 (dd,1H, J = 7·7, 2·0, $H_{arom}$), 6·74 (m,1H, $H_{arom}$). IR (neat) $\nu$ 2250, 1290 $cm^{-1}$. Calculated for $C_{12}H_{11}O_2N$: C, 71·62; H, 5·52; N, 6·96; found: C, 71·49; H, 5·50; N, 6·95.

*4-(1,4-Benzodioxin-5-yl)butanoic acid (**18**)*. A solution of cyano compound **17** (580 mg, 2·90 mmol) in 10% sodium hydroxide solution (20 mL) was heated under reflux for 20 h. The solvent was removed and the residue was dissolved in 1 M HCl (20 mL). The aqueous layer was extracted with ethyl acetate and the combined organic extracts were dried ($MgSO_4$) and concentrated in-vacuo. The acid **18** was obtained quantitatively as a white solid, mp 75–76°C. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·88 (q, 2H, J = 7·5, $CH_2$), 2·37 (t, 2H, J = 7·5, $CH_2$), 2·51 (t, 2H, J = 7·5, $CH_2$), 5·86 (d,1H, J = 3·5, =CH), 5·89 (d,1H, J = 3·5, =CH), 6·47 (dd,1H, J = 7·5, 1·8, $H_{arom}$), 6·66 (dd,1H, J = 7·5, 1·8, $H_{arom}$), 6·73 (m,1H, $H_{arom}$), 10·93 (br s,1H, $CO_2H$). IR (KBr) $\nu$ 3560–2560,

1705, 1290 $cm^{-1}$. Calculated for $C_{12}H_{12}O_4$: C, 65·44; H, 5·50; found: C, 65·29; H, 5·51.

N-*Propyl-4-(1,4-benzodioxin-5-yl)butyramide (**4b**)*. 4-Dimethylaminopyridine (330 mg, 3·54 mmol), and then acid **18** (410 mg, 2·36 mmol) were added at 0°C to a suspension of *n*-propylamine hydrochloride (290 mg, 2·60 mmol) in dry *N*, *N*-dimethylformamide (7 mL). EDCI (500 mg, 2·60 mmol) was then introduced. The reaction mixture was stirred at room temperature for 12 h under argon. After evaporation of the solvent, the residue was diluted with water and ethyl acetate (1 : 1). The aqueous layer was extracted with ethyl acetate and the combined organic extracts were dried ($MgSO_4$) and concentrated in-vacuo. Chromatography (ethyl acetate–petroleum ether, 7 : 3) gave the retroamide **4b** in 88% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0·90 (t, 3H, J = 7·3, $CH_3$), 1·49 (m, 2H, $CH_2$), 1·89 (m, 2H, $CH_2$), 2·15 (t, 2H, J = 7·5, $CH_2$), 2·47 (t, 2H, J = 7·5, $CH_2$), 3·20 (t, 2H, J = 7·2, $CH_2$), 5·57 (br s,1H, NH), 5·84 (d,1H, J = 3·7, =CH), 5·87 (d,1H, J = 3·7, =CH), 6·44 (dd,1H, J = 7·5, 2·0, $H_{arom}$), 6·64 (dd,1H, J = 7·5, 2·0, $H_{arom}$), 6·72 (m,1H, $H_{arom}$). IR (neat) $\nu$ 3410–3170, 1645, 1300 $cm^{-1}$. MS (CI/$NH_3$) m/z: 262 (M + 1). Calculated for $C_{15}H_{19}NO_3$: C, 68·93; H, 7·34; N, 5·36; found: C, 68·81; H, 7·34; N, 5·35.

*3-(2,3-Dihydro-1,4-benzoxathiin-5-yl)propyl acetate (**20**)*. Mercuric acetate (1·17 g, 3·66 mmol) was added to a solution of 5-(3-bromopropyl)-2,3-dihydro-1,4-benzoxathiin **19** (1·00 g, 3·66 mmol) in glacial acetic acid (20 mL). The reaction mixture was heated under reflux for 4 h, and concentrated in-vacuo. Ethyl acetate was added and the resulting precipitate was removed by filtration. The filtrate was washed with a saturated bicarbonate aqueous solution. The combined organic extracts were dried, and concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 1 : 3), and then trituration in dichloromethane–cyclohexane (1 : 1) to remove solid impurity gave the acetate **20** (83%) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1·96 (t, 2H, J = 7·1, $CH_2$), 2·65 (t, 2H, J = 7·1, $CH_2$), 3·13 (t, 2H, J = 4·7, $H_3$), 4·11 (t, 2H, J = 7·1, $CH_2$), 4·38 (t, 2H, J = 4·7, $H_2$), 6·69–6·76 (m, 2H, $H_6$ and $H_8$), 6·93 (m,1H, $H_7$). $^{13}$C NMR (62·9 MHz, $CDCl_3$), $\delta$ 19·2 ($CH_2$), 23·7 ($C_3$), 26·2 and 27·6 ($CH_2$), 62·1 and 62·9 ($C_2$ and $CH_2$) 114·5 (Ar), 115·2 ($C_{10}$), 120·1 (Ar), 123·0 ($C_7$), 136·7 ($C_5$), 150·1 ($C_9$), 169·4 (C = O). IR (neat) $\nu$ 1738, 1305, 1247, 1044, 1087 $cm^{-1}$.

*3-(2,3-Dihydro-1,4-benzoxathiin-5-yl)propanol (**21**)*. Potassium carbonate (986 mg, 7·13 mmol) was added to a solution of acetate **20** (1·20 g, 4·75 mmol) in methanol (48 mL) and water (12 mL). The reaction mixture was stirred for 2·5 h at room temperature. The salts were removed by filtration and the methanol evaporated. The residual aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), and concentrated in-vacuo. Filtration through silica gel ($CH_2Cl_2$) gave the alcohol **21** (94%) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3/D_2O$) $\delta$ 1·69 (t, 2H, J = 7·0, $CH_2$), 2·67 (t,1H, J = 7·0, $CH_2$), 3·13 (t, 2H, J = 4·4, $H_3$), 3·67 (t, 2H, J = 7·0, $CH_2$), 4·37 (t, 2H, J = 4·4, $H_2$), 6·69–6·77 (m, 2H, $H_6$ and $H_8$), 6·93 (m,1H, $H_7$). IR (neat) $\nu$ 3700–3100, 1300, 1242, 1076 $cm^{-1}$. Calculated for $C_{11}H_{14}O_2S$: C, 62·82; H, 6·72; S, 15·24; found: C, 62·65; H, 6·70; S, 15·10.

*3-(2,3-Dihydro-1,4-benzoxathiin-5-yl)propanal (**22**)*. A solution of dimethylsulphoxide (1·15 mL, 15·67 mmol) in dry dichloromethane (3·7 mL) was added to a solution of oxalyl chloride (680 $\mu$L, 7·85 mmol) in dry dichloromethane (18·8 mL) at −50°C under argon. The reaction mixture was stirred for 2 min, and a solution of alcohol **21** (1·50 g, 7·13 mmol) in dichloromethane (7·5 mL) was then added via a cannula at −50°C. After stirring for 30 min, triethylamine (5·0 mL, 35·7 mmol) was introduced. The reaction mixture was left to warm to room temperature, and stirring was continued for 2 h. The reaction mixture was diluted with dichloromethane. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 3 : 7) gave aldehyde **22** (84%) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2·75 (t, 2H, J = 7·8, $CH_2$), 2·85 (t, 2H, J = 7·8, $CH_2$), 3·13 (t, 2H, J = 4·4, $H_3$), 4·38 (t, 2H, J = 4·4, $H_2$), 6·70–6·78 (m, 2H, $H_6$ and $H_8$), 6·95 (m,1H, $H_7$), 9·85 (s,1H, CHO). IR (neat) $\nu$ 2870, 1738, 1310, 1247, 1080 $cm^{-1}$. Calculated for $C_{11}H_{12}O_2S$: C, 63·43; H, 5·82; S, 15·39; found: C, 63·35; H, 5·81; S, 15·24.

*3-(2,3-Dihydro-1,4-benzoxathiin-5-yl)propanoic acid (**23**)*. A solution of 3-(2,3-dihydro-1,4-benzoxathiin-5-yl)propanal **22** (190 mg, 0·91 mmol) in ethanol (4 mL) was added to a solution of silver nitrate (310 mg, 1·82 mmol) in distilled water (3·6 mL). The reaction mixture was stirred at room temperature, and a solution of sodium hydroxide (146 mg, 3·63 mmol) in distilled water (7·3 mL) was then added. The reaction mixture was stirred for 30 min and filtered. The solid residue was washed with ether and water. After separation, the aqueous layer was acidified. The

resulting precipitate was filtered, washed with water, and dried under high vacuum over $P_2O_5$. Acid **23** was obtained as a white solid in 64% yield. $^1$H NMR (250 MHz, DMSO-$d_6$) $\delta$ 2·40–2·50 (m, 2H, $CH_2$), 2·70 (t, 2H, J = 7·2, $CH_2$), 3·15 (t, 2H, J = 4·9, $H_3$), 4·28 (t, 2H, J = 4·9, $H_2$), 6·65 (m,1H, $H_{arom}$), 6·75 (m,1H, $H_{arom}$), 6·90 (m,1H, $H_7$), 12·16 (br s,1H, $CO_2H$). $^{13}$C NMR (62·9 MHz, DMSO-$d_6$) $\delta$ 23·8 and 26·8 ($CH_2$), 31·9 ($CH_2$), 63·5 ($C_2$), 115·2 ($C_{10}$), 116·0 (Ar), 120·4 (Ar), 123·7 ($C_7$), 136·8 ($C_5$), 150·7 ($C_9$), 172·7 (C = O).

N-*Methyl-3-(2,3-dihydro-1,4-benzoxathiin-5-yl)-propanamide* (**5**). This compound was prepared in 95% yield from acid **23** according to the procedure described for **4b**. Purification by column chromatography (chloroform–ethyl acetate, 7 : 3) gave the retroamide **5** as a white solid, mp 98–99°C. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 2·47 (m, 2H, $CH_2$), 2·79 (d, 3H, J = 4·9, $CH_3$), 2·93 (m, 2H, $CH_2$), 3·14 (m, 2H, $H_3$), 4·39 (m, 2H, $H_2$), 5·36 (br s,1H, NH), 6·71 (dd,1H, J = 8·1, 1·2, $H_{arom}$), 6·78 (br d,1H, J = 7·6, $H_{arom}$), 6·93 (m,1H, $H_7$). Calculated for $C_{12}H_{15}NO_2S$: C, 60·73; H, 6·37; N, 5·90; S, 13·51; found: C, 60·46; H, 6·35; N, 5·75; S, 13·53.

*4-(2,3-Dihydro-1,4-benzoxathiin-5-yl)butyronitrile* (***24***). Potassium cyanide (52 mg, 0·80 mmol) was added to a solution of 5-(3-bromopropyl)-2,3-dihydro-1,4-benzoxathiin **19** (200 mg, 0·73 mmol) in *N*, *N*-dimethylformamide (5 mL) under argon at room temperature. After stirring for 12 h, further potassium cyanide (52 mg, 0·80 mmol) was added, and stirring was continued for 12 h. The reaction mixture was the hydrolysed, the solvent evaporated, and the aqueous layer extracted with dichloromethane. The combined organic extracts were dried ($MgSO_4$), and concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 3 : 7) gave the cyano compound **24** (100%) as a colourless oil. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 1·99 (q, 2H, J = 7·2, $CH_2$), 2·36 (t, 2H, J = 7·2, $CH_2$), 2·74 (t, 2H, J = 7·2, $CH_2$) 3·13 (t, 2H, J = 4·6, $H_3$), 4·37 (t, 2H, H = 4·6, $H_2$), 6·71–6·76 (m, 2H, $H_6$ and $H_8$), 6·95 (m,1H, J = 7·9, $H_7$). $^{13}$C NMR (62·9 MHz, $CDCl_3$) $\delta$ 16·0 ($CH_2$), 24·1 ($CH_2$), 25·0 ($C_3$), 31·3 ($CH_2$), 64·0 ($C_2$), 116·2 ($C_6$ or $C_8$), 119·0 ($C_{10}$ or CN), 121·7 ($C_6$ or $C_8$), 124·3 ($C_7$), 137·5 ($C_5$), 152·1 ($C_9$). IR (neat) $\nu$ 2234, 1302, 1252, 1084, 1054. Calculated for $C_{12}H_{13}NOS$: C, 65·71; H, 5·99; N, 6·39; S, 14·62; found: C, 65·56; H, 5·98; N, 6·31; S, 14·53.

*4-(2,3-Dihydro-1,4-benzoxathiin-5-yl)butanoic acid* (***25***). This compound was prepared as a white solid, mp 93°C, in 71% yield, from the cyano compound **24** according to the procedure described for **10b** (heat under reflux for 24 h). $^1$H NMR (DMSO-$d_6$) $\delta$ 1·74 (t, 2H, J = 7·5, $CH_2$), 2·20 (t, 2H, J = 7·5, $CH_2$), 2·48 (t, 2H, J = 7·5, $CH_2$), 3·13 (t, 2H, J = 4·8, $H_3$), 4·27 (t, 2H, J = 4·8, $H_2$), 6·63 (d,1H, J = 7·1, $H_{arom}$), 6·71 (d,1H, 7·1, $H_{arom}$), 6·89 (m,1H, $H_7$), 12·00 (br s,1H, $CO_2H$). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta$ 23·6 and 24·3 ($C_3$ and $CH_2$), 31·3 and 32·8 ($CH_2$), 64·0 ($C_2$), 115·5 (CH), 116·4 ($C_{10}$), 121·2 (CH), 124·1 ($C_7$), 138·2 ($C_5$), 151·2 ($C_9$), 173·7 (C = O). IR (KBr) $\nu$ 3300–2790, 1712, 1040 cm$^{-1}$. Calculated for $C_{12}H_{14}SO_3$: C, 60·47; H, 5·93; S, 13·45; found: C, 60·28; H, 5·91; S, 13·39.

N-*Methyl-4-(2,3-dihydro-1,4-benzoxathiin-5-yl)butanamide* (**6a**). This compound was prepared as a white solid, mp 94°C, in 74% yield, from acid **25** and methylamine hydrochloride according to the procedure described for the retroamide **4b**. $^1$H NMR (300 MHz, $CDCl_3/D_2O$) $\delta$ 1·51–1·70 (m, 2H, $CH_2$), 1·96 (s, 3H, $CH_3$), 2·61 (t, 2H, J = 7·5, $CH_2$), 2·81 (t, 2H, J = 7·5, $CH_2$), 3·13 (t, 2H, J = 4·7, $H_3$), 4·38 (t, 2H, J = 4·7, $H_2$), 6·69–6·76 (m, 2H, $H_6$ and $H_8$), 6·93 (m,1H, $H_7$). $^{13}$C NMR (62·9 MHz, $CDCl_3$) $\delta$ 25·5 ($CH_2$), 26·0 ($CH_2$), 26·7 ($CH_3$), 32·9 ($CH_2$), 36·4 ($CH_2$), 65·2 ($C_2$), 116·7, 122·5 and 125·1 ($C_6$, $C_7$, $C_8$), 117·5 ($C_{10}$), 139·3 ($C_5$), 152·3 ($C_9$), 173·6 (C = O). IR (KBr) $\nu$ 3590–3270, 1722, 1274, 1116, 1075 cm$^{-1}$. MS (CI, $NH_3$), m/z 252 (M + 1). Calculated for $C_{13}H_{17}NO_2S$: C, 62·11; H, 6·83; N, 5·57; S, 12·75; found: C, 61·93; H, 6·80; N, 5·55; S, 12·66.

N-*Propyl-4-(2,3-dihydro-1,4-benzoxathiin-5-yl)-butanamide* (***6b***). This compound was prepared as a white solid, mp 93°C, in 96% yield, from acid **25** and propylamine hydrochloride, according to the procedure described for the retroamide **4b**. $^1$H NMR (300 MHz, $CDCl_3/D_2O$) $\delta$ 0·93 (t, 2H, J = 7·6, $CH_3$), 1·54 (q, 2H, J = 7·6, $CH_2$), 1·98 (q, 2H, 7·6, $CH_2$), 2·23 (t, 2H, J = 7·6, $CH_2$), 2·62 (t, 2H, J = 7·6, $CH_2$), 3·14 (t, 2H, J = 4·2, $H_3$), 3·22 (t, 2H, J = 7·6, $CH_2$), 4·39 (t, 2H, J = 4·2, $H_2$), 6·69–6·77 (m, 2H, $H_6$ and $H_8$), 6·94 (m,1H, $H_7$). IR (KBr) $\nu$ 3590–3260, 1668, 1274, 1120, 1075 cm$^{-1}$. MS (EI) m/z 279 (M). Calculated for $C_{15}H_{21}NO_2S$: C, 64·48; H, 7·58; N, 5·01; S, 11·48; found: C, 64·79; H, 7·59; N, 5·08; S, 11·39.

*Ethyl 5-(2,3-dihydro-1,4-benzoxathiin-5-yl)pent-2-enoate* (***26***). (Carbethoxymethylene)triphenylphosphorane (1·83 g, 5·28 mmol) was added to a solution of aldehyde **22** (500 mg, 2·40 mmol) in dry toluene. The reaction mixture was heated under reflux for 4 h. Triphenylphosphine oxide was precipitated by adding hexane to the ice-cooled reaction mixture, and removed by filtration. The filtrate was concentrated in-vacuo. Purification of the resi-

due by column chromatography (ethyl acetate–petroleum ether, 1 : 9) gave ester **26** (92%) as a colourless oil. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 1·28 (t, 3H, J = 6·1, $CH_3$), 2·52 (q, 2H, J = 5·6, $CH_2$), 2·71 (t, 2H, J = 5·6, $CH_2$), 3·15 (t,1H, J = 5·5, $H_3$), 4·20 (q, 2H, J = 6·1, $CH_2$), 4·41 (t, 2H, J = 5·5, $H_2$), 5·86 (d,1H, J = 11·1, =CH), 6·70–6·75 (m, 2H, $H_{arom}$ and =CH), 6·90–7·10 (m, 2H, $H_{arom}$). $^{13}$C NMR (62·9 MHz, $CDCl_3$) 14·3 ($CH_3$), 25·6 ($C_3$), 31·7 ($2CH_2$), 60·2 ($CH_2$), 64·8 ($C_2$), 116·5 (Ar), 117·0 ($C_{10}$), 121·8 (Ar and =CH), 124·9 ($C_7$), 138·2 ($C_5$), 148·0 (=CH), 152·0 ($C_9$), 166·6 (C = O). IR (neat) $\nu$ 1719, 1305, 1247, 1193, 1081. Calculated for $C_{15}H_{18}SO_3$: C, 64·71; H, 6·53; S, 11·52; found: C, 64·55; H, 6·49; S, 11·47.

*Ethyl 5-(2,3-dihydro-1,4-benzoxathiin-5-yl)pentanoate (**27**)*. Palladium on charcoal 10% (10% by weight) was added to a solution of unsaturated ester **26** (350 mg, 1·24 mmol) in ethanol (10 mL). The compound was hydrogenated, by means of a Parr apparatus, at 45 psig and room temperature for 12 h. The reaction mixture was filtered, and the filtrate concentrated in-vacuo. Purification by column chromatography (ethyl acetate–petroleum ether, 3 : 7) gave the saturated ester **27** (95%) as a yellow oil. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 1·24 (t, 3H, J = 7·1, $CH_3$), 1·59–1·75 (m, 4H, $CH_2$), 2·33 (t, 2H, J = 6·8, $CH_2$), 2·56 (t, 2H, J = 6·8, $CH_2$), 3·11 (t, 2H, J = 4·7, $H_3$), 4·11 (q, 2H, J = 7·1, $CH_2$), 4·36 (t, 2H, J = 4·7, $H_2$), 6·66–6·74 (m, 2H, $H_6$ and $H_8$), 6·91 (m,1H, $H_7$). IR (neat) $\nu$ 1732, 1305, 1247, 1081. Calculated for $C_{15}H_{20}SO_3$: C 64·25; H, 7·20; S, 11·43; found: C, 64·17; H, 7·18; S, 11·39.

*5-(2,3-Dihydro-1,4-benzoxathiin-5-yl)pentanoic acid (**28**)*. This compound was prepared as a white solid, mp 92–93°C, in 87% yield, from ester **27** according to the procedure described for **10a**. $^1$H NMR (250 MHz, DMSO-$d_6$) 1·30–1·50 (m, 4H, $CH_2$), 2·06–2·21 (m, 2H, $CH_2$), 2·34–2·48 (m, 2H, $CH_2$), 3·10 (t, 2H, J = 4·8, $H_3$), 4·22 (t, 2H, J = 4·8, $H_2$), 6·56 (d,1H, J = 7·3, $H_{arom}$), 6·67 (d,1H, J = 7·3, $H_{arom}$), 6·85 (m,1H, $H_7$), 11·95 (br s,1H, $CO_2H$). $^{13}$C NMR (62·9 MHz, DMSO- $d_6$) 24·3 and 24·7 ($CH_2$ and $C_3$), 28·2 ($CH_2$), 32·2 ($CH_2$), 33·4 ($CH_2$), 64·4 ($C_2$), 115·7 (Ar), 116·7 ($C_{10}$), 121·6 (Ar), 124·5 ($C_7$), 139·1 ($C_5$), 151·5 ($C_9$), 174·3 (C = O). IR (KBr) $\nu$ 3550–2500, 1706, 1305, 1232, 1082. Calculated for $C_{13}H_{18}SO_3$: C, 61·87; H, 6·40; S, 12·70; found: C, 61·79; H, 6·38; S, 12·65.

N-*Methyl-[5-(2,3-dihydro-1,4-benzoathiin-5-yl)]pentanamide (**7**)*. This compound was prepared as a white solid, mp 66°C, in 93% yield, from acid **28** according to the procedure described for retroamide **4b**. $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 1·60–1·83 (m, 4H, $CH_2$), 2·23 (t, 2H, J = 7·8, $CH_2$), 2·60 (t, 2H, J = 7·8, $CH_2$), 2·81 (s, 3H, $CH_3$), 3·15 (t, 2H, J = 4·6, $H_3$), 4·39 (t, 2H, J = 4·6, $H_2$), 6·63–6·80 (m, 2H, $H_6$ and $H_8$), 6·95 (m,1H, $H_7$). $^{13}$C NMR (62·9 MHz, $CDCl_3$) $\delta$ 24·9 and 25·6 ($C_3$ and $CH_2$), 25·6 ($CH_3$), 28·2 ($CH_2$), 32·1 ($CH_2$), 35·8 ($CH_2$), 64·1 ($C_2$), 115·4, 121·2 and 124·0 ($C_6$, $C_7$ and $C_8$), 138·8 ($C_5$), 151·2 ($C_9$ and $C_{10}$), 172·8 (C = O). IR (KBr) $\nu$ 3261–3590, 1720, 1276, 1113, 1075. MS (EI) m/z 265 (M). Calculated for $C_{14}H_{19}NSO_2$: C, 63·36; H, 7·22; N, 5·28; S, 12·08; found: C, 63·65; H, 7·19; N, 5·35; S, 12·40.

N-*Methyl-3-(4-dioxo-2,3-dihydro-1,4-benzoxathiin-5-yl)propanamide (**8**)*. Sodium dihydrogen phosphate buffer solution (0·16 M, 0·2 mL, 0·14 mmol) was added to a solution of aldehyde **22** (150 mg, 0·72 mmol). Potassium permanganate solution (1 M, 4·30 mL, 4·32 mmol) was then introduced. After stirring for 40 min at room temperature, the reaction was quenched with saturated sulphite sodium solution and manganese oxide was removed by filtration. The filtrate was washed with dichloromethane. Precipitation of the acid **29** occurred after acidification of the aqueous layer. Acid **29** (53%) was collected by filtration, dried under high vacuum and used in the next step without further purification. The retroamide **8** was then prepared as a white solid, mp 157°C, in 61% yield, from acid **29** according to the procedure described for compound **1**. $^1$H NMR (300 MHz, $CDCl_3/D_2O$) $\delta$ 2·57 (t, 2H, J = 7·3, $CH_2$), 2·78 (s, 3H, $CH_3$), 3·38 (t, 2H, J = 7·3, $CH_2$), 3·58 (t, 2H, J = 5·1, $H_3$), 4·73 (t, 2H, J = 5·1, $H_2$), 6·91 (d,1H, J = 8·0, $H_{arom}$), 7·01 (d,1H, J = 8·0, $H_{arom}$), 7·35 (m,1H, $H_7$). IR (KBr) $\nu$ 3292, 1652, 1286, 1230, 1177, 1134, 1075 cm$^{-1}$. MS (CI, $NH_3$) m/z 270 (M + 1). Calculated for $C_{12}H_{15}NO_4S$: C, 53·52; H, 5·61; N, 5·20; S, 11·91; found: C, 53·56; H, 5·57; N, 5·27; S, 11·91.

## *Pharmacology*

*Melatonin receptor-binding assays*. The affinity of the compounds was evaluated on ovine pars tuberalis membranes according to the method described by Morgan et al (1989a). Different concentrations of the new compounds were tested, in triplicate, for competitive inhibition of binding of 50 pM 2-[$^{125}$I]iodomelatonin to ovine pars tuberalis melatonin receptors under standard conditions (2 h, 37°C). Non-specific binding was assessed with 10 $\mu$M melatonin and data were analysed by computerized non-linear regression to determine affinity values

($K_i$, equilibrium dissociation constant). Experiments were repeated three times. Results are expressed as $-\log K_i \pm$ s.d.

*Cyclic AMP studies*. The functional activity of the compounds was determined by means of a single-dose cAMP inhibition bioassay, using cultured ovine pars tuberalis cells as described in detail by Morgan et al (1989b). Melatonin causes dose-dependant inhibition of forskolin stimulated cAMP production in these cells which can be compared with the melatonin-blocking or mimicking effects of a defined concentration of test compound. From these comparisons an activity index is calculated and provides a semi-quantitative measure of drug activity in this system. Drugs (10 $\mu$M) were tested, both alone and in combination with 1 nM melatonin, on cells stimulated by 10 $\mu$M forskolin; inhibitory effects were compared with that of forskolin or melatonin only. The forskolin/drug vs forskolin/melatonin index (F/D) provides a measure of the agonist-like activity of the drug and the forskolin/drug/melatonin vs forskolin/melatonin index (F/M/D) provides a measure of antagonist activity (Table 1). All experiments were conducted in triplicate for each treatment and the experiments were repeated three times.

## Results and Discussion

*Chemistry*

The synthesis of the novel melatonin analogues **1**–**8** was achieved by the routes depicted in Figures 2–7. Saponification of esters **9a** and **9c** (Guillaumet et al 1998) with aqueous sodium hydroxide in ethanol gave, after acidification, the corresponding acids **10a** and **10c**. Treatment of these compounds with methylamine in the presence of EDCI and 1-hydroxybenzotriazole (HOBt) in *N*, *N*-dimethylformamide led to the desired amides **1** and **3** (Figure 2).

Reduction of **9a** and **11** (Guillaumet et al 1998) with lithium aluminium hydride in ether gave the alcohols **12** and **13**, respectively. Tosylation then displacement of the resulting tosylates **14** and **15** with potassium cyanide gave the required nitriles **16** and **17**. Hydrolysis of the nitriles **16** and **17** with aqueous sodium hydroxide in ethanol provided, after acidification, the acids **10b** and **18** which react with appropriate amines to give the desired amides **2** and **4** (Figure 3).

Reaction of the bromide derivative **19** (Charton et al 1999) with mercury(II) acetate in acetic acid gave an ester that under basic conditions ($K_2CO_3$, $CH_3OH$, $H_2O$) afforded the corresponding alcohol **21**. Oxidation of this compound under Swern conditions led to aldehyde **22** and treatment of this product with silver nitrate in the presence of aqueous sodium hydroxide enabled access to acid **23**. The target compound **5** was prepared by reaction of acid **23** with methylamine hydrochloride in the presence of 4-dimethylaminopyridine (DMAP) and EDCI in *N*, *N*-dimethylformamide (Figure 4).

The bromide derivative **19** was converted into the nitrile **24** which was then hydrolysed with aqueous sodium hydroxide to the acid **25**. Treatment of **25** with methylamine in the presence of EDCI and HOBt or with *n*-propylamine hydrochloride in the presence of DMAP and EDCI, led to the desired amides **6** (Figure 5).

The aldehyde **22** enabled access to compound **27** by successive use of Wittig's reaction with (carbethoxymethylene)triphenylphosphorane and catalytic reduction, in the presence of palladium on carbon, of the intermediate unsaturated ester **26** (*E* isomer only). Saponification (10% NaOH, $C_2H_5OH$) and amidification (methylamine hydrochloride, DMAP, EDCI) gave the desired amide **7** (Figure 6).

The target sulphone **8** was synthesized by oxidation of the aldehyde **22** with potassium permanganate then treatment of the acid obtained, **29**, with methylamine, as before (Figure 7).

*Pharmacology*

The two best compounds of the series were (**2a**) and (**6a**) with identical *N*-methylbutyramide side-

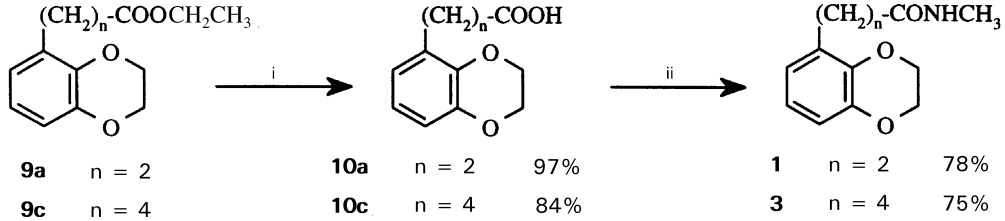


Figure 2. Synthesis of 2,3-dihydro-1,4-benzodioxin derivatives **1** and **3**. Reagents and conditions: i. 10% NaOH in $C_2H_5OH$, reflux, then HCl; ii. $CH_3NH_2$, HOBt, EDCI, DMF, room temp.

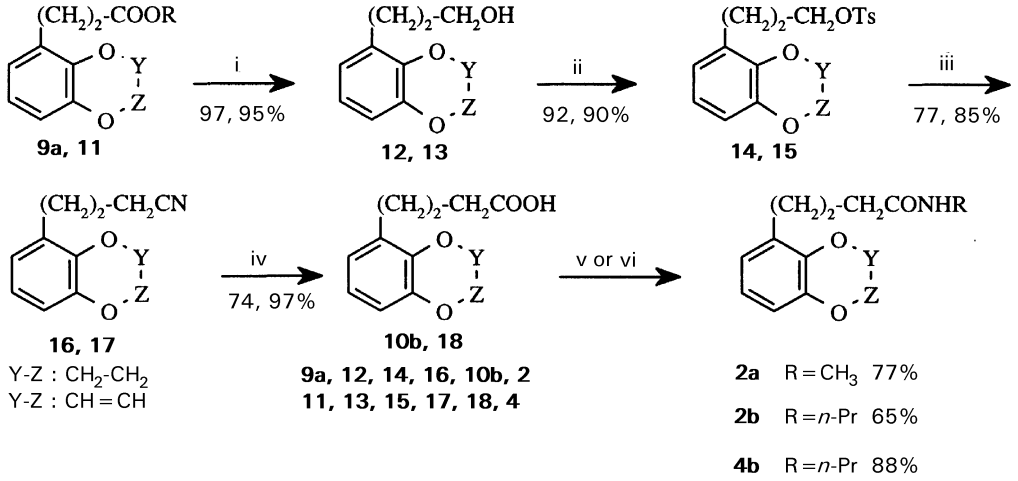


Figure 3. Synthesis of derivatives **2a**, **2b** and **4b**. Reagents and conditions: i. $LiAlH_4$, $(C_2H_5)_2O$, room temp., then reflux; ii. TsCl, $(C_2H_5)_3N$, $CH_2Cl_2$, room temp.; iii. KCN, DMF, 100°C; iv. 10% NaOH in $C_2H_5OH$, reflux, then 1 M HCl, room temp.; v. $CH_3NH_2$, HOBt, EDCI, DMF, room temp.; vi. $n$-$PrNH_3^+Cl^-$, EDCI, DMAP, DMF, room temp.

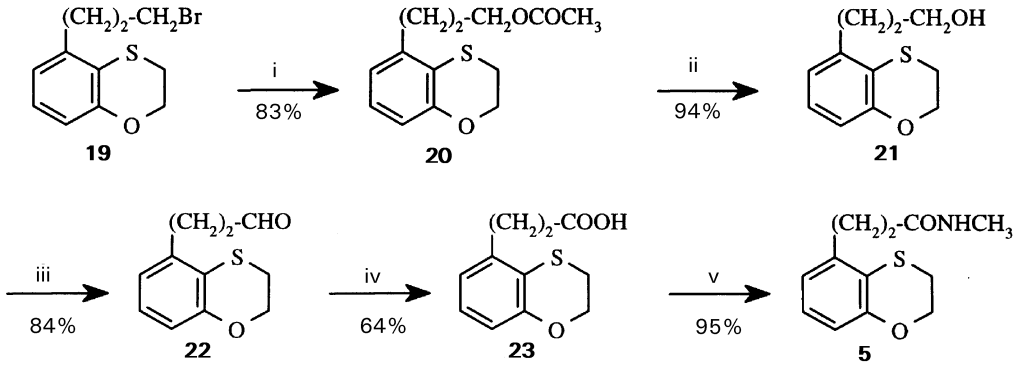


Figure 4. Synthesis of 2,3-dihydro-1,4-benzooxathiin analogue **5**. Reagents and conditions: i. mercury(II) acetate, $CH_3COOH$, reflux; ii. $K_2CO_3$, $CH_3OH$, $H_2O$, room temp.; iii. $(ClCO)_2$, DMSO, $CH_2Cl_2$, −50°C, then $(C_2H_5)_3N$, −50°C; iv. $AgNO_3$, aq. NaOH, $C_2H_5OH$, room temp.; v. $CH_3NH_3^+Cl^-$, DMAP, EDCI, DMF, room temp.

chains (Table 1). They were better melatoninergic ligands than **A** (Charton et al 1999).

For each skeleton tested, butyramide (**2a**, **2b**, **4b**, **6a**, **6b**) and pentanoylamide (**3**, **7**) side-chains resulted in similar affinities. This was not true of the propionamide side-chain (**1**), which led to loss of affinity. The worst affinity was obtained with the 4,4-dioxo-2,3-dihydro-1,4-benzoxathiin skeleton (compound **8**).

The biological activity of the molecules was most influenced by variation of the length of the side-chain. Indeed, irrespective of the type of skeleton, we obtained either agonist compounds with a butyramide side-chain (**2a**, **2b**, **6a**) or partial agonist or antagonist compounds with a pentanoylamide side-chain (**3**, **7**).

Therefore, even though these compounds have similar binding interactions with the receptor, subsequent activation of biological effects is dependent on side-chain length. Similar discrepancies in binding and activation have been reported (Leclerc et al 1998) for other retroamide compounds.

In this retroamide series, propyl substitution of the retroamide did not lead to the increase in affinity usually observed in amide series (Copinga et al 1993; Depreux et al 1994; Garratt et al 1996; Leclerc et al 1998; Teh & Sugden 1998). Accommodation of this alkyl group in the putative hydrophobic binding pocket possibly differs because the retroamide group induces a change of conformation at the binding site and reduced hydrophobic interaction compared with the usual amide function.

If the ratio F/D (an estimate of agonist activity) is compared for compounds in the 2,3-dihydro-1,4-benzodioxin, 1,4-benzodioxin, and 2,3-dihydro-1,4-benzoxathiin series with side-chains of the

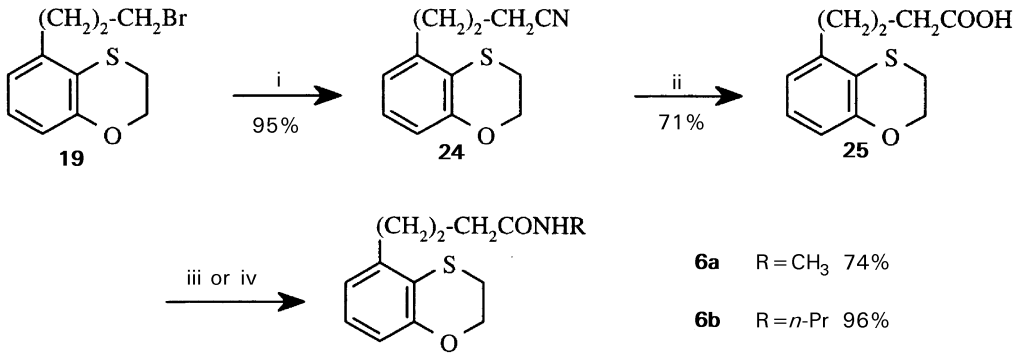


Figure 5. Synthesis of retroamides **6a** and **6b**. Reagents and conditions: i. KCN, DMF, room temp.; ii. 10% NaOH in $C_2H_5OH$, reflux, then 1 M HCl, room temp.; iii. $CH_3NH_2$, HOBt, EDCI, DMF, room temp.; iv.*n*-$PrNH_3^+Cl^-$, EDCI, DMAP, DMF, room temp.

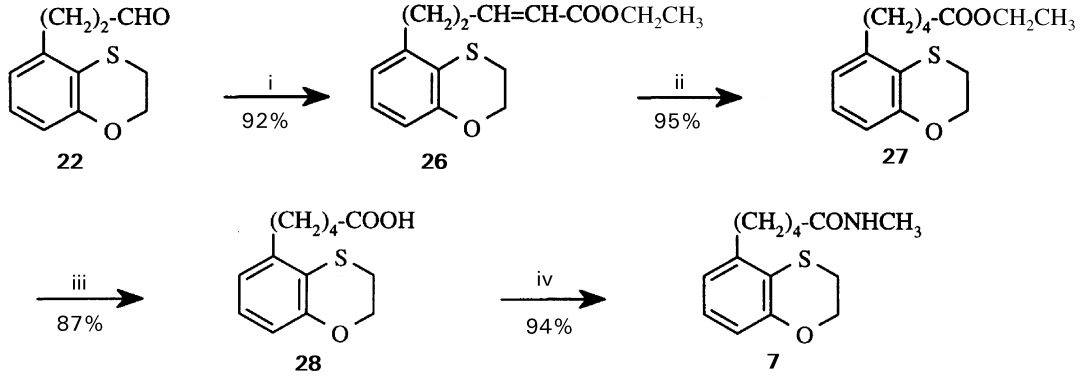


Figure 6. Synthesis of pentanoylamide analogue **7**. Reagents and conditions: i. $(C_6H_5)_3P=CHCOOC_2H_5$, toluene, reflux; ii. $H_2$, Pd/C, $C_2H_5OH$, 45 psig, room temp.; iii. 10% NaOH in $C_2H_5OH$, reflux, then 1 M HCl, room temp.; iv. $CH_3NH_3^+Cl^-$, DMAP, EDCI, DMF, room temp.

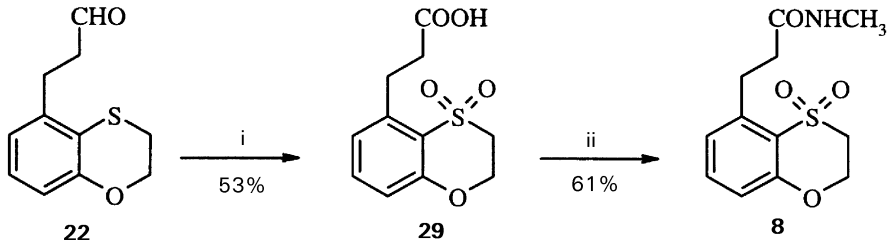


Figure 7. Synthesis of sulphone derivative **8**. Reagents and conditions: i. $KMnO_4$, $NaH_2PO_4$, *t*-BuOH; ii. $CH_3NH_2$, HOBt, EDCI, DMF.

same length it seems that a double bond (**4b**) or a sulphur atom in the *ortho* position of the side-chain (**6b**) lead to partial or total loss of agonist activity (reduced value of F/D).

Despite the similar binding values, in the 2,3-dihydro-1,4-benzodioxin series the butyramide **2b** (F/D = 0·90) and pentanoylamide **3** (F/D = 0·56) are agonist and partial agonist, respectively, whereas in the 2,3-dihydro-1,4-benzoxathiin series the butyramide **6b** (F/D = 0·47) and pentanoylamide **7** (F/D = 0·24) are partial agonist and antagonist, respectively, and in the 1,4-benzodioxin series the butyramide **4b** (F/D = 0·53) is a partial agonist.

Only **2a**, **2b** and **6a** have the same full agonist activity, with F/D = 0·97, 0·90 and 0·86, respectively. These three compounds have the main features required if the activity of these retroamide compounds is to be high—oxygen in the position *meta* to the side-chain and a butyramide side-chain.

In conclusion, the series of melatoninergic ligands with a retroamide group in common confirms the importance of the length of the side-chain in both binding to and activity at the melatonin

Table 1. Pharmacological evaluation of melatonin analogues on ovine pars tuberalis membranes.

| | Binding Activity | | | |
|---|---|---|---|---|
| | Ovine pars tuberalis $-\log Ki \pm$ s.d. (n = 3) | Agonist index F/D $\pm$ s.d. (n = 3) | Antagonist index F/M/D $\pm$ s.d. (n = 3) | Comment |
| Melatonin | 9·52 ± 0·10 | 1 | – | – |
| **1** | 5·10 ± 0·02 | 0·07 ± 0·19 | 0·94 ± 0·12 | No activity |
| **2a** | 8·08 ± 0·13 | 0·97 ± 0·26 | 0·86 ± 0·34 | Full agonist |
| **2b** | 7·57 ± 0·06 | 0·90 ± 0·05 | 0·87 ± 0·02 | Full agonist |
| **3** | 7·58 ± 0·04 | 0·56 ± 0·09 | 0·48 ± 0·12 | Partial agonist |
| **4b** | 7·60 ± 0·01 | 0·53 ± 0·01 | 0·76 ± 0·03 | Partial agonist |
| **6a** | 8·23 ± 0·07 | 0·86 ± 0·10 | 0·87 ± 0·13 | Full agonist |
| **6b** | 7·84 ± 0·15 | 0·47 ± 0·07 | 0·80 ± 0·01 | Partial agonist |
| **7** | 7·71 ± 0·12 | 0·24 ± 0·05 | 0·35 ± 0·24 | Antagonist |
| **8** | < 4 | 0·06 ± 0·01 | 0·98 ± 0·21 | No activity |
| **A** | 7·11 ± 0·01 | 0·85 ± 0·21 | 0·94 ± 0·25 | Full agonist |

receptor, and shows the influence of the skeleton on the activity of the compounds.

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