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Design and synthesis of 1-(2-alkanamidoethyl)-6-methoxy-7-azaindole derivatives as potent melatonin agonists

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ABSTRACT

A series of 7-azaindolic ligands bearing a methoxy group and a *N*-acetyl chain as melatoninergic pharmacophores were synthesized and their binding affinities towards MT_1 and MT_2 receptors were evaluated. Compounds **7a–c** and **12** (cyclohexyl ring connected at C-2 and C-3 position) appears as important melatonin MT_2 and MT_1 receptors agonists. On the other hand, the presence of basic groups (amines) at position C-3 was detrimental to the melatoninergic affinities.

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone secreted mainly by the pineal gland.[1] In animals, secretion (only during the dark period) and circulating levels of melatonin vary in a daily cycle, thereby regulating the circadian rhythms of several biological functions.[2] One of the first applications of melatonin was for the treatment of diseases associated with the desynchronization of biological clock, such as jet-lag. However, melatonin is involved in many others physiological processes within our bodies and therefore, the possible therapeutic applications for melatonin ligands are significant. Because of its short half-time, the clinical use of melatonin is limited and a great attention have been drawn to the design of novel agonist or antagonist ligands for melatonin receptors. Only two melatonin agonists are on the market: ramelteon approved for the treatment of sleep disorders, and agomelatine for the treatment of depression. Agomelatine has the particularity to be also a 5-HT_{2C} antagonist.[3]

Melatonin acts on specific high affinity G-protein coupled receptors. Two mammalian melatonin receptor subtypes (MT_1 and MT_2) have been identified by molecular cloning studies.[4] Both receptors are expressed mainly in the suprachiasmatic nucleus but they are also expressed in others parts of the brain and even in some peripheral organs.[5] More recently, some studies allowed the identification of a third binding site called MT_3.[6] This binding site has been described in hamster as homologue of the cytoplasmic quinone reductase 2.[7]

Although several teams have worked on melatonin receptors, the knowledge of the physiological roles and significance of each melatonin receptors are still not fully characterized. To gain a deeper knowledge about these receptors and to broaden their pharmacological interest, a considerable attention has been devoted to the design of novel selective melatonin ligands,[8] discovery of agonist ligands representing a more challenging milestone.[9] Rapidly, it was proved that the methoxy group as well as the *N*-acetyl chain of melatonin were the crucial pharmacophores to retain agonist activities.[10] Therefore, the indole ring of melatonin can be considered only as a linker and can be replaced easily by a wide range of others heterocyclic structures without significant loss of binding affinity to MT_1 and MT_2 receptors.[11] This discovery led, for example, to the conception of agomelatine.[12]

In this Letter, we disclose the design and the synthesis of a new class of 7-azaindole MT_1 and MT_2 ligands with agonist activities. Studies in regards to the possible functionalization at position C2 or C2–C3 without losing affinities over MT_1/MT_2 receptors lead to the conception of a highly potent tricyclic ligand.

According to Spadoni et al.[13] the indole core of melatonin can be flipped over without impacting dramatically the binding affinity of the resulting 1-(2-acetamidoethyl)-6-methoxyindole **1**. Indeed, the affinity of compound **1** for the melatonin receptor isolated from quail optic tecta was similar to melatonin (K_i of 3.4 nM and 0.6 nM for compound **1** and melatonin, respectively; Fig. 1). In addition, we have reported that unlike 7-azamelatonin, 4-azamelatonin was a good melatonin receptors ligand (Table 1).[14] We assumed that the flip of the 7-azaindole core could be the starting point for the development of a new class of melatoninergic ligands

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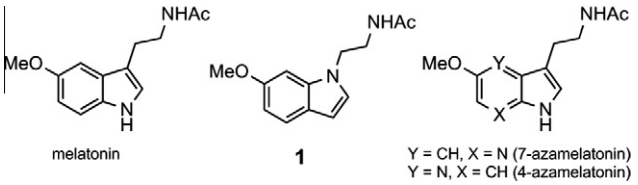


Figure 1. Structures of some potent melatonin ligands.

considering the similar arrangement of the methoxy, pyridine nitrogen and ethylacetamido functions compared to the 4-azamelatonin. In order to validate this pharmacological hypothesis, we synthesized the compound **7a** as depicted in Scheme 1.

The commercially available 7-azaindole was N-oxidized by treatment with *m*CPBA. According to the procedure described by Storz et al.[15] the N-oxide function was O-methylated by treatment with dimethyl sulfate and after addition of sodium methanolate, the desired 6-methoxy-7-azaindole was isolated in good yield. Introduction of the *N*-acetyl chain was achieved in four steps. Compound **4** was first N-alkylated using 1,2-dibromoethane. After reaction of the resulting bromo derivative **5** with sodium azide, compound **6** was converted into amine by Raney nickel catalyzed hydrogenation. The desired amide **7a** was prepared in situ by adding acetic anhydride at the end of the hydrogenation step. This synthetic approach allowed the easy pharmacomodulation of the amide group. Compounds **7b** and **7c** were rapidly prepared by adding the corresponding acyl chloride instead of the acetic anhydride in the last step.

These three novel 7-azaindole derivatives were evaluated (Table 1) for their binding affinity for membranes prepared from human MT_1 and MT_2 receptors stably transfected in Human Embryonic Kidney (HEK 293) cells or Chinese Hamster Ovarian (CHO) cells, using 2-[^{125}I]iodomelatonin as radioligand. The intrinsic activity of the most interesting compounds has been evaluated only on both melatonin receptor subtypes. The results are shown in Table 2. The [^{35}S]GTPγS binding assay used to determine the functional activity of the compounds was performed using Chinese Hamster Ovarian (CHO) cell lines stably expressing the human MT_2 receptors. An agonist stimulates [^{35}S] GTPγS binding, and this stimulation is proportional to the efficacy and intrinsic activity of the molecule. By convention, the natural ligand melatonin has an efficacy (EC_{max}) of 100%. Full agonists stimulate [^{35}S]GTPγS binding with a maximum efficacy, close to that of melatonin itself. If EC_{max} is between 30% and 70%, the compound is considered as partial agonist. Whereas if EC_{max} is inferior to 30%, the compound is considered as antagonist. Compounds **7a–c** show very high binding affinity for MT_1 and MT_2 receptors closed to those of melatonin for MT_2 receptors. All these compounds exhibit evident agonistic activity. It is noteworthy that the length of the acyl function (Me, Et, *n*Pr) has almost no impact on the MT affinities.

These results showed that flipping the 7-azaindole core of 7-azamelatonin allow to keep affinities at nanomolar concentrations for MT receptors. Improvement of the biological properties of these new ligands was investigated mainly by substituting the pyrrole ring. Considering the remain biological affinities reported for the N-1 functionalized endogenous melatonin,[16] we started our investigation by functionalize the C-3 position of **7a**.

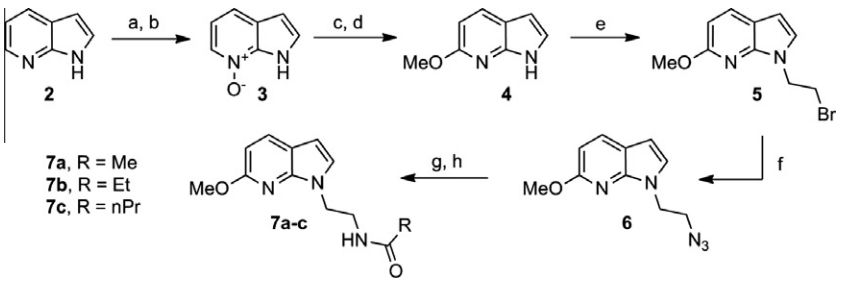
Taking advantage of the reactivity of the C3 position of azaindole towards strong electrophiles, we generated a small library of C-3 substituted 7-azaindole derivatives (compounds **8a–g**, Table 2) using the Mannich reaction. All these novel compounds have been evaluated on MT_1/MT_2 receptors but, unfortunately, a dramatic negative effect on affinity occurred. None of these new ligands kept a reasonable activity.

The presence of an extra basic nitrogen atom in compounds **8a–g** was though to be responsible of the drop of binding affinities. The synthesis and evaluation of compounds **8h–i** gave this hypothesis credit (Scheme 2, Table 3). Indeed, the introduction of trifluoromethyl group (compound **8h**) or iodine (compound **8i**) at C-3 position did not impact dramatically the binding affinities.

Following these considerations, we decided to introduce an additional cycloalkyl chain connected to the C-2 and the C-3 position. We hypothesized that this bis functionalisation should not be prejudicial for the melatoninergic affinity by analogy with the remain biological affinities reported for the N-1 or C-2 functionalized endogenous melatonin.[8d,17] The synthesis of this original target **12**

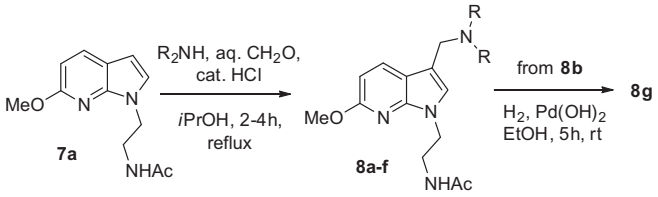
Table 1
MT_1 and MT_2 binding affinities (K_i) and functional activities (EC_{50} and E_{max}) of compounds **7a–c**

Compounds	MT_1			MT_2			K_{iMT_1}/K_{iMT_2}
	K_i ± SEM (nM)	EC_{50} ± SEM (nM)	EC_{max} ± SEM (%)	K_i (nM)	EC_{50} (nM)	EC_{max} (%)	
Melatonin	0.25 ± 0.09	1.5 ± 0.64	101 ± 18	0.34 ± 0.09	0.42 ± 0.17	102 ± 2	0.7
7-Azamelatonin	40 ± 9.4	1 100 ± 104	92 ± 4	117 ± 15.6	380 ± 125	101 ± 5	0.3
4-Azamelatonin	0.2 ± 0.004	12 ± 1.3	96 ± 3	0.3 ± 0.6	1.1 ± 0.2	99 ± 4	0.7
7a	1.4 ± 0.1	4.0 ± 0.5	99 ± 3	0.6 ± 0.05	2.1 ± 0.6	72 ± 4	2.3
7b	3.3 ± 1.0	5.1 ± 0.6	87 ± 4	0.28 ± 0.01	1.4 ± 0.5	105 ± 4	11.8
7c	1.3 ± 0.1	2.1 ± 0.3	93 ± 3	0.3 ± 0.04	0.7 ± 0.1	77 ± 10	4.3



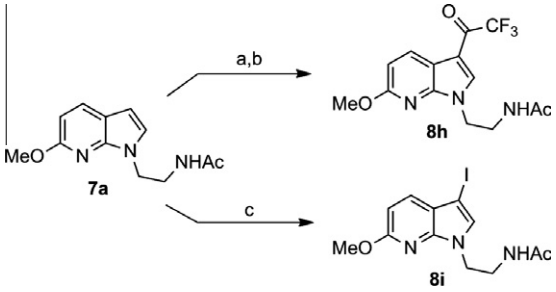
Scheme 1. Reagents and conditions: (a) mCPBA, AcOEt, 0 °C–rt, 18 h; (b) aq K_2CO_3, 6 h, 4 °C, 57% over two steps; (c) Me_2SO_4, MeCN, 15 h, 65 °C; (d) MeONa, 18 h, 60 °C, 61% over two steps; (e) 1,2-dibromoethane, aq NaOH 50%, Bu_4NI, 38 h, rt, 90%; (f) NaN_3, water/dioxane, 40 h, reflux, 99%; (g) H_2, Raney Ni, AcOEt, 2 h, rt; (h) K_2CO_3, Ac_2O or corresponding acyl chloride, 1–3 h, rt, 76–83% over two steps.

Table 2
Synthesis, MT_1, MT_2 binding affinities (K_i) and functional activities (EC_{50} and E_{max}) of C-3 substituted 7-azaindolic derivatives bearing an extra basic nitrogen atom



R_2N-, compounds no.	Yield	K_i MT_1 (nM)	K_i MT_2 (nM)	K_{iMT_1}/K_{iMT_2}
8a	81%	>10,000	>10,000	/
8b	91%	10,460 ± 613	4007 ± 318	2.6
8c	81%	510 ± 75	742 ± 38	0.7
8d	70%	>10,000	550 ± 28	>20
8e	52%	1210 ± 255	929 ± 60	1.3
8f	59%	9490 ± 211	7440 ± 117	1.3
8g	30% from **8b**	8295 ± 397	1090 ± 24	7.6

is depicted in Scheme 3. The pyridylhydrazine **10** was synthesized from the commercially available 2-chloro-6-methoxypyridine **8** by nucleophilic aromatic substitution of the chlorine with hydrazine. The Fischer indole methodology was then used as a key step for the construction of the azaindole core. Although the Fischer cyclization is known to be tricky with this specific pyridylhydrazine,[18] the tricyclic core **10** was obtained with moderate yield under microwave activation. The *N*-acetyl chain was then introduced using the synthetic sequence described in Scheme 1.



Scheme 2. Reagents and conditions: (a) TFAA, CH_2Cl_2, MW activation, 100 °C, 20 min; (b) MeOH, aq Na_2CO_3, 86% over two steps; (c) NIS, THF, 2 h, rt, 72%.

The biological evaluation of **12** gave good satisfaction (Table 3). This new derivative appears to be an interesting melatonin ligand with affinity in the nanomolar range. This result shows that C-2 and C-3 positions can be substituted without losing biological activities and opens the way to the design of original tricyclic melatonin ligands.

In summary, we have identified a novel series of melatonin agonist for both MT_1 and MT_2 receptors, built on a 7-azaindole core. We have shown that extending the terminal alkyl chain of the amide had a benefic effect on the biological affinities. Basic groups (amine) at position C-3 were detrimental to the melatonin affini-

Table 3
MT_1 and MT_2 binding affinities (K_i) and functional activities (EC_{50} and E_{max}) of compounds **7a**, **8h–i** and **12**

Compounds	MT_1			MT_2			K_{iMT_1}/K_{iMT_2}
	K_i ± SEM (nM)	EC_{50} ± SEM (nM)	EC_{max} ± SEM (%)	K_i (nM)	EC_{50} (nM)	EC_{max} (%)	
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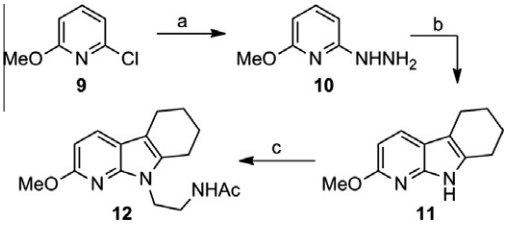
7-Azamelatonin	40 ± 9.4	1100 ± 104	92 ± 4	117 ± 15.6	380 ± 125	101 ± 5	0.3
4-Azamelatonin	0.2 ± 0.004	12 ± 1.3	96 ± 3	0.3 ± 0.6	1.1 ± 0.2	99 ± 4	0.7
7a	1.4 ± 0.1	4.0 ± 0.5	99 ± 3	0.6 ± 0.05	2.1 ± 0.6	72 ± 4	2.3
8h	91 ± 12	93 ± 9.9	82 ± 4	30 ± 1	10 ± 0.5	93 ± 3	3.0
8i	16 ± 1.4	32 ± 7	37 ± 5	5 ± 0.5	11 ± 1.7	37 ± 7	3.2
12	9.0 ± 1.4	16 ± 4.3	83 ± 7	0.68 ± 0.05	1.3 ± 0.1	89 ± 1	13.2



Scheme 3. Reagents and conditions: (a) hydrazine, MW activation, 160 °C, 20 min, 70%; (b) cyclohexanone, aq H_2SO_4 4%, MW activation, 160 °C, 20 min, 31%; (c) see Scheme 1 for reagents and conditions, 33% over four steps.

ties while a cyclohexyl ring connected at C-2 and C-3 positions affords good affinities for MT_2/MT_1 receptors with agonist potency.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.097.

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