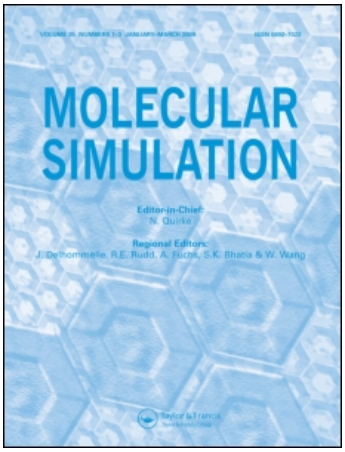


Molecular Simulation
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644482

A review of the molecular conformations of melatonin ligands at the melatonin receptor

Paul W. R. Harris[a]; Helmut M. Hügel[a]; Faizul Nurlawis[a]
[a] Department of Applied Chemistry, RMIT University, Melbourne, Vic., Australia

Online publication date: 26 October 2010

To cite this Article Harris, Paul W. R. , Hügel, Helmut M. and Nurlawis, Faizul(2002) 'A review of the molecular conformations of melatonin ligands at the melatonin receptor', Molecular Simulation, 28: 10, 889 — 902
To link to this Article: DOI: 10.1080/089270204000002557
URL: http://dx.doi.org/10.1080/089270204000002557

Molecular Simulation, 2002 Vol. 28 (10–11), pp. 889–902

A REVIEW OF THE MOLECULAR CONFORMATIONS OF MELATONIN LIGANDS AT THE MELATONIN RECEPTOR

PAUL W.R. HARRIS, HELMUT M. HÜGEL* and FAIZUL NURLAWIS

Department of Applied Chemistry, RMIT University, G.P.O. Box 2476,Melbourne, Vic. 3001, Australia

(Received May 2001; In final form November 2001)

This review examines the 1992–2000 literature on studies of the molecular conformations of melatonin ligands at the melatonin receptor. In order to investigate quantitative structure-affinity relationships between different chemical classes of melatonergic ligands binding to the melatonin GPCR, CoMFA has been applied to extended sets of compounds, to obtain 3D-QSAR agonist/antagonist models. The results of several authors have suggested that the active conformation of the C-3 aminoethyl side chain of melatonin and related compounds is in a folded form, orthogonal to the aromatic ring. Positive steric potentials were found in the C-2 region, surrounding the C-5 methoxy group and near the *N*-acyl group of the side chain, while substituents in positions C-6 and C-7 cause a decrease in affinity. Negative steric regions were found between indole N-1 and C-2. Receptor binding affinities have been predicted for a range of structurally diverse compounds for the sheep brain melatonin receptor considering steric, electrostatic and lipophilic fields.

Keywords: Melatonin receptor-binding; Melatonin pharmacophore model; CoMFA analysis; 3D-QSAR; Molecular modeling

INTRODUCTION

The earth in which we live continuously cycles from light to dark once every 24 h and most forms of life have adapted to this regular change in the environment by developing a variety of circadian rhythms. Each day our bodies adjust themselves in a variety of ways to meet the demands of the two environments in which we

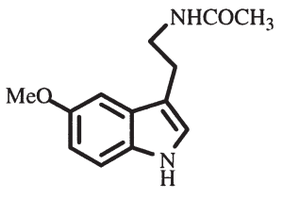
*Corresponding author.

ISSN 0892-7022 © 2002 Taylor & Francis Ltd
DOI: 10.1080/0892702021000002557

live our lives. Our circadian cycles are synchronized by these environmental light–dark cycles called zeitgebers that can control the timing of circadian rhythms. Indeed light is acting as a drug to alter neurochemical activity in the brain. Melatonin (*N*-acetyl-5-methoxytryptamine, aMT) (Fig. 1) is a neurohormone produced and secreted by the pineal gland and its biosynthesis reaches a peak about six hours after dark and passes through a minimum about six hours after re-exposure to light. This effect, known as a circadian rhythm [1,2] is generated from the suprachiasmatic nuclei, responsive to the photoperiodic environment.

The usefulness of melatonin for human health cannot be underestimated given the growing acceptance that circadian rhythm disorders underlie a number of medical disorders [3,4]. Therapeutic applications have been developed for the treatment of delayed sleep phase syndrome and melatonin exhibits good sleep-inducing effects. Melatonin has been advocated as a natural product capable of resetting the circadian rhythm in individuals with jet-lag syndrome and seasonally affective disorders. Administered at the beginning of the night, melatonin advances the sleeping phase, an effect similar to that obtained by bright light. The melatonin receptor belongs to the superfamily of guanine nucleotide-binding protein (G protein—coupled receptors, GPCR) and melatonin is assumed to dock to a binding domain within the seven transmembrane proteins of the receptor and inhibit cAMP accumulation as their common signaling mechanism. For melatonin, the primary structures of some 23 GPCR have been determined [5]. At the present time, three types of high affinity receptors have been found: Mel_{1a}, Mel_{1b}, Mel_{1c}, the first two receptors have been cloned from mammals, Mel_{1c}, has only been cloned from *Xenopus laevis*, *zebrafish* and *chickens*.

This review examines the recent literature concerning the conformational studies that have been reported on melatonin receptor ligands. Whilst the applications and uses of melatonin have received a lot of attention and interest, at the molecular level, the interaction between melatonin and the high affinity receptor is poorly understood [5–7].



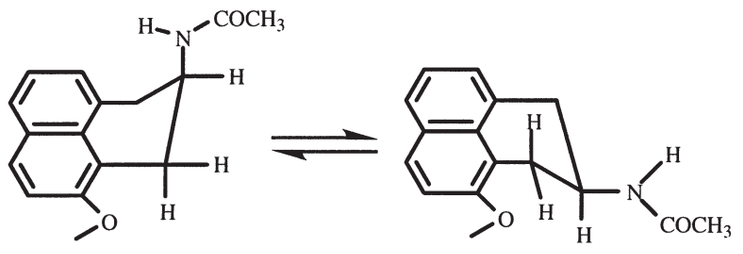
Melatonin

FIGURE 1 Structure of the neurohormone produced by the pineal gland.

CoMFA THEORY

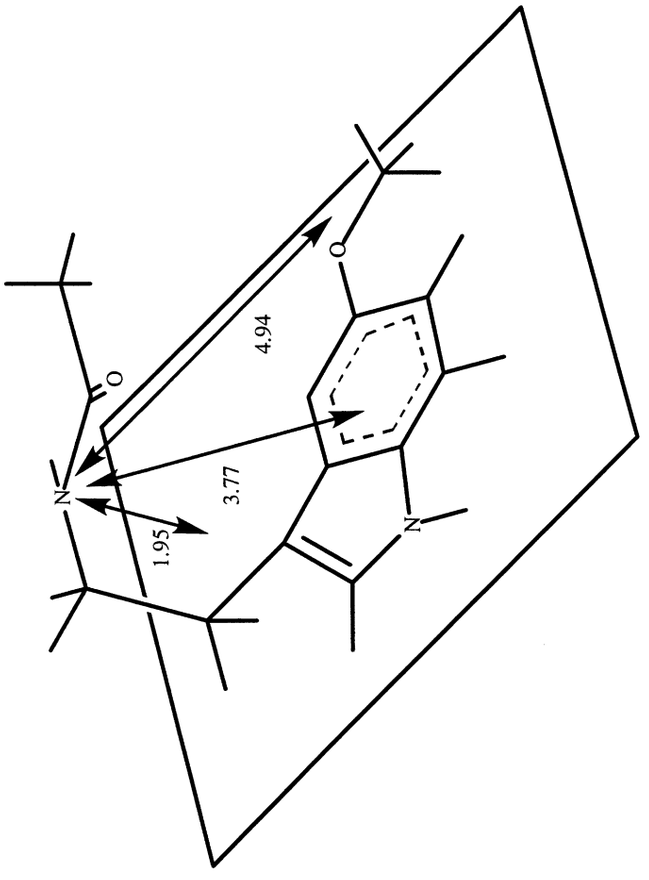
The objectives of Conformational Molecular Field Analysis (CoMFA), is that changes in a dependent variable are often related to differences in the shape of the non-covalent fields surrounding the set of molecules to be tested. For the shape of a molecule to be put into a QSAR table, the magnitudes of its steric, electrostatic and lipophilic fields are sampled at regular intervals throughout a defined region. Of the many adjustable parameters in CoMFA, one of the most important is the relative alignment of all the individual molecules in a way that properly aligned molecules have a comparable conformation and a similar orientation in space. The QSAR is then generated by partial least square analysis of the data to construct the models. The value of the resulting QSAR can be determined through the cross-validated q^2 reported by the partial least squares. If the data is acceptable, the CoMFA QSAR can then be manipulated using various graphics techniques to obtain figures that represent the most relevant regions of space where the variations of the fields are largest. If the data is unsatisfactory, the alignment of one or more molecules can be changed, or some other parameters altered and the analysis repeated. Once an acceptable QSAR has been derived, the prediction of the dependent variable for new compounds can be obtained. This approach has been widely applied to different classes of compounds and receptors.

Sicsic and coworkers [8] used the CoMFA method that was pioneered by Cramer [9,10] in 1988, to develop a three dimensional model pharmacophore describing the common geometry of a series of compounds and its relation to receptor activity. In order to use the CoMFA approach, a tricyclic conformationally constrained compound (Fig. 2), which exhibited better binding



Tricyclic rigid compound used as template structure

FIGURE 2 Structure of the tricyclic conformationally constrained compound selected as the template for the CoMFA alignment rules.



Sicsic melatoninergic CoMFA model pharmocophore

FIGURE 3 Structure from CoMFA analysis of melatonin in which the C-3 side chain is folded towards the ring in a syn conformation.

affinities than other compounds in the training set was selected as the basic template for the CoMFA alignment rules.

Using the axial conformation of the tricyclic template structure for CoMFA analysis, the model produced the best pharmacophore illustrated in Fig. 3(distances in Å) whereby the C-3 side chain of the active conformation of melatonin is folded towards the indole ring in a syn conformation. What are the implications of this finding towards understanding melatonin—receptor interactions? It may be of benefit having a compact melatonin conformation so that the side chain N–H group can maximize H-bonding with complementary receptor amino acids.

This could explain why ligands with an alicyclic amide group may not be able to adopt this folded bioactive pharmacophore conformation and therefore serve as receptor antagonists. The best CoMFA model had a ratio of 52/48 for electrostatic/steric fields, no lipophilic fields were included. A 60° rotation of the conformation of the methyl group of the methoxy-aryl substituent of the compounds studied should also have been included in the pharmacophore analysis.

Navajas and coworkers [11] used the secondary structure of rhodopsin to construct a model for melatonin recognition at its GPCR. The criteria upon which they proposed their model were: (i) that the oxygen and methyl group of 5-methoxy substituent are specifically recognized, (ii) that the amide oxygen of the *N*-acetyl group is also specifically identified and that this recognition results in high binding affinity at a distance of about 10.80 Å from the methoxy group, (iii) a π-aromatic interaction between the receptor and the indole ring stabilizes the docking of melatonin at its receptor, (iv) the energetically minimized conformation of melatonin occupies a tilted L form, the methoxy and *N*-acetyl groups are recognized in a different plane from the indole ring. Unlike the previous pharmacophore by Sicsic, the melatonin model structure had the side chain extended. A melatonin conformation was obtained that fits to the

TABLE I Range of distances (Å) of the d_1–d_4-pharmacophoric points shown in Fig. 4 for the conformers superimposed by DISCO for models A and B and corresponding distances for melatonin reference

	All compounds		*Melatonin (gauche/anti)*	
Distance	*Model A*	*Model B*	*Model A*	*Model B*
d_1	6.7–8.48	7.51–10.07	6.7	9.88
d_2	7.47–10.72	7.08–10.72	9.71	8.03
d_3	7.64–8.67	7.96–9.46	7.64	9.40
d_4	6.34–8.92	7.01–8.91	8.37	7.32

hydrophilic binding pocket formed by the seven transmembrane helices. This model and that of other researchers [12,13] have suggested that the methoxy group is involved in H-bonding interaction with a fully conserved histidine residue in transmembrane 5 at the Mel_{1a} receptor, which has been supported by previous mutagenesis studies [14,15]. However such support for the other proposed docking sites reported by Navajas are more speculative and hypothetical.

Two pharmacophoric models have been devised by Spadoni *et al.* [16] for melatonin and conformationally restrained melatonin analogues by searching for a common set of distances among the pharmacophoric points using the DISCO module of SYBIL 6.3 (Table I). The methoxy and amide groups were considered in fixed positions and the most informative pharmacophoric points used are shown in Fig. 4.

The presence of a flexible side chain in *N*-[3-(2-carboxymethyl-1H-indol-4-yl)propyl] acetamide presented problems as despite meeting the two pharmacophore model structural requirements, this compound showed no binding affinity $pIC_{50} < 4$. This weakened the validity of their proposed two pharmacophore ligand models. In another paper, by the same research group [17],

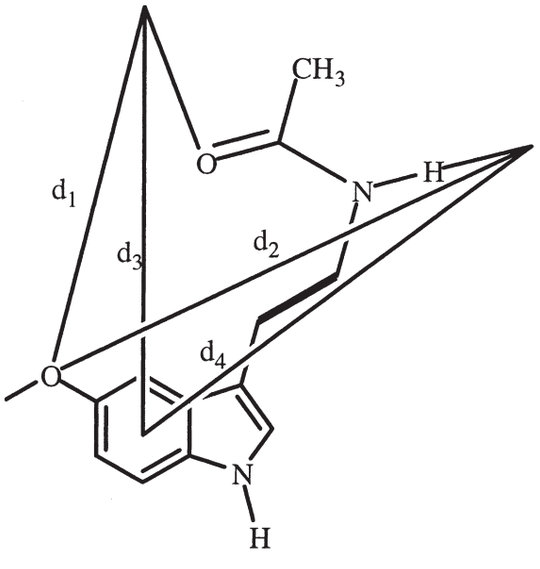


FIGURE 4 Illustration of the common distances d_1-d_4 among the pharmacophoric points used to estabish the melatonin pharmacophore using the DISCO model of SYBIL.

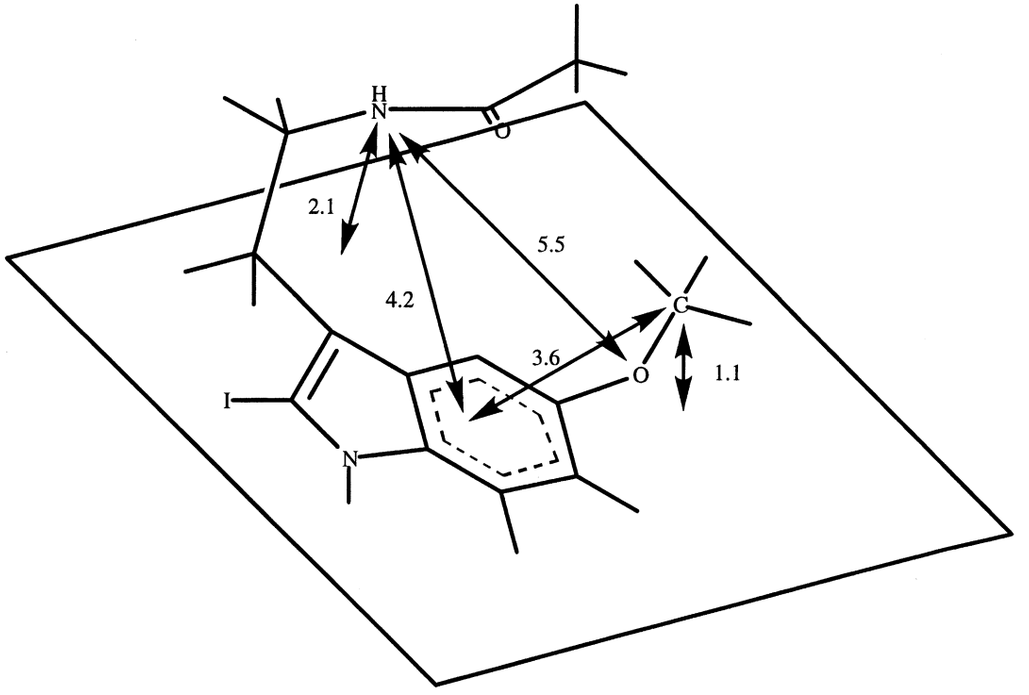
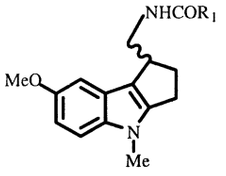
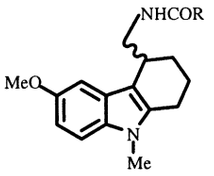
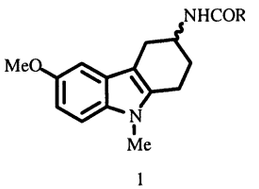
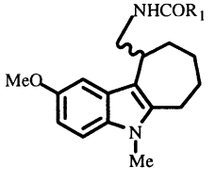


FIGURE 5 The folded structure of 2-iodomelatonin characterized by the five inter-atom dimensions (Å units) used to determine the common molecular geometry which provided the best comparison between experimental and predicted pIC_{50} values.

TABLE II Binding and melanophore assays for annelated ring compounds

				
	3	2	1	4
R_1	CH_3	CH_3	CH_2CH_3	CH_2CH_3
K_i (nM)	5350	0.97, (+) 48.4, (−) 0.372	16, (+) 243, (−) 1.7	7, (+) 201.5, (−) 6.5
Agonist EC_{50-}	N/A	0.7, 14.8, 0.30	408, 789, 3.4	91, N/A, 6.5
Antagonist IC_{50}	64			

the CoMFA analysis was applied to 133 melatonergic compounds using the previously proposed two putative pharmacophore models to elucidate SAR information about the less explored positions. The inclusion of a second bromine at C-6 as in 2,6-dibromomelatonin partially negated the activating effect of 2-bromomelatonin, whereas with 2,4-dibromomelatonin reversal of activity was observed. The most significant positive steric regions were found to be situated in the vicinity of the C-2 position of melatonin which can be considered as an additional receptor binding point and surrounding the methyl of the methoxy group which is oriented so that the oxygen lone pair can interact with the receptor for high affinity binding. The negative steric regions encompassed C-6, C-7 and the position between N-1 and C-2 above the indole plane of melatonin.

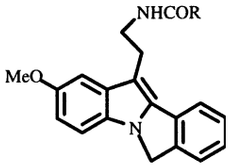
Marot and coworkers [18] determined the common molecular geometry of a 142 compounds which were selected on binding studies using 2-[^{125}I]-iodomelatonin on ovine pars tuberalis membrane homogenates expressed as pIC_{50} values. The compounds chosen represented 16 different ring systems, some of which were conformationally constrained and exhibited a wide spectrum of affinities which were necessary for the quantitative 3D-QSAR analysis by the CoMFA methodology. The folded structure of 2-iodomelatonin was used as the pharmacophore which was characterized by five inter-atom dimensions (Å units) as shown in Fig. 5. This model compares favorably with the Sicsic melatonin conformation shown in Fig. 3.

The model that provided the best comparison between experimental and predicted pIC_{50} values for the 64 compounds in the training set ($r^2 = 0.96$, $F = 248$ and $s = 0.28$) suggested a good correlation existed between the molecular fields with relative contributions: steric (28%), electrostatic (35%) and lipophilic (37%). The potential predictive power of their proposed model was applied to 78 compounds of the test set and its usefulness evaluated in relation to the gap between the experimental and predicted pIC_{50} values. Of the 39 test compounds that were in the pIC_{50} 7–9.5 range, 21/39 (54%) were overestimated, 15/39 (38%) understated and 3/39 (8%) were exactly predicted.

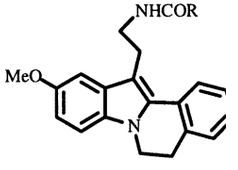
Garratt and coworkers [19] have investigated the conformation of the C-3 amidoethane side chain on binding to the active site of the receptor as a function of the size of the annelated ring connected to the side chain. Furthermore, they have also studied the selective binding affinity of the individual enantiomers and some representative data is presented in Table II.

A wide range of compounds with variable R_1 substituents were prepared and tested. All products without the 5-methoxy group generally showed weaker receptor binding. The binding studies indicate that when the conformation of the C-3 amidoethane side chain was present in the fixed extended form as in the *N*-acyl-3-aminotetrahydrocarbazoles, structure 1, the compounds have lower

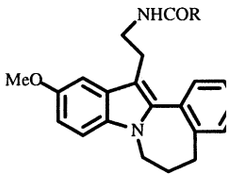
TABLE III Binding and melanophore assays for N1-C-2-phenyl linked ring compounds



5



6



7

	K_i (nM)		*Selectivity MT_2/mt_1*	*Agonist EC_{50} (nM)*	*K_i (nM)*		*Selectivity MT_2/mt_1*	*Agonist EC_{50} (nM)*	*K_i (nM)*		*Selectivity MT_2/mt_1*	*Antagonist IC_{50} (nM)*
R	mt_1	MT_2			mt_1	MT_2			mt_1	MT_2		
Me	1.82	0.06	30	2.29	7.24	0.51	14	12.6	275	6.31	44	37100
Et	4.37	0.17	26	21.9	5.89	0.12	49	6.03	52.5	1.41	37	3980
Pr	4.47	0.05	89	5.75	4.07	0.20	20	9.33	66.1	0.50	132	380

binding affinity relative to the position of the side chain in *N*-acyl-4-aminotetrahydrocarbazoles, structure 2 and the other annelated ring compounds, structures 4 and 5. Substances with a six membered annelated ring, structure 2, showed stronger receptor affinity than those with five or seven membered annelated rings. From X-ray crystallographic structure analysis of several compounds, it was deduced that compounds with the (−)S enantiomeric configuration also showed the highest receptor binding affinities. This again reflects the view that the spatial relationship between the methoxy group and the aminoethyl side chain are critical elements in determining receptor potency. The large differences in binding capacity between the (+)R and (−)S enantiomeric forms enhances the view that the 3D orientation as well as the distances between the functional groups influences optimal binding and interaction of the ligands with their receptor. Whether all receptors exhibit the same chiral selectivity in binding has yet to be determined.

The effect of a ring connection between the indole N-1 and a C-2 phenyl ring on receptor binding and agonist/antagonist activity was investigated by Garratt and coworkers [20]. Increasing the carbon chain length between N-1 of indole and the C-2 phenyl groups as represented in Fig. 3, structures 5–7, leads to a reduction in binding affinity and a transformation from melatonin agonist activity of compounds 5 and 6 to antagonist activity for compound 7. These three series of compounds shown in Table III, illustrate that the conformational changes between the indole ring and the adjacent phenyl ring, induce steric restrictions which negate receptor binding.

All of the compounds tested had either similar or a greater selectivity for the MT_2[†] receptor compared to the mt_1 receptor (range 1:1–1:132). The MT_2 receptor can more readily accommodate the steric requirements of compounds 6 and 7 than the mt_1 receptor. Importantly, PM3 minimized energy structures for compounds 5, 6 and 7 ($R = CH_3$) showed very similar spatial proximities for the methoxy group and the aminoethyl side chain.

In our conformational studies of the ligand-receptor interactions, we are currently preparing [21] the compounds shown in Fig. 6.

We have combined in structure 8, the previously established receptor binding enhancement of C-2 substituents and the selective S enantiomeric higher binding affinity of the constrained aminoethyl side chain, with the objective of finding a ligand conformation which will further increase binding affinity with the receptor. In previous SAR studies [22], we showed that 2,3-dihydromelatonin

[†]According to the International Union of Pharmacology, on receptor nomenclature, the mel_{1a} melatonin receptor is referred to as the mt_1 receptor and the mel_{1b} melatonin receptor as the MT_2 receptor.

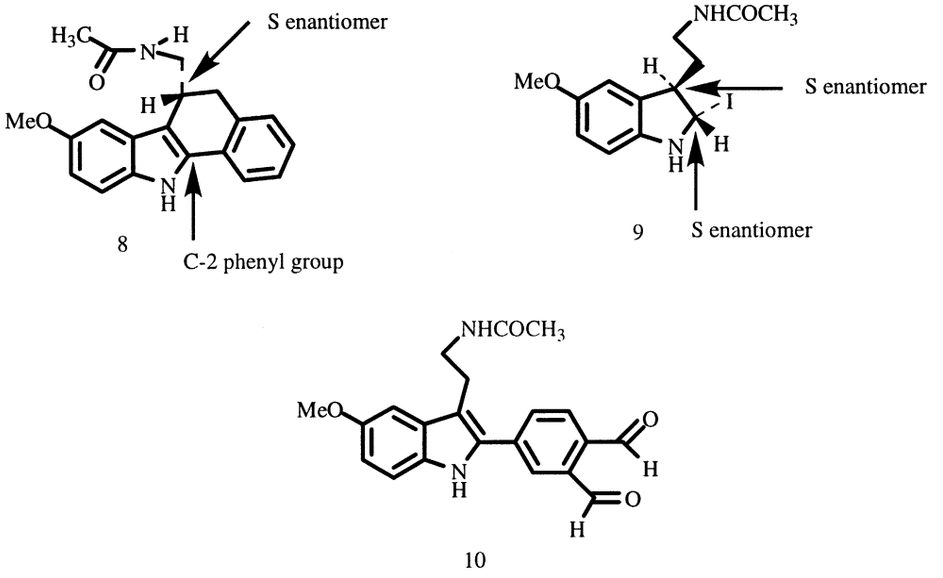


FIGURE 6 Structures of compounds 8 and 9 for studies to determine the importance of chiral carbons on receptor affinity. Compound 10 illustrates the *o*-phthaldehyde functional group on the benzene ring to probe receptor binding with electron rich amino acid residues.

remained biologically active. Asymmetric reduction of 2-iodomelatonin provides compound 9 and binding studies will determine the importance of the presence of C-2 and C-3 chiral functional groups on receptor affinity. In compound 10, the C-2 phenyl ring is transformed into an electrophilic *o*-phthaldehyde moiety. The intention is to demonstrate whether covalent binding to the GPCR is possible in this receptor region, which amino acid residues are involved and could this translate into increased agonist potency of C-2 substituted melatonin analogues? Furthermore, we use covalent crosslinking approaches in order to map the contact sites with the receptor.

SUMMARY

Many melatonin analogues have been designed to measure their receptor binding potency and CoMFA analysis have been used to define possible pharmacophore models for various receptors. The CoMFA method has suggested that the C-3 aminoethyl side chain in melatonin and related compounds adopts a folded structure in which the C-5 methoxy group-C-3 aminoethyl side chain distance is critical in binding to the receptor. The difficulty has been in representing the alignment of the molecules to reflect the ligand-receptor binding geometry. A range of structurally variable classes of conformationally constrained compounds have been produced to explore the potential relative spatial orientations of the functional atoms interacting with the receptor. It is therefore not surprising that a range of pharmacophore models have also been suggested and the entropic effects arising from the C-3 aminoethyl side chain has presented difficulties. The CoMFA method is useful for the evaluation of SAR of melatonin agonists and antagonists, but cannot as such be used for receptor mapping. X-ray crystallographic analysis of ligands has provided insights into the 3D spatial selectivity surrounding the receptor. We are investigating the spatial conformations of ligands that enhance binding affinity and are also exploring the outcomes of stronger chemical interactions with the receptor transmembrane protein residues.

References

[1] Young, M.W. (2000) "The tick-tock of the biological clock", *Sci. Am.* **March**, 46, Genetic research has made a fundamental contribution to our understanding of the molecular basis of circadian time and the molecular mechanism of the biological clock.
[2] Sassone-Corsi, P. (1998) "Molecular clocks: mastering time by gene regulation", *Nature* **392**, 871, Genetic research has made a fundamental contribution to our understanding of the molecular basis of circadian time and the molecular mechanism of the biological clock.
[3] Penev, P.D., Zee, P.C. and Turek, F.W. (1997) "Serotonin in the spotlight", *Nature* **385**, 123.

[4] Weibl, L., Rettori, M.C., Lesieur, D., Delagrange, P., Renard, P. and Van Reeth, O. (1999) "A single oral dose of S 22153, a melatonin antagonist, blocks the phase advancing effects of melatonin in C3H mice", *Brain Res.* **829**, 160.
[5] Gubitz, A.K. and Reppert, S.M. (2000) "Chimeric and point-mutated receptors reveal that a single glycine residue in transmembrane domain 6 is critical for high affinity melatonin binding", *Endocrinology* **141**, 1236.
[6] Kennaway, D.J. and Hügel, H.M. (1992) "Mechanisms of action of melatonin within the central nervous system", *Animal Rep. Sci.* **30**, 45.
[7] Kennaway, D.J. and Hügel, H.M. (1992) "Melatonin binding sites: are they receptors?", *Mol. Cell. Endocrinol.* **88**, C1.
[8] Sicsic, S., Serraz, I., Andrieux, J., Bremont, B., Mathe-Allainmat, M., Poncet, A., Shen, S. and Langlois, M. (1997) "Three-dimensional quantitative structure-activity relationship of melatonin receptor ligands: a comparative molecular field analysis study", *J. Med. Chem.* **40**, 739.
[9] Cramer, III, R.D., Patterson, D.E. and Brunce, J.D. (1998) "Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins", *J. Am. Chem. Soc.* **110**, 5959.
[10] Kubinyi, H., (1993) In: For an outline of the practice of CoMFA refer to "3D QSAR in Drug Design" (ESCOM, ISBN 90-72199-14-6, p 443.
[11] Navajas, C., Kokkola, T., Poso, A., Honka, N., Gynther, J. and Laitinen, J.T. (1996) "A Rhodopsin-based model for melatonin recognition at its G protein-coupled receptor", *Eur. J. Pharmacol.* **304**, 173.
[12] Garratt, P.J., Travard, S., Vonhoff, S., Tsotinis, A. and Sugden, D. (1996) "Mapping the melatonin receptor. 4. Comparison of the binding affinities of a series of substituted phenylalkyl amides", *J. Med. Chem.* **39**, 1797.
[13] Grol, C.J. and Jansen, J.M. (1996) "The high affinity melatonin binding site probed with conformationally restricted ligands. II. Homology modeling of the receptor", *Bioorg. Med. Chem.* **4**, 1333.
[14] Conway, S., Canning, S.J., Barrett, P., Guardiola-Lemaitre, B., Delagrange, P. and Morgan, P. (1997) "The roles of valine 208 and histidine 211 in ligand binding and receptor function of the ovine $Mel_{1a\beta}$ melatonin receptor", *Biochem. Biophys. Res. Commun.* **239**, 418.
[15] Kokkola, T., Watson, M.A., White, J., Dowell, S., Foord, S.M. and Laitinen, J.T. (1998) "Mutagenesis of human Mel_{1a} receptor expressed in yeast reveals domains important for receptor function", *Biochem. Biophys. Res. Commun.* **249**, 531.
[16] Spadoni, G., Balsamini, C., Diamantini, G., Di Giacomo, B., Tarzia, G., Mor, M., Plazzi, P.V., Rivara, S., Lucini, V., Nonno, R., Pannacci, M., Fraschini, F. and Stankov, B.M. (1997) "Conformationally restrained melatonin analogues: synthesis, binding affinity for the melatonin receptor, evaluation of the biological activity, and molecular modeling study", *J. Med. Chem.* **40**, 1990.
[17] Mor, M., Rivara, S., Silva, C., Bordi, F., Plazzi, P.V., Spadoni, G., Diamantini, G., Balsamani, C., Tarzia, G., Fraschini, F., Lucini, V., Nonno, R. and Stankov, B.M. (1998) "Melatonin receptor ligands: synthesis of new melatonin derivatives and comprehensive comparative molecular field analysis (CoMFA) study", *J. Med. Chem.* **41**, 3831.
[18] Marot, C., Chavatte, P., Morin-allory, L., Viaud, M.C., Guilluamet, G., Renard, P., Lesieur, D. and Michel, A. (1998) "Pharmacophoric search and 3D-QSAR comprehensive comparative molecular field analysis studies on agonists of melatonin sheep receptors", *J. Med. Chem.* **41**, 4453.
[19] Davies, D.J., Garrat, P.J., Tocher, D.A., Vonhoff, S., Davies, J., Teh, M-T. and Sugden, D. (1998) "Mapping the melatonin receptor. 5. Melatonin agonists and antagonists derived from tetrahydrocyclopent[b]indoles, tetrahydrocarbazoles and hexahydrocyclohept[b]indoles", *J. Med. Chem.* **41**, 451.
[20] Faust, R., Garratt, P.J., Jones, R., Yeh, L.-K., Tsotinis, A., Panoussopoulou, M., Calogeropoulou, T., Teh, M.-T. and Sugden, D. (2000) "Mapping the melatonin receptor. 6. Melatonin agonists and antagonists derived from 6H-Isoindolo[2,1-α]indoles, 5,6-Dihydroindolo[2,1-α]isoquinolines, and 6,7-dihydro-5H-benzo[c]azepino[2,1-α]]indoles", *J. Med. Chem.* **43**, 1050.
[21] Harris, P.W.R., Hügel, H.M. and Nurlawis, F. Work in progress.
[22] Kennaway, D.J., Hügel, H.M., Clarke, S., Tjandra, A., Johnson, D.W., Boyles, P., Webb, H.A. and Carbone, F. (1998) "Structure-activity studies of melatonin analogues in prepubertal male rats", *Aust. J. biol. Sci.* **41**, 393.