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Binding affinity and biological activity of oxygen and sulfur isosteres at melatonin receptors as a function of their hydrogen bonding capability

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Abstract

Analogues of melatonin (**1**) and of *N*-acetyl 5-ethoxytryptamine (**3**) in which the oxygen atoms are replaced by sulfur have been prepared and tested against human and amphibian melatonin receptors. All sulfur analogues show a decreased binding affinity at human MT_1 and MT_2 receptors and a reduced potency as melatonin agonists on the *Xenopus* melanophore assay. The 5-methoxy oxygen of melatonin is significantly more important for receptor binding than the amide oxygen. *N*-Acetyl 5-ethoxytryptamine shows a decrease in both binding affinity and potency in comparison with melatonin. In this series, replacing either the ethoxy or amide oxygen by sulfur has a similar but smaller effect on both binding affinity and potency. Using K_B^H values from Abraham's equations we have assessed the possibility of estimating EC_{50} values for sulfur isosteres from the EC_{50} values of their oxygen analogues.
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Keywords: Melatonin; Hydrogen bonds; Melanophores; Lawesson's reagent

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1. Introduction

The identification of three distinct melatonin receptor subtypes by molecular cloning experiments [1–4] has made possible a comparison of the melatonin and serotonin receptors. A consideration of the changes in the nature of the ligand from serotonin to melatonin (hydroxy to methoxy: amine to amide) led us to suggest that a histidine in putative transmembrane domain 5 (TM5) conserved in all melatonin receptors, which replaces serine in serotonin receptors, acts as a proton donor for the C-5 methoxy group of melatonin [5]. Molecular modelling came to a similar conclusion [6,7]. This concept has been supported by mutagenesis studies [8,9] which indicated that this histidine residue in the putative TM5 is important for binding, for access of melatonin to the binding site and for maintaining the required receptor conformation. Gubbitz and Reppert [10], in a study of chimeric receptor proteins between human $MT_1$and the melatonin-related H9 receptor, have reported that substitution of serine 20 in TM6 renders the receptor inactive. Serine residues in TM3 have also been shown to be critical for melatonin binding, but not for the binding of the antagonist luzindole [11]. The melatonin receptor has been subjected to a number of modelling studies based on both the amino acid sequence [6,12] and pharmacore models [13–16], and a number of active conformations for melatonin have been proposed. These models have been compared and assessed in a review by Mor et al. [17].

It has previously been shown that the 5-methoxy and amide groups are major factors of binding melatonin to its receptors [17]. Whereas replacing the 5-methoxy group by other electron donors, such as halogens, give compounds with significant agonist activity, replacement by poor electron donor groups, such as methyl, gave weak agonists [17]. An exception is *N*-[2-(5-ethylbenzo[b]thien-3-yl)ethyl]acetamide (S 22153), which is reported to have a high affinity for melatonin receptors although it has antagonist effects [18]. The binding affinity of both the 5-methoxy and amide groups probably consists of a number of factors such as dipole–dipole interactions, van-der-Waals attractive forces and hydrogen bonding. In order to estimate the hydrogen bonding component of binding, we have substituted sulfur for oxygen in both the methoxy and acetyl groups of the melatonin molecule and have also prepared the sulfur analogues of *N*-acetyl 5-ethoxytryptamine. The electron donating potential of sulfur is comparable to chlorine and it would be expected to make a similar contribution to any dipole–dipole interaction. It is, however, a much less efficient electron-donor to hydrogen than oxygen, and forms only weak hydrogen bonds. A comparison of the binding affinities of the sulfur and oxygen isosteres should give some indication of the contribution of hydrogen bonding to receptor binding. The binding affinity of all these compounds at the human MT_1 and MT_2 receptors was determined and their biological activity was then examined using the pigment aggregation response of a clonal *Xenopus laevis* melanophore cell line [19,20]. We have used the K_B^H values from Abraham's equations [21,22] to assess whether the EC_{50} values for the sulfur compounds can be estimated from the EC_{50} values of their oxygen analogues.

2. Materials and methods

2.1. Chemistry

Melting points were measured on a Gallenkamp MFB apparatus and are uncorrected. EI mass spectra were recorded on a Concept 32 Kratos or Fison V.G. Platform LCMS system mass spectrometers, and FAB mass spectra on a MS50 Kratos mass spectrometer. Only molecular ions (M^+) or $[M+1]^+$ ions and base peaks are reported. IR spectra were recorded on a Bruker IFS66 or Bio-Rad FTS155 spectrometer. NMR spectra were taken in $CDCl_3$ unless otherwise stated. ^1H NMR spectra were recorded using Bruker 360, 400 or 200 MHz spectrometers and the spectra are reported in δ. ^{13}C NMR spectra were recorded at 90 MHz on a Bruker 360 MHz spectrometer, or at 100 MHz on a Bruker 400 spectrometer and signals are reported in δ. Column chromatography was performed with Merck silica gel 9385. Microanalyses were carried out by the Butterworth Laboratories, Teddington, Middlesex.

2.1.1. Conversion of amides to thioamides using Lawesson's reagent

The amide (1 M equivalent) and Lawesson's reagent (0.5 M equivalents) were refluxed in toluene until HPLC indicated that no amide remained. The solvent was removed under reduced pressure, the residue chromatographed and the product triturated with ether to give a solid.

2.1.2. N-[2-(5-methoxyindol-3-yl)ethyl]thioacetamide (**2**)

White solid, 340 mg, 69%, mp 89–90 °C; ^1H NMR δ 2.48 (s, 3H), 3.10 (t, $J = 7.0$ Hz, 2H), 3.87 (s, 3H), 3.98 (m,2H), 6.88 (dd, $J = 8.8$, 2.2 Hz, 1H), 7.02 (s, 1H), 7.09 (d, $J = 2.2$ Hz, 1H), 7.26 (d, $J = 8.8$ Hz, 1H), 7,38 (br s, 1H), 8.12 (br s, 1H); ^{13}C NMR δ 23.1, 33.8, 45.8, 55.5, 99.9, 111.6, 112.2, 112.6, 122.4, 127.4, 131.2, 153.8, 200.4; IR 3403, 3284, 2925, 1653, 1559, 1545, 1457, 1369, 1230, 1103, 801 cm^{-1}; MS m/e 249 (100), 174. $C_{13}H_{16}N_2SO$ requires C, 62.87; H, 6.49; N, 11.28. Found: C, 62.68; H, 6.40; N, 11.14.

2.1.3. N-[2-(5-ethoxyindol-3-yl)ethyl]thioacetamide (**4**)

White solid, 24 mg, 23%, mp 103–104 °C; ^1H NMR δ 1.45 (t, $J = 7.0$ Hz, 3H), 2.48 (s, 3H), 3.10 (t, $J = 7.0$ Hz, 2H), 3.99 (m, 2H), 4.09 (q, $J = 7.0$ Hz, 2H), 6.89 (dd, $J = 8.8$, 1.8 Hz, 1H), 7.04 (s, 1H), 7.09 (d, $J = 1.8$ Hz, 1H), 7.28 (d, $J = 8.8$ Hz, 1H), 7.30 (br s, 1H), 7.99 (br s, 1H); ^{13}C NMR δ 15.5, 24.0, 34.8, 46.7, 64.7, 101.9, 112.4, 113.6, 113.7, 123.2, 128.2, 132.6, 154.1, 201.3; IR 3331, 2974, 1599, 1535, 1481, 1389, 1298, 1205, 1142, 1034, 953, 827, 800 cm^{-1}; MS m/e 263 (305), 188 (100%). $C_{14}H_{18}N_2OS$ requires C, 64.09; H, 6.92; N, 10.68. Found: C, 63.88; H, 6.85; N, 10.53.

2.1.4. N-[2-(5-ethylthioindol-3-yl)ethyl]thioacetamide (**6**)

White paste, 27 mg, 28%; ^1H NMR δ 1.22 (t, $J = 7$ Hz, 3H), 2.41 (s, 3H), 2.89 (q, $J = 7.0$ Hz, 2H), 3.02 (t, $J = 7.0$ Hz, 2H), 3.90 (q, 2H), 6.99 (d, $J = 1.8$ Hz, 1H), 7.20–7.30 (m, 2H), 7.69 (s, 1H), 8.52 (br s, 1H); ^{13}C NMR δ 14.7, 23.4, 30.2, 34.0,

46.4, 111.8, 111.9, 122.4, 122.9, 125.2, 126.2, 127.9, 135.4, 200.6; IR 3398, 3175, 2980, 1552, 1460, 1441, 1367, 1220, 1135 cm^{-1}; MS *m/e* 279 (100%).

*2.1.5. N-[2-(5-ethylthioindol-3-yl)ethyl]acetamide (**5**)*

Melatonin (696 mg, 3 mmol) was dissolved in CH_2Cl_2 (5 mL) and added dropwise to an ice-cold mixture of $AlCl_3$ (600 mg, 4.5 mmol) and ethanethiol (3.73 g, 60 mmol). The resulting mixture was stirred for 2 h at 0 °C and then a mixture of $AlCl_3$ (600 mg, 4.5 mmol) and ethanethiol (3.73 g, 60 mmol) was added. Stirring was continued at 0 °C for a further 3 h and then another mixture of $AlCl_3$ (600 mg, 4.5 mmol) and ethanethiol (3.73 g, 60 mmol) was added. The mixture was stirred and allowed to come to room temperature over 18 h. when HPLC indicated that the starting material had been consumed. The mixture was cooled to 0 °C and 1 M HCl (6 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (4×25 mL). The organic layers were combined, washed with brine and dried ($MgSO_{4)}$. The $MgSO_4$ was removed by filtration, the filtrate evaporated under reduced pressure and the resulting red, viscous oil chromatographed, eluting with EtOAc. Trituration with ether, followed by lyophilisation from dioxane gave **5** as a white solid, 114 mg, 15%, mp 95–96 °C; ^1H NMR δ 1.27 (t, $J = 7.0$ Hz, 3H), 1.95 (s. 3H), 2.82–3.00 (m, 4H), 3.59 (q, $J = 7.0$ Hz, 2H); 5.62 (br s, 1H), 7.01 (d, $J = 1, 8$ Hz, 1H), 7.28–7.32 (m, 2H), 7,68 (s, 1H), 8.42 (br s, 1H); ^{13}C NMR δ 14.6, 23.3, 25.1, 30.4, 39.7, 111.6, 112.6, 122.6, 125.2, 126.5, 128.0, 135.5, 170.1; IR 3403, 3284, 2925, 1653, 1545, 1457, 1369, 1230, 1103, 801 cm^{-1}; MS *m/e* 525 (40, $[M+1]_2^+$) 263 (100, M^+); $C_{14}H_{18}N_2OS$ requires 263.1218. Found: 263.1218. $C_{14}H_{18}N_2OS$ requires C, 64.09; H, 6.92; N, 10.68. Found: C, 63.74; H, 6.76; N, 10.48.

*2.1.6. 3-(2-Nitrovinyl)-5-methylthioindole (**9**)*

1-Dimethylamino-2-nitroethene (854 mg, 7.36 mmol) was added at 0 °C to trifluoroacetic acid (10 mL) under nitrogen. 5-Methylthioindole (**8**) [23] (1.20 g, 7.36 mmol) was then added and the mixture was stirred and allowed to warm to 45 °C. Stirring was continued for 10 min and the mixture was then cooled in ice and then poured into ice-cold water (40 mL). The mixture was extracted with EtOAc (3×30 mL), the organic extracts combined and washed with saturated $NaHCO_3$ (2×30 mL), brine (2×30 mL) and dried ($MgSO_4$). The $MgSO_4$ was removed by filtration and the filtrate evaporated under reduced pressure to give an orange oil. Trituration with CH_2Cl_2 gave **9** as yellow needles, 600 mg, 35%, mp 184–185 °C (dec); ^1H NMR (acetone-d_6) δ 2.58 (s, 3H), 7.29 (dd, $J = 8.4$, 2.0 Hz, 1H), 7.53 (d, $J = 8.4$ Hz, 1H), 7.88 (d, $J = 1.6$ Hz, 1H), 7.92 (d, $J = 13.0$ Hz, 1H), 8.14 (s, 1H), 8.16 (d, $J = 13.0$ Hz, 1H), 12.2 (br s, 1H); ^{13}C NMR δ 16.7, 107.9, 113.4, 118.9, 123.6, 125.8, 131.0, 131.5, 134.5, 136.0, 136.2; IR 3410, 1618, 1559, 1555, 1459, 1310, 1285, 1237, 1110, 884; MS *m/e* 234 (40), 218 (100). $C_{11}H_{10}N_2SO_2$ requires C, 56.93; H, 4.30; N, 11.96. Found: C, 56.88; H, 4.28; N, 11.92.

*2.1.7. N-[2-(5-Methylthioindol-3-yl)ethyl]acetamide (**7**)*

Compound 9 (800 mg, 3.43 mmol) was added in portions to a stirred suspension of $LiAlH_4$ (190 mg, 5 mmol) in dry THF (20 mL) at 0 °C. The mixture was then heated

to reflux with stirring for 3 h and then cooled in ice. Water (2 mL) was added, followed by aqueous NaOH (2 M, 6 mL) and then water (2 mL). The mixture was filtered and EtOAc (30 mL) was added to the filtrate. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×20 mL). The combined organic layers were extracted with 0.5 M HCl (2×25 mL). The aqueous extracts were combined, washed with EtOAc (25 mL) and then treated with aqueous NaOH (2 M) until the mixture was basic. The mixture was then extracted with EtOAc (4×50 mL) and the combined organic extracts were dried ($MgSO_4$). The solvent was removed under reduced pressure and the resulting orange oil was chromatographed, eluting with EtOAc. Evaporation of the solvent under reduced pressure gave 2-(5-methylthioindol-3-yl)ethylamine as a colourless gum, 191 mg, 27%, which was acetylated without further purification. The amine (150 mg, 0.73 mmol) was dissolved in dry CH_2Cl_2 (12 mL), stirred and acetic anhydride (86 mg, 0.85 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise over 15 min. The mixture was stirred for 2 h and water (10 mL) was then added. The mixture was separated and the aqueous layer was washed with CH_2Cl_2 (2×10 mL) and the organic layers combined and washed with water (10 mL), aqueous HCl (2 M, 10 mL), saturated aqueous $NaHCO_3$ (2×10 mL), brine (10 mL) and then dried ($MgSO_4$). After filtration the filtrate was evaporated under reduced pressure to give a grey solid. The solid was chromatographed, eluting with EtOAc, to give a white solid which was triturated with ether and recrystallised from EtOAc to give 7, white crystals, 67 mg, 37% (from amine), mp 109–111 °C; 1H NMR δ 1.91 (s, 3H), 2.49 (s, 3H), 2.90 (t, $J = 7.0$ Hz, 2H), 3.49 - 3.57 (t, $J = 6.4$ Hz, 2H), 5.94 (br t, $J = 5.4$ Hz, 1H), 6.96 (s, 1H), 7.17–7.23 (m, 1H), 7.25–7.29 (m, 1H), 7.58 (s, 1H), 8.90 (br s, 1H); ^{13}C NMR δ 18.3, 22.7, 24.6, 39.4, 111.5, 111.7, 118.9, 122.5, 123.4, 126.7, 127.5, 134.7, 170.0; IR 3273, 2918, 1657, 1643, 1536, 1545, 1434, 1276, 1104, 895 cm^{-1} MS m/e 249 (20), 190 (100) 143. $C_{13}H_{16}N_2SO$ requires C, 62.87; H, 6.49; N, 11,28. Found: C, 62.58; H, 6.39; N, 11.16.

2.2. Pharmacology

The affinity of the analogues was determined in competition radioligand binding assays using 2-[^{125}I]iodomelatonin (specific activity 2000 Ci/mol, Amersham, UK) as described previously [24] on the recombinant human MT_1 and MT_2 subtypes expressed in NIH 3T3 cells, kindly provided by Dr. S.M. Reppert (Harvard Medical School, Boston, MA). Biological activity was assessed in a well-established, specific model of melatonin action, the pigment aggregation response of *X. laevis* melanophores [19,20]. In these cells many thousands of black pigment granules are normally distributed throughout the cell, and addition of melatonin induces their rapid movement towards the centre of the cell. This response can be quantified by measuring the changes in light absorbance of the cells as the pigment concentrates near the cell centre. In the present study, a clonal melanophore line, generously provided by Dr. Michael Lerner (Department of Dermatology, University of Texas), was used. Melanophore cells were grown in 96-well tissue culture plates, and growth medium [25,26] was replaced with $0.7 \times$ L-15 culture medium 18 h before analogues were tested. Initial absorbance of cells (A_i, 630 nm) was measured in each well using a

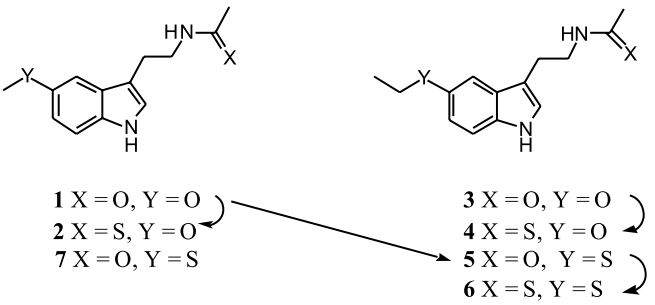
Bio-Tek microtiter plate reader (model EL311, Anachem, UK), then the cells were treated with a specific concentration of the analogue. All experiments used quadruplicate wells at six different concentrations. The final absorbance (A_f) was measured after 60 min, and the fractional change in absorbance ($1 - A_f/A_i$) was calculated. Vehicle did not alter pigment granule distribution itself or inhibit responses to melatonin. The concentration of analogue producing 50% of the maximum agonist response (EC_{50}) was determined using a curve-fitting program [27]. Data are reported as the negative log EC_{50} (pEC_{50}).

3. Results and discussion

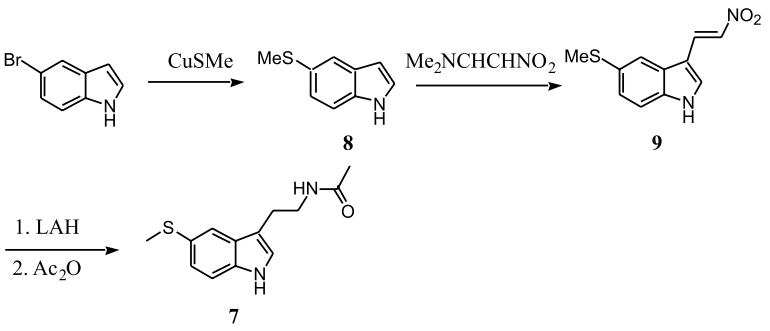
3.1. Synthesis

The conversion of the amide to the thioamide was carried out with Lawesson's reagent, 2,4-bis(4-methoxyphenyl)-1,3-dithiadiphosphetaine-2,4-disulfide [28,29]. Thus reaction of melatonin (**1**) with Lawesson's reagent (2×0.5 molar equivalents) gave the desired thioamide (**2**) in 69% yield (Scheme 1). The ^{1}H NMR spectrum of **2** showed the expected downfield shift of the *N*-acetyl side chain methyl group from δ 1.93 to δ 2.5 in the *N*-thioacetyl derivative, and a smaller downfield shift is also observed for the C-9 methylene protons. The acetamide NH proton at δ 5.66 is shifted downfield to δ 7.4 for the thioacetamide NH proton. The IR spectrum showed no amide stretching band at ca. 1650 cm^{-1} and the ^{13}C NMR spectrum showed a signal at δ 200 assigned to the C=S carbon. Treatment of the corresponding ethoxy derivative **3** in the same way gave the thioamide **4** in 23% yield.

Although there did not seem to be available a convenient method to convert the methoxy group of melatonin into the methylthio analogue, Caubere et al. [30] have reported a method for converting methoxy groups into the ethylthio analogue. Treatment of melatonin (**1**) with aluminium trichloride and ethane thiol gave the ethylthio derivative **5** in 15% yield. The ^{1}H NMR spectrum of **5** showed the ethyl proton signals at δ 1.24 and 2.9 and the presence of a C=O stretching frequency at 1650 cm^{-1} in the IR spectrum indicated that the acetyl group had not been affected.



Scheme 1.



Scheme 2.

The 5-ethylthio derivative **5** was now available for conversion into the corresponding thioamide by the application of Lawesson's reagent, and this proceeded to give **6** in 28% yield. The low yield in this and similar reactions appears to occur primarily during the purification procedures, examination of the course of the reaction by NMR spectroscopy indicating that only identifiable products were obtained. No optimisation of the processes was, however, attempted.

We then returned to the problem of preparing the methylthio analogue **7** of melatonin. There was a literature report [31] for the synthesis of 5-methylthiotryptamine which had found subsequent use, but the overall yield was only 6%. More recently, Yang et al. [32] published a method for the preparation of methylthioindole using a halogen-metal exchange procedure. A similar method had previously been reported by Guillaume et al. [23] using methylthiocopper and we elected to use this. Treatment of 5-bromoindole with methylthiocopper gave the desired 5-methylthioindole (**8**) in 55% yield (Scheme 2). This could be converted into the nitrovinylindole **9** in 35% yield by treatment with 1-dimethylamino-2-nitroethylene by the procedure of Büchi and Mak [33], this being superior to the two step Vilsmeier-Haack: Henry sequence in this case.

Reduction of **9** with LAH and acylation with acetic anhydride gave the desired methylthiomelatonin analogue **7**, albeit in very poor overall yield from 5-bromoindole (ca. 2%). An attempt was made to convert a small amount of **7** into the thioacetyl derivative with Lawesson's reagent but although mass spectroscopic evidence was adduced for the preparation of the desired compound, none could be isolated.

3.2. Pharmacology

Table 1 shows the pK_i values for the binding affinities of melatonin, *N*-acetyl 5-ethoxytryptamine and their analogues to the human MT_1 and MT_2 receptors. Fig. 1 shows the pigment aggregation produced in *X. laevis* melanophores by varying the concentration of melatonin and the various sulfur derivatives, and Table 2 shows the calculated pEC_{50} values for these analogs.

Table 1
Binding affinity of melatonin (**1**), *N*-acetyl 5-ethoxytryptamine (**3**) and their sulfur analogues on human MT_1 and MT_2 receptor subtypes

Compound	Y	X	MT_1 $pK_i \pm SEM$	MT_2 $pK_i \pm SEM$
Melatonin, **1**	O	O	9.31 ± 0.12	9.43 ± 0.18
2	O	S	8.88 ± 0.03	9.16 ± 0.08
7	S	O	7.77 ± 0.08	7.79 ± 0.04
N-Acetyl 5-ethoxytryptamine, **3**	O	O	8.76 ± 0.05	8.73 ± 0.05
4	O	S	8.34 ± 0.01	8.01 ± 0.08
5	S	O	8.68 ± 0.05	8.13 ± 0.06
6	S	S	7.34 ± 0.07	7.66 ± 0.10

pK_i values are the mean ± SEM of six concentration–response curves. See Scheme 1 for structures of compounds.

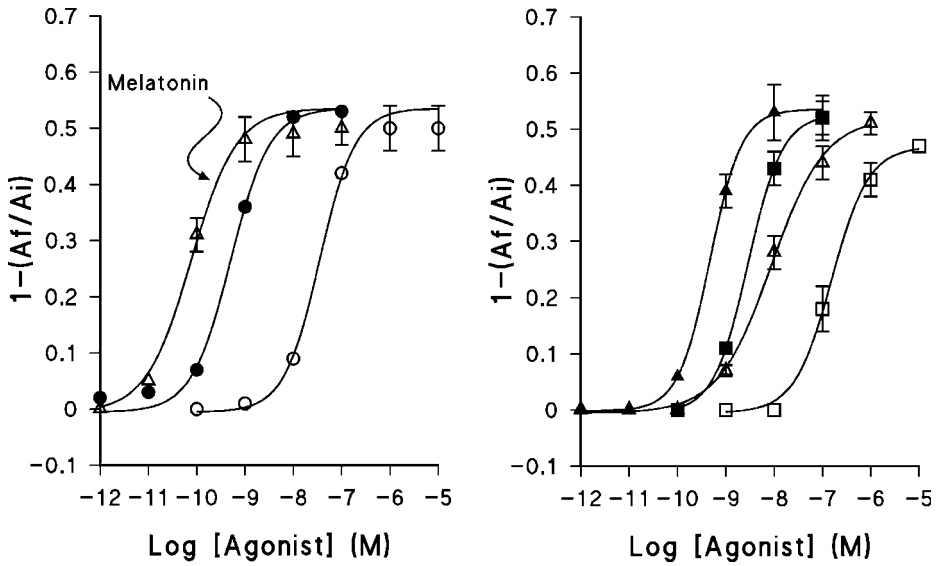


Fig. 1. Pigment aggregation produced in *X. laevis* melanophores by melatonin and the sulfur analogues. The change in pigment distribution was determined by measuring cell absorbance before (A_i) and 60 min after (A_f) addition of melatonin or analogues **2** (●), **7** (○), **3** (▲), **4** (△), **5** (■), **6** (□). Each point is the mean ± SEM of triplicate wells. Error bars have been omitted where the SEM was less than 0.02.

4. Discussion

Introduction of sulfur for the amide oxygen, compound **2**, (Table 1) results in an ca. 2.5-fold reduction in binding affinity to the human MT_1 and an ca. 2-fold reduction in binding affinity to the MT_2 receptor subtype. Introducing sulfur for the methoxy oxygen, compound **7**, results in a ca. 35-fold reduction in binding affinity to

Table 2
Potency of melatonin (**1**) and its sulfur analogues and *N*-acetyl 5-ethoxytryptamine (**3**) and its sulfur analogues on *X. laevis* melanophores

Compound	*Y*	*X*	$pEC_{50} \pm SEM$
Melatonin, **1**	O	O	10.30 ± 0.06
2	O	S	9.36 ± 0.14
7	S	O	7.58 ± 0.04
N-Acetyl 5-ethoxytryptamine, **3**	O	O	9.44 ± 0.12
4	O	S	8.24 ± 0.24
5	S	O	8.55 ± 0.15
6	S	S	6.72 ± 0.18

pEC_{50} values are the mean ± SEM of six concentration–response curves. See Scheme 1 for structures of compounds.

$MT_1$and an ca. 44-fold reduction in binding affinity to MT_2. The oxygen of the 5-methoxy group of melatonin is thus significantly more important in binding to both human melatonin receptor subtypes than is the amide oxygen. If one assumes that the change in binding affinities are only dependent on the change in hydrogen bond forming propensities of the two elements, then it might be possible to take Abraham and co-workers' K_B^H values [22] for appropriate model compounds. Assuming the binding affinities are inversely proportional to the K_B^H values gives Eq. (1).

$$EC_{50}(S) = K_B^H(\text{reference O compound})/K_B^H(\text{reference S compound})EC_{50}(O). \quad (1)$$

Taking their log K_B^H data for tetramethylurea (2.346) and for tetramethylthiourea (1.283) as models for the amide to thioamide conversion (**1** to **2**) gives a value of 1.83 for $K_B^H(O)/K_B^H(S)$. One then derives EC_{50} values for **2** from equation 1 of 1.56×10^{-9} (pK_i 8.81) for MT_1 and 1.10×10^{-9} (pK_i 8.96) for MT_2, in reasonable accord with the observed values. Taking their log K_B^H data for Et_2O (0.988) and for Et_2S (0.220) for the conversion of OMe into SMe (**1** to **7**) gives a value of 4.5 for $K_B^H(O)/K_B^H(S)$. Introducing this into equation 1 gives pK_i values of 8.42 for MT_1 and 8.57 for MT_2. These values clearly have greatly overestimated the binding affinity of the sulfur derivatives. This probably indicates that equation 1 does not depict the change of oxygen to sulfur in a valid manner in this case, although it is also conceivable that the models we have used are not appropriate. The K_B^H value of methylphenylether (0.708), a more suitable model, would, however, require a very low value for the K_B^H of methylthioether (0.02) to reproduce the correct EC_{50} for **7**. A value for methylthioether was not determined by Abraham [34], and there is no K_B^H value of magnitude comparable to 0.02 in the tables [21].

Sulfur substitution in melatonin has a much greater effect on potency in the functional pigment aggregation assay in melanophores than it does on binding affinity in the human receptors. Compound **2** is ca. 9-fold less potent than melatonin while compound **7** is ca. 500-fold less potent. One possible explanation is that the Mel_{1c} receptor, which mediates pigment aggregation in *Xenopu*s melanophores is more reliant on hydrogen bonding to the 5-methoxy oxygen than are the human MT_1 and MT_2 receptor subtypes.

Introduction of the 5-ethoxy group, compound **3**, for the 5-methoxy group of melatonin leads ca. 4-fold decrease in binding at MT_1 and a 5-fold decrease in MT_2 (Table 1) with a 7-fold reduction in potency (Table 2). The steric sensitivity of this binding site has been observed previously [35]. The effect of replacing ethyl for methyl is similar in magnitude to that for replacing the *N*-acetyl oxygen of melatonin for sulfur (Table 1, compound **2**). Compound **3** can now be used as a model for the effects on the system of replacing the oxygens by sulfur. Replacing the acetyl oxygen in compound **3** by sulfur, compound **4**, leads to ca. 3-fold reduction in binding at MT_1 and ca. 5-fold reduction at MT_2 (Table 1) with a 16-fold reduction in potency (Table 2). The loss in potency is larger, but of a similar magnitude, to the 8-fold effect of changing the acetyl oxygen of melatonin for sulfur. Replacing the oxygen of the 5-ethoxy group by sulfur, compound **5** has virtually no effect binding to MT_1 and an ca. 4-fold decrease in affinity to MT_2. These effects are much smaller than those for the corresponding changes to melatonin. The potency is reduced by only 8-fold (Table 2), again much less than the equivalent substitution in the melatonin series. Part of this difference between the changes to melatonin and **3** may be ascribed to the lower binding affinity of the latter. The 5-ethoxy group has less affinity for the receptor and this diminution may occur from a less favourable conformation for hydrogen bond formation. Substitution of the oxygen for sulfur would then be less disruptive. Although this may be so, comparison of the potency of **5** with melatonin shows only a 56-fold decrease of potency, an order of magnitude less than for the substitution of sulfur for the 5-methoxy oxygen of melatonin. When both oxygen atoms of **3** are substituted by sulfur, compound **6** (Table 2), the potency falls by a factor of 525. The magnitude of this reduction again suggests that the effect of substituting the oxygen of the 5-ethoxy group in **3** for sulfur is markedly less than the effect for the corresponding substitution in melatonin. Compound **6** is ca. 4000 times less potent than melatonin, showing the combined effects of the ethyl for methyl and sulfur for oxygen substitutions.

5. Conclusions

The 5-methoxy group of melatonin is a major factor in the binding of melatonin to its receptor as had previously been shown [17]. The binding of the oxygen is likely to involve both electrostatic interactions and hydrogen bonding, and mainly the latter should be affected by substitution of sulfur for oxygen. The ca. 40-fold diminution in binding affinity on this exchange can be reasonably be ascribed to the decrease in hydrogen bonding. The 5-methoxy methyl group appears to occupy a sterically demanding pocket, since exchanging it for ethyl leads to a considerable decrease in binding. Exchanging sulfur for oxygen in the 5-ethoxy series has much less effect on the binding and this is only partially explained by the prior disruption of the hydrogen bond by substitution of ethyl for methyl. The amide oxygen may also act as an electron donor to the formation of a hydrogen bond with a receptor amino acid, but this is weaker and of smaller significance to the stability of the receptor–melatonin complex.

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