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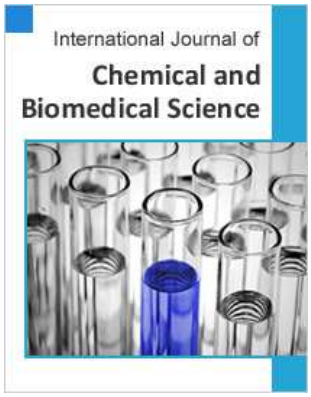

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MT3 Receptor Selective Ligands in the Treatment of Glaucoma: Achievements and Prospects

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Abstract

Glaucoma is a neurodegenerative disease characterized by a continuous or periodic increase in intraocular pressure (IOP). The modern drug therapy of glaucoma basically consists in IOP reduction. Recent studies have shown that the endogenous hormone melatonin and its analogs, especially the melatonin receptor MT3 agonist MCA-NAT, have great potential in reducing the intraocular pressure. Data are discussed indicating that MT3 receptor ligands, being an promising antioxidant and neuroprotectors, may become a good alternative to the existing drugs using for improving quality of life of people with glaucoma.

1. Introduction

Glaucoma is a large group of eye diseases characterized by a continuous or periodic increase in intraocular pressure (IOP) caused by the violation of the outflow of aqueous humor from the eye. The increase in the pressure results in the gradual development of visual function disorders characteristic of glaucoma and atrophy of the optic nerve. The optic nerve damage is a long-term consequence of the disease that can be prevented by the timely execution of the operation. It should be noted that clinically manifested changes in the optic nerve and in the field of vision of patients occur only after the loss of a significant proportion (about 40%) of nerve fibers. Increased IOP and atrophy of the optic disc are the causes of the violation of visual functions: impaired dark adaptation, reduced contrast and color sensitivity of the eye, visual acuity. However, the most characteristic feature is the occurrence of glaucomatous visual field defects [1].

There are a number of types of glaucoma: primary congenital, juvenile, or secondary glaucoma. Primary glaucoma occurs without syndromic features, whereas secondary glaucoma is often a result of an anterior chamber defects that confer a 50% chance of developing glaucoma. Subtypes of glaucoma can be further divided according to the age of onset: congenital glaucoma (CG) occurs within the first two years of life, and juvenile glaucoma (JG) develops before the age of 40. These subtypes can be further classified according to the status of the iridocorneal angle of the anterior chamber, which regulates the flow of aqueous humour. These subtypes are referred to as open-angle and angle-closure glaucoma. Open-angle glaucoma, commonly seen in patients of African descent, has a normal anterior chamber angle, although the flow of fluid is restricted by malformations in the conventional outflow pathways. Angle-closure glaucoma, commonly seen in East Asian populations (especially Chinese) is the closure of this angle, impeding aqueous humour flow [2].

2. Glaucoma Treatment

The goal of glaucoma therapy is to reduce intraocular pressure up to the tolerance level, referred to as the target pressure. This is the top level of the tonometer intraocular pressure, at which it is possible to stop or slow down the damage of the internal structures of the eyeball and the deterioration of visual function [2].

Drugs, which are most widely used in the therapy of glaucoma, are divided into the following three types (Figure 1). The combination therapy of introduced drugs is also used.

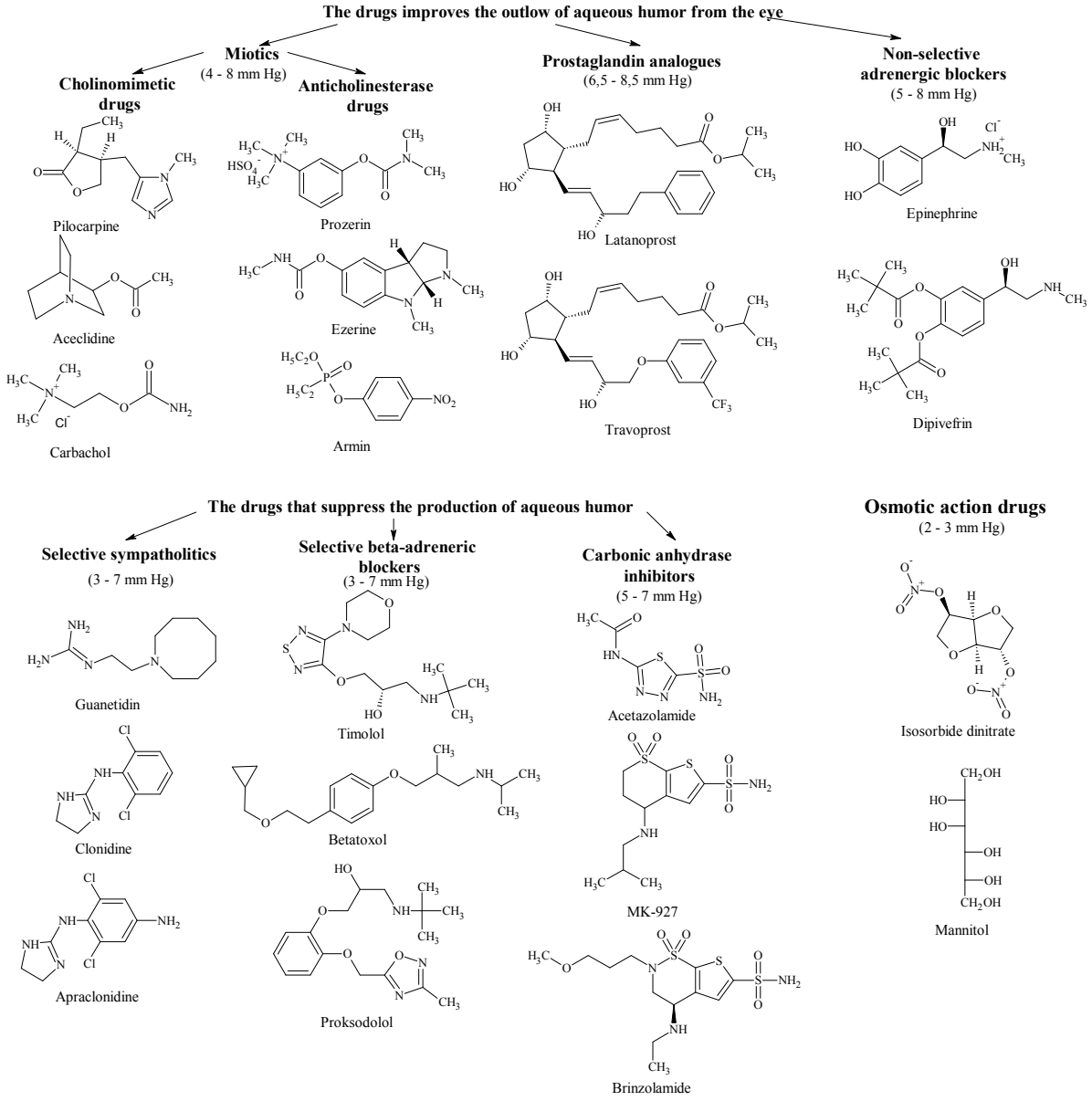
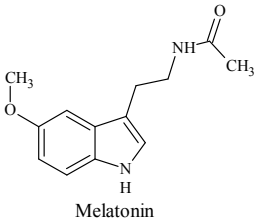


Figure 1. *Classification of drugs regulating IOP. Range of IOP reduction is given in brackets for all groups.*

3. Melatonin Action

Melatonin is the epiphyseal hormone that performs many functions in the human body, including regulating circadian rhythms and seasonal rhythms of many animals, increasing the efficiency of the immune system, antioxidant and neuroprotective activities, inhibiting aging processes, lowering blood pressure, etc [3, 4, 5, 6].



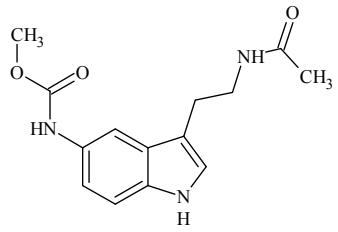
The effect of melatonin occurs due to the three subtypes of the melatonin receptor MT1, MT2, MT3.The MT1 and MT2 subtypes are the 7TM, G-coupled receptors, which are well characterized at the molecular level (Ki (MT1) = 0.12 nM, Ki (MT2) = 0.31 nM [7]). The binding site of MT3 has very peculiar characteristics, particularly regarding its fast association/dissociation kinetics [8]. However, antioxidant and neuroprotective effects and the protection against toxic compounds are most likely associated with the MT3 subtype [9, 10, 11].

4. Quinone Reductase 2 (QR2 or NQO2)

The MT3 receptor subtype is the least studied melatonin receptor. Many researchers believe that the MT3-melatonin receptor subtype is the enzyme quinone reductase 2 (QR2) [4, 12, 13, 14, 15, 16, 17]. Further studies on this enzyme firmly established that MT3 and QR2 are the same protein. Indeed, transfection of QR2 in Chinese hamster ovary cells revealed an MT3-type binding site. Similarly, tissues from various species showed a correlation between the QR2 activity and the MT3 binding [18].

The crystal structures of QR2 show constituent of homodimers with two independent and equivalent active sites each containing a FAD cofactor. The analysis of the crystal structure of QR2 revealed a specific metal-binding site, which is not present in QR1. The crystal structure was described and a specific metal-binding site was found. This is most likely a copper (I) site, which is near the protein surface, is solvent accessible and is separated from the FAD cofactor by a distance of about 13 Å [19].

The enzyme QR2 functions via a ping-pong mechanism involving two steps: the oxidation of FAD by the co-substrate followed by the reduction of the substrate (mostly quinones) by the enzyme [16]. The first electron transfer between the substrate and the co-substrate occurs in the deep hydrophobic pocket of the protein, whereas the QR2 metal-binding site may be involved in the second electron-transfer reaction, assuming that it is a redox-active site [copper (I)] [19]. Taking into account its close similarity to QR1 (49% [19]), it was believed that QR2 might also serve as a detoxifying enzyme that can produce hydroquinones, which are less toxic to cells compared with the parent quinones [20]. Melatonin displays weak activity toward the MT3-binding site (Ki = 56.9 nM [7]) in contrast to the affinity for MT1 and MT2 receptors that is lower than 1 nM [18]. Melatonin reduces IOP (max 25%) in a dose-dependent manner [21]. Currently, 5-MCA-NAT (5-methoxycarbonylamino-N-acetylthryptamine) is the only known MT3 selective receptor agonist (Ki = 65 nM [7], IC_{50} = 58 nM [22]), which effectively reduces IOP in a dose-dependent manner (max 45%) [21, 23] and has only μM affinity for MT1 and MT2 [18]. Moreover, it was shown that MCA-NAT has antidepressant and neuroprotection activity [24]. Despite the effective IOP reduction 5-MCA-NAT is not used in therapy of glaucoma yet. This may be due to poor solubility of this compound in H_2O [9].



5-Methoxycarbonylamino-N-acetylthryptamine

Crystallographic data [20] showed that MCA-NAT binds at the catalytic site and nowhere else on QR2. QR2 exists as a dimer in solution and crystallizes with one dimer containing two equivalent catalytic sites per asymmetric unit [20] It was also shown that QR2 does not use melatonin as a substrate or a co-substrate [8], i.e. melatonin acts as the inhibitor of QR2. However, there is no consensus on this hypothesis. Alternatively, it was suggested that melatonin acts as a co-substrate donating an electron to FAD, resulting in the reduction of the latter to FADH and then to $FADH_2$. This process involves two melatonin molecules and is described by figure 2.

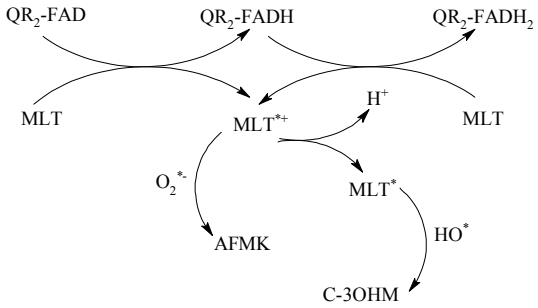


Figure 2. *Scheme of interactions between melatonin (MLT) and QR2.*

Nevertheless, different properties of melatonin reported to involve MT3 cannot be explained so far according to what is known today about QR2. For example, MT3 was originally described as a membrane-bound binding site, while QR2 is a cytosolic enzyme which has never been reported to be membrane-associated. Furthermore, specific MT3 binding at the membrane level could be detected in wild-type mice but not in QR2-/- mice [26]. Moreover, the hypothesis that other inhibitors of quinone reductase should decrease IOP was refused by a series of experiments with chloroquine, menadione, resveratrol, etc. [27]. These pharmacological differences might be attributed to the presence of QR2 monomers at the membrane level. The monomer would not have any catalytic activity because the native enzyme exists as a homodimer and both catalytic sites of the QR2 homodimer are formed of amino acids belonging to each monomer, but it would have a large portion of its sequence available for association with membranes [18].

To determine the mechanism of inhibition of QR2 activity by melatonin, steady-state kinetic studies were performed by varying the concentrations of either the substrate (menadione)

or the co-substrate (NMeH). From the various fits and the patterns of lines in the double-reciprocal plots, it was determined that melatonin is a competitive inhibitor towards the co-substrate NMeH (Ki = 7.2 μM) and a non-competitive inhibitor towards the substrate menadione (Ki = 92 μM) [20].

Besides, it was shown that the hypotensive effect of the high-affinity ligand 5-MCA-NAT on the ocular MT3 receptor is not associated with quinone reductase 2 and, presumably, there is another MT3 subtype melatonin receptor localized in the eye, which is also a G-protein coupled enzyme (phospholipase C) [25, 28, 27, 29].

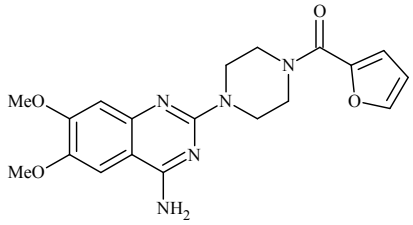
Interestingly, MT3 binding is temperature-dependent. Thus, at temperatures higher than 37°C neither melatonin nor the specific MT3 receptor agonist, MCA-NAT, binds to the MT3 site [25].

In addition, there is the assumption that melatonin ligands have an indirect effect on intraocular pressure through the central nervous system [30]. Furthermore, it was shown that carbonic anhydrase (CA) genes seem to be good targets through which 5-MCA-NAT exerts its long-term hypotensive effect. Reduction of the expression of CA may account for the long-term hypotensive effect of 5-MCA-NAT [31].

The mechanism of the hypotensive action of melatonin and its analogues is not fully understood. However, it was shown that melatonin and its synthetic analogues regulate gene expression of both α-adrenergic receptors, which are responsible for the production of the intraocular fluid, and carbonic anhydrase affecting its outflow [32]. Thus, the mechanism of melatonin receptor activation implements both mechanisms IOP regulation simultaneously.

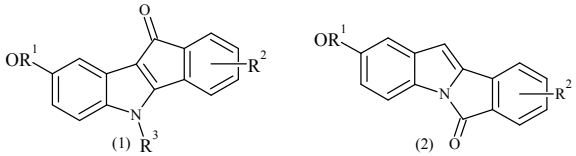
There are a number of approaches to the synthesis of melatonin analogues. The following are the most common examples of modifications of the endogenous ligand, which have been done to be selective to the MT3 subtype. It is believed that the MT3 receptor is an enzyme quinone reductase 2, however, since clarity on this issue has not yet been achieved, this review includes only the works, in which the QR2 ligand binding affinity were studied in comparison with melatonin or other melatonin receptors.

New analogues with high-affinity ligands for the MT3 melatonin receptor subtype were prepared by the bioisosteric replacement of the indole moiety [9] The melatonin analogue prazosin is a widely used a α,β-adrenoceptor antagonist, although it has been claimed to be a melatonin MT3 receptor antagonist when used at nanomolar concentrations (IC_{50} = 6 nM [33], Ki = 3.3 nM [7] in contrast to 7 μM affinity for MT1 and MT2 [33]) [28]. Prazosin is also a good antidepressant agent [24]. It may be considered as a melatonin analogue, in which the indole scaffold is bioisosterically replaced by the quinazoline moiety. It was also shown that prazosin can improve the action of melatonin and MCA-NAT by increasing cAMP at micromolar concentrations [34]. On the other hand, prazosin may be considered as a melatonin analogue, in which the carbon chain in position 3 of the endogenous ligand is homologized and the functional acetamide group is replaced by the furan-2-carbonyl moiety.



Prazosin

It was shown that using tetracyclic compounds can lead to an increase in the affinity for the MT3 subtype against MT1/MT2. The compound of general formula 1 has a higher affinity [35].



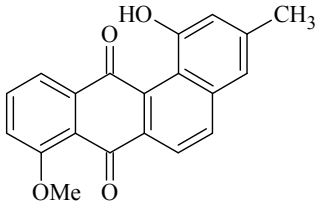
Tetracyclic analogues

The tetracyclic analogue S29434 showed the best selectivity to the MT3 subtype against MT1 and MT2 and has nanomolar affinity among the other ligands for the MT3/QR2 binding site (Table 1).

Moreover, the selective QR2 inhibitor S29434 was shown to protect neurons against the death induced by the deprivation of nutrients or by menadione, an enzyme substrate known to be very damaging to cells after being reduced and activated by the catalytic activity of QR2. The inhibitor S29434 is able to reduce menadione-induced apoptosis probably through the inhibition of the mitochondrial pathway [11, 37].

This compound prevents paraquat (PQ)-induced toxicity in vitro and in vivo in rat models of systemic and brain toxicity and it is several times more potent than melatonin and apocynin, which have been previously shown to antagonize PQ-induced toxicity [38].

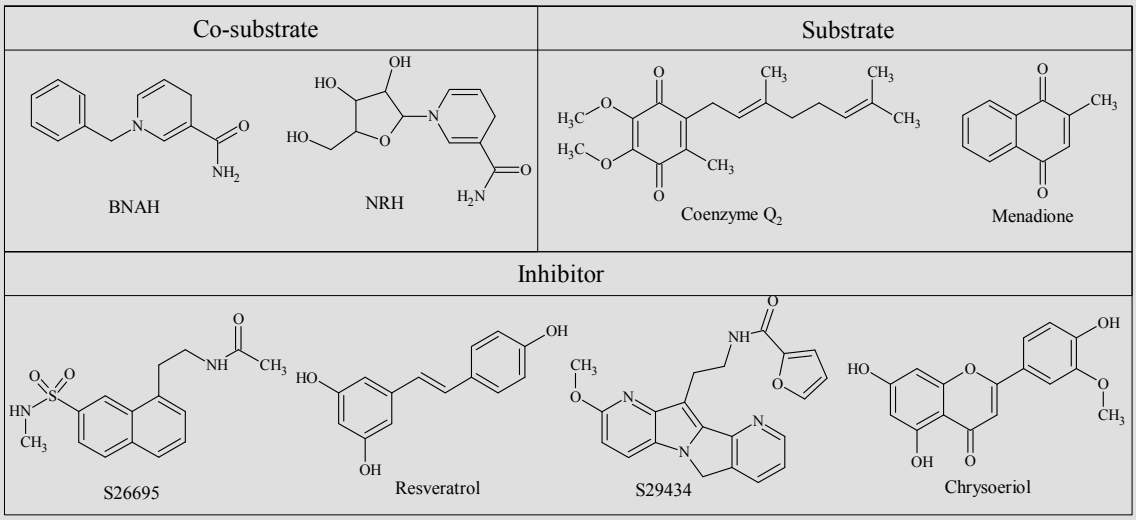
Another tetracyclic compound (tetrangulol methyl ether) was found by screening natural products for inhibitors of quinone reductase 2. This compound was obtained from Actinomyces sp. isolated from marine sediments [39]. However, this compound has only micromolar affinity for the enzyme (IC_{50} = 0.16 μM [39]).



Tetrangulol methyl ether

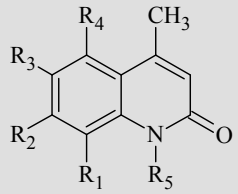
Furthermore, quinolinone analogues of melatonin were also shown to inhibit QR2 in a micromolar concentration range (Table 2). It can be seen that the presence of the methylgroup at the R_5 position results in an increase in the affinity for the enzyme.

Table 1. *IC_{50} values of tetracyclic analogues to menadione and QR2 with different co-substrates.*



Substrate	Inhibitor QR_2	IC_{50}, µM	
		BNAH	**NRH**
Menadione	Melatonin	41.5±1.5	2.8±0.3
	Resveratrol	0.143±0.05	0.129±0.02
	S29434	0.0067±0.0007	0.0007±0.0001
	Chrysoeriol	0.16±0.01	0.016±0.003
	S26695	15.1±3.5	1.2±0.06
Coenzyme Q_2	Melatonin	>100	39.7±5
	Resveratrol	70±11	2.9±0.2
	S29434	0.079±0.01	0.005±0.0009
	Chrysoeriol	0.82±0.015	0.076±0.01
	S26695	2.5±0.1	12.6±1.9

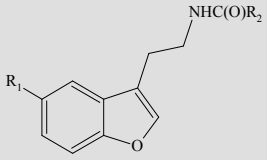
Table 2. *IC_{50} values of quinolone analogue to QR2.*



R_1	R_2	R_3	R_4	R_5	IC_{50} (QR_2), µM
-OCH_2O-		H	H	H	10.8±2.1
-OCH_2O-		H	H	CH_3	6.2±0.8
H	H	H	H	H	>500
H	H	H	H	CH_3	18.2±3.1
OCH_3	H	H	H	H	24.1±3.4
OCH_3	H	H	H	CH_3	5.8±0.9
OCH_3	OCH_3	H	H	H	29.3±3.4
OCH_3	OCH_3	H	H	CH_3	9.3±2.3
OCH_3	H	OCH_3	H	H	8.8±1.1
OCH_3	H	OCH_3	H	CH_3	1.9±0.2
OCH_3	H	H	OCH_3	H	10.8±1.5
OCH_3	H	H	OCH_3	CH_3	4.1±0.6
OCH_3	OCH_3	OCH_3	H	H	10.0±1.4
OCH_3	OCH_3	OCH_3	H	CH_3	7.0±1.1
OCH_3	H	OCH_3	OCH_3	H	6.0±0.6
OCH_3	H	OCH_3	OCH_3	CH_3	10.8±1.3
OCH_3	OCH_3	OCH_3	OCH_3	H	>500
OCH_3	OCH_3	OCH_3	OCH_3	CH_3	>500
Melatonin					11.3±2.1

A few analogues selective to the MT3 subtype were synthesized based on benzofuran as scaffold [22] (Table 3).

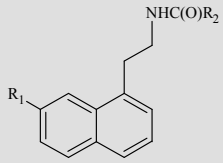
Table 3. *IC_{50} values of benzofuran analogues to melatonin receptors.*



R_1	R_2	IC_{50} (MT_1), nM	IC_{50} (MT_2), nM	IC_{50} (MT_3), nM
OCH_3	CH_3	0,6±0,02	0,7±0,01	64±1
$COOCH_3$	CH_3	-	-	14±0,9
$COOCH_3$	$CH(CH_3)_2$	-	-	65±1
$COOCH_3$	c-C_5H_9	3880±42	389±6	18±1,1
$NHCOOCH_3$	CH_3	-	-	16±0,4
$NHCOOCH_3$	$CH(CH_3)_2$	5350±32	4580±15	23±0,3
$NHCOOCH_3$	c-C_3H_5	8180±5	5900±22	24±1,1
$NHCOCF_3$	CH_3	>10000	2000±54	-
Melatonin		0,2±0,03	0,53±0,06	64,6±0,9
5-MCA-NAT		1000±44	4000±53	58±0,1

The effective naphthalene analogues of MCA-NAT were synthesized and evaluated as melatonin receptor ligands [41] (Table 4).

Table 4. *IC_{50} values of naphthalene analogues to melatonin receptors.*

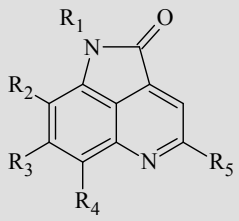


Naphthalenic analogues

R_1	R_2	IC_{50} (MT_1), nM	IC_{50} (MT_2), nM	IC_{50} (MT_3), nM
$NHCO_2Me$	Me	85.4±3	26.3±11	0.39±0.1
SMe	Me	0.875±0.07	0.955±0.04	18.0±1
SOMe	Me	77.6±7	97.0±16	37.0±6
SO_2Me	Me	477±13	879±272	21±4
SO_2NH_2	Me	310±50	371±31.5	32±3
SO_2NHMe	Me	5000±1730	>10000	4.9±03
SO_2NHMe	Furyl	2610±45	6840±130	9.1±0.2

A number of ammosamide analogues were synthesized and evaluated as inhibitors of QR2. The IC_{50} values for these compounds are given in Table 5. It can be seen that the second formationally restricted melatonin analogues have nanomolar affinity for quinone reductase 2.

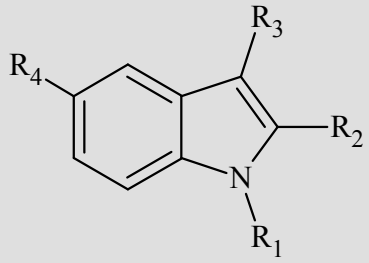
Table 5. *IC_{50} values of formationally restricted analogues to QR2.*



R_1	R_2	R_3	R_4	R_5	IC_{50}, μM
CH_3	NH_2	Cl	NH_2	$C(O)NH_2$	0.061±0.005
CH_3	Cl	H	Cl	$C(O)NH_2$	5.8±1.2
CH_3	Cl	H	Cl	$C(O)NHCH_2Ph$	7.8±1.2
CH_3	Cl	H	Cl	$C(O)NH(CH_3)_2$	25.2±9.4
CH_3	NH_2	Cl	$NHCH_3$	$C(O)NH_2$	0.0041±0.0002

Another way to modify the melatonin molecule is to change substituents in any position of the indole moiety. For example, new melatonin analogues were synthesized and tested for reducing IOP [14] (Table 6).

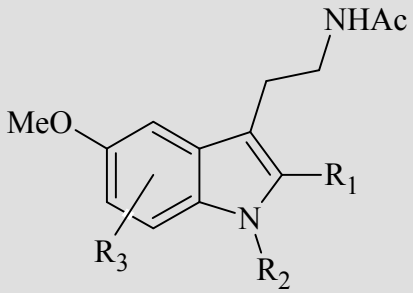
Table 6. *IOP reducing of 1-, 2-, 3-, 5-substituted analogues of melatonin.*



R_1	R_2	R_3	R_4	Maximum percentage of IOP reduction, %
H	H	$-(CH_2)_2NHC(O)CH=CHPh$	OMe	33
Bn	H	$-(CH_2)_2NHAc$	NHC(O)OMe	26
H	H	$-(CH_2)_2NHS(O)_2Bu$	OMe	25
H	H	$-CH_2NHP(O)(OPh)_2$	OMe	22
H	H	$-(CH_2)_2NHCOEt$	NHC(O)OMe	22
H	Br	$-(CH_2)_2NHAc$	NHC(O)OMe	20
H	Me	$-(CH_2)_2NHAc$	NHC(O)OMe	20
H	H	$-(CH_2)_2NHAc$	$NHC(O)OCH_2CH=CH_2$	20
H	H	$-(CH_2)_2NHCOEt$	OMe	20
H	H	$-(CH_2)_2NHCOiPr$	OMe	20
H	H	$-(CH_2)_2NHC(O)Ph$	OMe	18
H	H	$-(CH_2)_2NHC(O)OMe$	NHC(O)OMe	11
H	H	$-(CH_2)_2NHS(O)_2Me$	OMe	10
Me	H	$-(CH_2)_2NHAc$	NMeC(O)OMe	8

New nitroindole derivatives with high affinity and selectivity for MT3 against MT1/MT2 were also synthesized. The binding affinity of these compounds was measured (Table 7). It was shown that the introduction of the nitrogroup in position 4 leads to a significant increase in the selectivity to the MT3 subtype especially in the presence of the 1-Me substituent. In other examples, the selectivity to the MT3 subtype was not achieved.

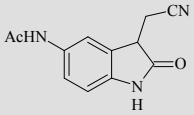
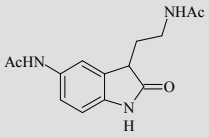
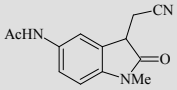
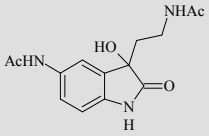
Table 7. *Affinity of ring-NO_2-substituted analogues to melatonin receptors.*



R_1	R_2	R_3	K_i (MT_1), nM	K_i (MT_2), nM	K_i (MT_3), nM
H	CH_3	H	2.6±0.3	3.1±0.2	2.8±0.2
I	H		0.013±0.001	0.16±0.01	6.5±0.03
I	CH_3		0.080±0.001	0.047±0.002	0.088±0.004
H	H	4-NO_2	1060±14	820±25	1.1±0.1
H	CH_3		8560±427	194±3	0.310±0.003
I	H		19.2±0.2	23.7±2.1	0.18±0.01
I	CH_3		1880±67	39±0	0.130±0.006
H	H	6-NO_2	14.6±1.0	5.3±0.4	>1000
I	H		0.30±0.03	0.170±0.005	70±2
I	CH_3		1.10±0.04	0.77±0.05	6.1±0.5
H	H	7-NO_2	49.0±0.2	29.0±0.2	>1000

2-Oxindole derivatives were also shown to have higher affinity to QR2 [44]. The IC_{50} values for quinone reductase 2 are given in Table 8.

Table 8. *IC50 values of 5-acetamid analogues to QR2.*

Compound	IC_{50}(QR2), μM
Melatonin	30.3 ± 3.4
	14.1 ± 0.8
	20.7 ± 2.6
	7.0 ± 0.5
	0.2 ± 0.04

It can be seen that the presence of the methyl group in position 1 leads to an increase in the receptor binding affinity.

Furthermore, the new QSAR model was shown to predict the affinities for the MT3/QR2 melatonin binding site and the ligand, and it would be expected to facilitate the design and development of new selective MT3/QR2 ligands [45]. Taking into account a great potential of selective MT3 receptor ligands, the development of an approach to the synthesis of new melatonin derivatives, the preparation of new melatonin receptor ligands of different subtypes and investigation of their activity are important problems to be addressed.

5. Conclusion

It is clear that melatonin and its receptors play important roles in ocular physiology. Over the past three decades there were synthesized and tested many of molecules, which specifically bind to melatonin receptors. Nevertheless, research in this area is not complete. Despite the many successful works in the synthesis of selective melatonin receptor ligands, a systematic study of the impact of effective MT3 ligands to the IOP is still rare. The exhaustive study of effective MT3 ligand in vitro and in vivo on mammalian model can lead to more effective treatment of glaucoma.

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