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STRUCTURE-ACTIVITY RELATIONSHIPS OF MELATONIN ANALOGUES

MELATONIN ANALOGLARININ YAPI-AKTİVİTE İLİŞKİLERİ

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ABSTRACT

This paper includes how melatonin and its related analogs interact with melatonin receptors with the hope of developing important tools and agents of significant clinical and scientific importance. The review provides currently published melatonergic ligands and their relative affinities for melatonin receptors and discusses the importance of developing reversible, high-affinity, and subtype selective melatonin receptor antagonists. Further design, synthesis, and application of melatonergic ligands will lead us to a clearer understanding of the role that melatonin and its receptors play in humans.

Key words: Melatonin, melatonin analogues, melatonin receptor and melatonin ligands.

ÖZET

Bu makale klinik ve bilimsel önemi olan ajanların geliştirilmesi amacı ile, melatonin ve ilgili analoglarının melatonin reseptörleri ile etkileşimlerini içermektedir. Derleme son zamanlarda yayınlanan melatonerjik ligandlar ve onların ilgili melatonin reseptörlerine olan affinitelerini vermekte ve geri dönüşümlü, yüksek affiniteli ve seçici melatonin alt reseptörlerinin gelişimini tartışmaktadır. İleri tasarım, sentez ve melatonerjik ligandların kullanılması melatonin ve reseptörlerinin insanlardaki rolünü daha iyi anlamamıza olanak sağlıyacaktır.

Anahtar kelimeler: Melatonin, melatonin analogiarı, melatonin reseptörü ve melatonin ligandları

INTRODUCTION:

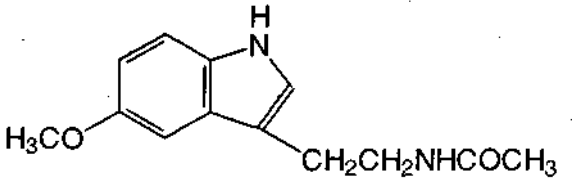


FIGURE 1: Melatonin

Melatonin (N-acetyl 5-methoxytryptamine) (Fig.l) is a neurohormone, first isolated by Lerner et al. (1) in 1958 from bovine pineal tissue, has a central role in the regulation of daily rhythms and seasonal cycles in vertebrates. Its potential usefulness to a number of therapeutic areas such as those related to the desynchronization of biological rhythms, such as jet-lag, disturbed sleep-wake cycles (2), seasonal disorders and depression are known (3). Melatonin may also play a role in the cardiovascular system (4). This is supported by recent findings which show that 2-[^{125}I] iodomelatonin-binding sites are localized in both the caudal and cerebral arteries of the rat. In addition, melatonin binding has been reported at many other sites including the retina (propably related to resynchronization role) and peripheral tissues such as the spleen (related to a role immune system), gastrointestinal tract, blood platelets and the harderian gland (5). Furthermore antioxidant properties of melatonin have recently been proposed (6,7). Although the effects of melatonin in oncogenesis are unclear; majority of the studies conclude that the hormone has a protective role in the modulation of cancer or cancerous cells (8,9). Melatonin may also play a role in brain function (10) but the mechanisms underlying such functions are not known (11). Despite its potential involvement in the regulation in many possible physiological processes, two problems limit its the therapeutic use at present. The first is its very short biological half-life (15-30 min), due to its rapid catabolism to 6-hydroxymelatonin and N-acetylkynurenamines. The second problem is the lack of selectivity of melatonin at target sites. The development of novel analogues of provides a strategic approach to overcome both of these limitations (5).

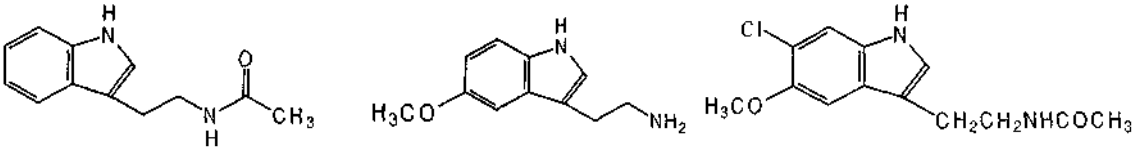
Moleculer Biology of Melatonin Receptors

A high-affinity melatonin receptor was first cloned from frog dermal melanophores in 1994. Since then, 20 distinct full-length or partial melatonin receptor DNA sequences from a variety of species have been reported. Phylogenetic analyses of the predicted amino acid sequences of melatonin receptors support their division into three subtypes: mel_{1a}, mel_{1b} and $meli_{1c}$ families. However, a fourth family, typified by a partial Xenopus laevis cDNA clone, may exist (12).

ML_1-type receptor:

SAR studies on derivatives of melatonin have elucidated several key interactions between the ligand and the ML_1-type receptor. Importantly, both the methoxy and the amide functional groups are critical to melatonin's affinity. N-acetyltryptamine possesses over a thousand-fold

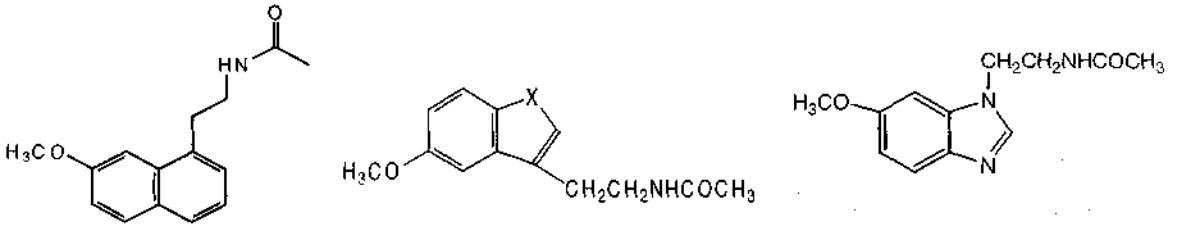
lower affinity (Ki=730 nM) for the receptor in chicken brain compared to the melatonin (Ki=0.24 nM) while 5-methoxy tryptamine (Ki=2528 nM) exhibits no significant affinity for the ML_1-type receptors (Fig.2) (13). Both the methoxy and the amide groups are therefore presumed to be involved in critical hydrogen bonds to the receptor recognition site. While the methoxy group is a major factor in binding melatonin to its receptor, it is not a necessary criteria for agonist activity as had been suggested previously (14).



N-acetyl tryptamine 5-methoxy tryptamine 6-chloromelatonin

FIGURE 2: The chemical formula of N-acetyl tryptamine, 5-methoxy tryptamine and 6-chloromelatonin

Additional features of the ML_1-type receptor include a small, hydrophobic pocket which can accommodate some increase in the size of group, R (alkyl), attached to the amide carbonyl. Increasing the length of R up to three carbons increases affinity for the receptor in ovine pars tuberalis but groups longer than three carbons, or those with branching, are not well-tolerated and result in a significant decrease in receptor affinity (15). Substituents on the indole ring are well-tolareted at both the 2- and 6- positions. 6-Chloromelatonin (Fig.2) demonstrates comparable affinity (K_I=0.58 nM) to melatonin for the receptor in chicken brain (13). Substitution at the 2-position of the indole by halogen, methyl or phenyl groups enhances receptor affinity, either by influencing the conformation of the ethylamido side chain or accessing an auxiliary binding site (16).



6 **7** X=O **8** X=S **9**

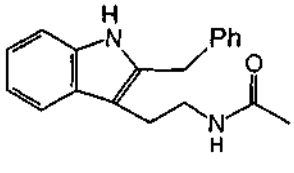
FIGURE 3: Naphthalene, benzofuran, benzothiophene and benzimidazole derivatives

The indole ring of melatonin appears to be a scaffold which is not critical for melatonin receptor recognition. The naphthalene derivative 6 possesses essentially equivalent affinity (K_i=0.035 nM) to melatonin, for the receptor in ovine pars tuberalis (Fig.3) (17).

The indole ring of melatonin can also be replaced with a benzofuran (7) or benzothiophene (8) but these bio-isosteric replacements result in a slight loss in affinity. However, replacement of indole ring with the benzimidazole ring (9) dramatically attenuates activity (Fig.3) (5).

ML_1-Antagonist:

Potent, selective antagonist for the ML_1-type receptors are still lacking. Although a few compounds are functional antagonists **(23-27)** (Fig.4), they have a modest affinity for ML, receptors (18). Luzindole, N-acetyl-2-benzyltryptamine (23) antagonizes the melatonin-induced inhibition of dopamine release from rabbit retina (19). A recent report has demonstrated an excellent correlation between the $meli_{1b}$ binding affinity of compounds **24-26** and their activity in the same functional assay. The naphthalene derivative 27 blocks the inhibitory action of melatonin on forskolin-stimulated cyclic AMP accumulation in sheep pars tuberalis cells and reverses the melatonin-induced pigment aggregation in Xenopus melanophores (20).



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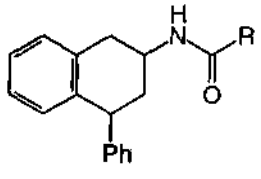

24 R=Me
25 R=Et
26 R=CH_2Cl

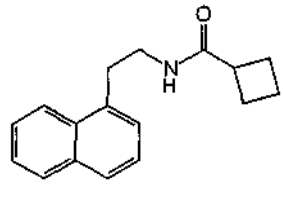

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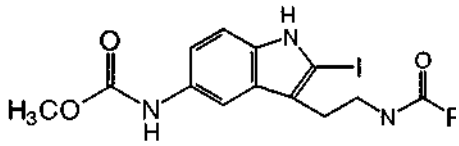
FIGURE 4: ML_1-Antagonist compounds

ML_2-binding site:

SAR data for the ML_2-binding site is limited. In simple derivatives of melatonin, lengthening the group on the carbonyl increases affinity for the ML_1 receptor, but decreases affinity for the ML_2 site, thereby increasing selectivity for the ML_1 site over the ML_2 (20). Compounds which demonstrate good selectivity for the ML_2 site include, 2-iodo-5-methoxycarbonylamino-N-acetyltryptamine 28, and a series of benzimidazoles 29 (Fig.5) (21).



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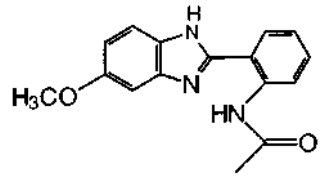

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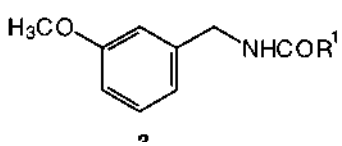
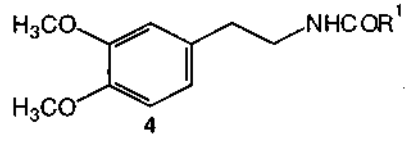
FIGURE 5: Effective compounds for ML_2 binding site

A series of 2-,3- and 4-substituted phenylalkyl amides were prepared as potential melatonin analogs in order to investigate the nature of the binding site of the melatonin receptor in chicken brain. The length of the alkyl chain-was systematically varied from n=l to 4, and methoxy substituents were incorporated into the phenyl ring at the 2-, 3-, and 4-positions. The maximum binding affinity was found to occur when n=3 and when the methoxy substituent was in the 3-position, the direct analog of the carbon framework of melatonin in which the first and second atoms of the indole ring have been removed. Whereas there was only a relatively small decrease in binding affinity for the corresponding 2-methoxy derivatives, 4-methoxy substitution led to a large decrease in binding affinity, suggesting that the binding sites for the side chain and methoxy group could not now be occupied at the same time. As in the indole analogs of melatonin, replacement of the methyl group of the amide by a longer alkyl chain led to an increase in binding affinity for ethyl and propyl with a subsequent decrease in binding affinity for butyl chains. Thus N-propanoyl-3-(3-methoxyphenyl)propanamide (6f) has a binding affinity of 5.6 nM, a remarkably high affinity for a simple compound in structure (22) (Table 1).





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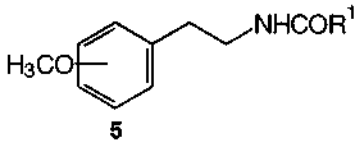

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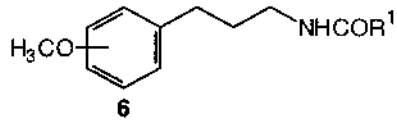

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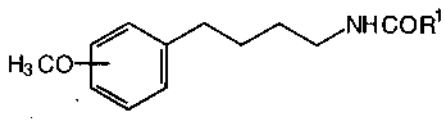

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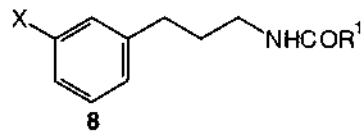

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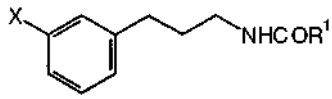
Compound	**R[1]**	**rec. binding, Ki (nM)**
Melatonin		0.59±0.06
3a	Me	57000±8100
3b	Et	22000±2500
3c	Pr	13000± 1500
4a	Me	870± 130
4b	Et	130±21
4c	Pr	59±9
5 a, 2-OMe	Me	573±68 420[a]
5b, 2-OMe	Et	135± 21789[a]
5c, 2-OMe	Pr	69±12 748[a]
5d, 2-OMe	Bu	16000± 1600
5e, 3-OMe	Me	958± 108 581[a]253[b]
5f, 3-OMe	Et	62±7
5g, 3-OMe	**Pr**	40±6
5h, 3-OMe	Bu	741±63
5i, 4-OMe	Me	> 100000 NE,[a]>1000[b]
5j, 4-OMe	Et	19200±5100
5k, 4-OMe	Pr	17900±5200
51, 4-OMe	Bu	70800±4100
6a, 2-OMe	Me	1430± 310
6b, 2-OMe	Et	374±80
6c, 2-OMe	**Pr**	442± 122
6d, 2-OMe	Bu	>100000
6e, 3-OMe	Me	63±4
6f, 3-OMe	Et	5.6± 1.7
6g, 3-OMe	Pr	5.5±1.8
6h, 4-OMe	Me	14000± 1600
6i, 4-OMe	Et	2900±800
6j, 4-OMe	Pr	860±210
7a, 2-OMe	Et	339±54
7b, 2-OMe	Pr	116±29
7c, 2-OMe	Bu	9190± 1260

7d, 3-OMe	Et	119±25
7e, 3-OMe	Pr	208±55
7f, 3-OMe	Bu	9670±2660
7g, 4-OMe	Me	7300±1300
7h, 4-OMe	Et	1400±300
7i, 4-OMe	Pr	822±192
7j, 4-OMe	Bu	7000±1100

TABLE 1. Binding affinity in chicken brain assay of (methoxyphenyl) alkyl amides (22)

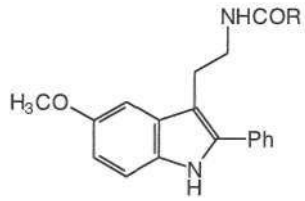
A number of propyl amides 8 were prepared in which the 3-methoxyl group was replaced by halogen, compounds similar to the 5-halo-substituted tryptamides (23). The binding affinities for these compounds are shown in Table 2. In all cases there is a reduction of the binding affinity compared to the corresponding methoxy system, but nevertheless, some of these compounds show considerable affinity for the melatonin receptor. The 3-chloro derivatives have the highest binding affinities, and these again show the effect of changing the acylating group, butanoyl being the most effective, whereas in the 3-bromo series the propanoyl and the butanoyl show similar affinities. Unlike with the bromo and chloro derivatives, no maximum was observed in the 3-flouro series (22) (Table 2).

Two series of 2-phenyltryptamides were prepared as melatonin analogues to investigate the nature of the binding site of the melatonin receptor in chicken brain and in Xsenopus laevis melanophore cells. The 5-methoxy-2- phenyltryptamides **(6a-j)** (Table 3) have high binding affinities for the chicken brain receptor, in some cases **(6a-d)** greater than that for melatonin, confirming and extending the work of Spadoni et al. (16) and act as agonists in the Xsenopus melanophore assay. Analogues lacking the 5-methoxyl group **(2a-n)** had a considerably lower affinity for the chicken brain receptor. In the Xsenopus melanophore assay the compounds acylated on nitrogen by an alkyl group **(2a-d)** were agonist whereas the compounds acylated on nitrogen by an alicyclic group **(2f-i)** (Table 4) were antagonist (24).



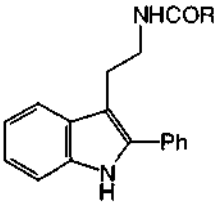
Compound	X	R[1]	rec. binding [Ki] nM
Melatonin			0.59±0.06
8a	F	Me	1920±330
8b	F	Et	740±110
8c	F	Pr	434±66
8d	Cl	Me	636±134
8e	Cl	Pr	113±33
8f	Cl	Bu	15100±2700
8g	Br	Me	852±93
8h	Br	Et	318±30
8İ	Br	Pr	354±74

TABLE 2. Binding affinity in chicken brain assay of 3-(3-halophenyl)propyl amides (22)



Compound	R	rec. binding [Ki] nM	Xenopus melanophores action, conc,M
Melatonin		0.59±0.06	Agonist, [10^{-8}]
6a	Me	0.0596±0.0074	Agonist, [10^{-8}]
6b	Et	0.0466±0.0066	Agonist, [10^{-8}]
6c	Pr	0.0558±0.012	Agonist, [10-^{8}]
6d	CF_3	0.0190±0.003	Agonist, [10-^{8}]
6e	cyclo-C3H5	0.3047±0.066	Agonist, [10^{-8}]
6f	Cyclo-C_4H_7	2.7±0.66	Agonist, [10-^{6}]
6g	Cyclo-C_5H_9	32.8±7.8	Agonist, [10^{-6}]
6h	cyclo-C_6H_{11}	216±31	Agonist, [10^{-6}]
6İ	1 -adamantil	1100±300	Agonist, [10^{-6}]
6j	$(CH_2)_2Ph$	263±40	Agonist, [10^{-6}]

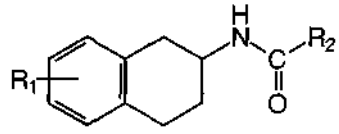
TABLE 3. Binding affinity in chicken brain assay and response of Xsenopus laevis melanophores to 5-methoxy-2- phenyltryptamine derivatives (24).



Compound	R	rec. Binding [Ki] nM	Xenopus melanophores action, conc, M
Melatonin		0.59±0.06	Agonist, [10^{-8}]
2a	Me	100±12	Agonist, [10^{-6}]
2b	Et	70±9	Agonist, [10^{-6}]
2c	Pr	112±13	Agonist, [10^{-6}]
2d	CF_3	148±37	Agonist, [10^{-6}]
2e	C_2H_5	6500±1060	NA, [10^{-5}]
2f	cyclo-C_3H_5	213±46	Antagonist$^{\alpha}$
2g	cyclo-C_4H_7	565±126	$^{\alpha}$
2h	cyclo-C_5H_9	633±113	Antagonist$^{\alpha}$ Antagonist$^{\alpha}$
2I	cyclo-C_6H_{11}	1550±240	Antagonist$^{\alpha}$
2j	$(CH_2)_2Ph$	7000	NA, [10^{-5}];Nant[10^{-5}]
2k	CH_2N_3	410±79	Agonist, [10^{-6}]
21	CH_2Br	55±12	Agonist, [10^{-6}]
2m	CHBrMe	1900±400	NA, [10^{-5}]
2n	1-adamantil	1100±190	NA, [10^{-5}];Nant[10^{-5}]

TABLE 4. Binding affinity in chicken brain assay and response of Xsenopus laevis melanophores to 2-phenyltryptamine derivatives (24).

A series of unsubstituted and methoxy-substituted 2-amidotetralins **(4a-q)** were prepared and evaluated for their ability to compete for 2-[^{125}I] iodomelatonin binding to chicken retinal membranes and for their potency to inhibit the calcium-dependent release of [^{3}H] dopamine from rabbit retina. The lead compound, 2-acetamido-8-methoxytetralin (4j), showed a moderate affinity (Ki=46 nM) and potency (IC_{50}= 1.4 nM) at the melatonin receptor. The structural requirements necessary for optimal agonistic activity at the melatonin receptor are as follows. First, the amido group, which should have a small, nonbranched alkyl group, is essential for affinity, and second, the methoxy substituent at the 8-position of the 2-amidotetralin ring is essential for optimal agonistic activity at the melatonin receptor. The researchers concluded that this series of unsubstituted and methoxy-substituted 2-amidotetralins constitutes a class of nonindolic melatonin-like agents that can be used as pharmacological tools to further characterize melatonin receptors and to elucidate the mode of action of melatonin (25) (Table 5).



4a-4q

Compound	R_1	R_2	Ki, nM [b]	**Relative affinity**[c]	IC_{50}, nM[e]	**relative potency**
Melatonin			0.57	1.00	0.017	1.0
4a	H	Me	665	1170	52	3060
4b	H	Et	145	254	8.1	476
4c	H	n-Pr	44.4	78	1.0	59
4d	H	CH_2Cl	95.1	167	1.3	76
4e	H	CH_2Ph	NE[h]		ND1	
4f	H	Ph	NE		ND	
4g	5-OCH_3	Me	1950	3420	6.5	382
4h	6-OCH_3	Me	NE		ND	
4İ	7-OCH_3	Me	121	212	1.6	94
4j	8-OCH_3	Me	46.3	81	1.4	82
4k	8-OCH_3	Et	7.40	13	0.48	28
4l	8-OCH_3	n-Pr	3.60	6.3	1.2	71
4m	8-OCH_3	i-Pr	312	547	7.9	465
4n	8-OCH_3	C-C_3H_5	141	247	2.5	147
4o	8-OCH_3	CH_2Cl	3.75	6.6	0.063	3.7
4p	8-OCH_3	CH_2Ph	NE		NE	
4q	8-OCH_3	Ph	23100	40500	22	1290

TABLE 5. Pharmacological evaluation of the amides 4a-q (25).

CONCLUSION

It has been demonstrated that the indole ring of melatonin is not an essential characteristic of the molecule for either its affinity for the melatonin receptor or for its biological activity, as it can be replaced by a naphthalene bioisostere. Whilst substitution of the nitrogen in the indolic ring by either S (benzothiophen) or O (benzofuran) can be tolerated, they both reduce binding affinities to some extent, and the later substitution elicits effects which cannot be presently explained. Homologous extension of the N-acetyl side chain of the naphthalenic analogue together with other modifications can increase the affinity of the compounds for the melatonin receptor over that of melatonin itself.

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