Melatonin Receptor Agonists: New Options for Insomnia and Depression Treatment

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SUMMARY

The circadian nature of melatonin (MLT) secretion, coupled with the localization of MLT receptors to the suprachiasmatic nucleus, has led to numerous studies of the role of MLT in modulation of the sleep-wake cycle and circadian rhythms in humans. Although much more needs to be understood about the various functions exerted by MLT and its mechanisms of action, three therapeutic agents (ramelteon, prolonged-release MLT, and agomelatine) are already in use, and MLT receptor agonists are now appearing as new promising treatment options for sleep and circadian-rhythm related disorders. In this review, emphasis has been placed on medicinal chemistry strategies leading to MLT receptor agonists, and on the evidence supporting therapeutic efficacy of compounds undergoing clinical evaluation. A wide range of clinical trials demonstrated that ramelteon, prolonged-release MLT and tasimelteon have sleep-promoting effects, providing an important treatment option for insomnia and transient insomnia, even if the improvements of sleep maintenance appear moderate. Well-documented effects of agomelatine suggest that this MLT agonist offers an attractive alternative for the treatment of depression, combining efficacy with a favorable side effect profile. Despite a large number of high affinity nonselective MLT receptor agonists, only limited data on MT₁ or MT₂ subtype-selective compounds are available up to now. Administration of the MT₂-selective agonist **IIK7** to rats has proved to decrease NREM sleep onset latency, suggesting that MT₂ receptor subtype is involved in the acute sleep-promoting action of MLT; rigorous clinical studies are needed to demonstrate this hypothesis. Further clinical candidates based on selective activation of MT₁ or MT₂ receptors are expected in coming years.

Introduction

Melatonin (MLT, Figure 1) is the main biologically active substance secreted by the pineal gland, an organ long considered as a vestigial structure, until Lerner, a dermatologist from Yale University, in 1958 isolated an amphibian skin-lightening molecule, and identified it as *N*-acetyl-5-methoxytryptamine [1]. Its isolation marked the start of an increasing research effort to identify the endogenous role of MLT and the mechanism by which it regulates the physiology of both mammalian and nonmammalian species.

The pineal production of MLT shows a circadian rhythm with low circulating levels during the day and high plasmatic concentrations of hormone at night. This rhythmicity is controlled by the suprachiasmatic nucleus (SCN) of hypothalamus (the body master clock controlling circadian rhythms), whose activity is synchronized to the 24 h period by the light/dark cycle [2].

Although circulating MLT in mammals is mainly of pineal origin, the hormone is also produced by neuroendocrine cells [3]

in the retina, Harderian glands, pancreas, gastrointestinal tract, and in minor amounts by nonendocrine cells such as bone marrow, thymus, and lymphocytes, where it may be of some local importance.

The biosynthesis of MLT in the pineal gland involves several steps that can be summarized as follows: pinealocytes take up L-tryptophan from the cerebral vessels and convert it to serotonin through 5-hydroxylation and decarboxylation; serotonin is then converted to *N*-acetyl-serotonin by the rate-limiting enzyme arylalkylamine *N*-acetyl transferase, before finally being converted into MLT by hydroxyindole-*O*-methyl transferase.

Once synthesized, MLT is not stored within the pineal gland, but diffuses out into the bloodstream, rapidly reaching all tissues of the body. Circulating MLT is metabolized by cytochrome P-450 which catalyzes hydroxylation of the hormone at the C-6 indole position to yield 6-hydroxymelatonin. This reaction is followed by conjugation with sulfuric acid (or to a lesser extent with glucuronic acid) to produce the principal urinary

Figure 1 Melatonin (MLT) and clinically advanced MLT receptor agonists.

metabolite, 6-sulfatoxymelatonin. In the brain MLT can be metabolized to the kynurenine derivative *N*₁-acetyl-5-methoxykynurenine by oxidative pyrrole-ring cleavage, or to a cyclic 3-hydroxymelatonin derivative (1-{3 α -hydroxy-5-methoxy-3,3 α ,8,8 α -tetrahydropyrrolo[2,3-*b*]indol-1(2*H*)-yl}ethanone).

The absolute bioavailability of MLT in humans after exogenous administration of deuterated D₇-MLT was low (from 1% to 37%) due to first pass metabolism, and a short half-life (30–45 min) was observed [4].

Much of the research in the first 30 years after the discovery of MLT was related to its ability to modulate reproductive physiology in photoperiod-dependent seasonally breeding mammals [5]. As a result of these findings, MLT has been successfully used as a pharmacological agent to advance the breeding season of sheep and other photoperiodic animals [6].

During the past four decades MLT has been claimed to have an effect in almost all the main physiological functions of the body. Among the actions attributed to MLT are those concerning circadian rhythms and sleep regulation [7], control of mood and behavior [8], neuroimmunomodulation [9], blood pressure control [10], pain perception [11], antiinflammatory [12], antioxidant [13], neuroprotectant [14], and antitumor [15]. Of particular interest are also the reports on its putative role in the events leading to the development of type 2 diabetes [16,17].

Although many of the miraculous claims made about MLT are matter of speculation, clinical trials, and results of preclinical studies in validated animal models suggest that some therapeutic options exist for MLT, particularly for the treatment of insomnia, circadian-rhythm sleep disorders (CRSDs), and depression.

Melatonin Receptors

The therapeutic potential of MLT, and its synthetic analogs, depends critically on our understanding of its target sites and mechanisms of action. Initial attempts to identify receptors using radioligand binding techniques involved the use of [³H]-MLT [18]. The discovery of different MLT binding sites was facilitated by the synthesis in 1984 of the high-affinity agonist radioligand 2-[¹²⁵I]-

iodoMLT [19] that has made possible autoradiographic studies revealing a widespread distribution of binding sites throughout the nervous system [20]. MLT binding sites are particularly abundant in the retina, the SCN, the pars tuberalis of the pituitary gland, cerebral, and tail arteries [20], but as data continue to accumulate, it seems that few tissues are devoid of MLT membrane receptors [21]. Knowledge of the structure and function of MLT receptors has evolved over the past 15 years, since the cloning of two well-characterized receptors, now designated by IUPHAR as MT₁ and MT₂ [22–24]. These receptors are members of a new subfamily of G-protein-coupled receptors (GPCRs) that show little sequence homology with other known receptors. Both MT₁ and MT₂ receptors exhibit subnanomolar binding affinity for MLT and are negatively coupled to adenylate cyclase, even if they can also interact with other intracellular signaling pathways [25]. A third MLT receptor subtype (termed Mel_{1c}), has been cloned from *Xenopus laevis*, chicken and zebrafish, but it is not expressed in mammals [26]. In addition to these high affinity GPCRs MLT receptors, a distinct binding site (termed MT₃), displaying lower affinity for MLT (*K*_i $\cong$ 10–60 nM), has been purified and characterized as the human enzyme quinone reductase 2 [27]. MLT has also been described as a ligand for the retinoid related orphan nuclear hormone receptor family (RZR/ROR) [28], but the existence of these MLT binding sites is highly controversial, as the direct binding to these receptors has never been reproduced [29,30]. Finally, a MLT related orphan receptor (GPR50) has been cloned from humans, sheep and rats [31]. Interestingly, this orphan receptor is structurally similar to GPCRs MLT receptors, with a 45% homology at the amino acid level, but it does not bind MLT or related compounds. In mammals, MLT receptors are expressed in the brain, with considerable variation in location and density of expression among species, and also in some peripheral tissues [21,32]. The diversity in tissue distribution suggests different functional physiological roles for each MLT receptor subtype, but a clear functional distinction among these subtypes needs an intensive further investigation. For example, there is evidence suggesting that activation of MT₁ receptors in mouse inhibits neuronal firing within the SCN, inhibits prolactin

Figure 2 Strategies leading to melatonin receptor agonists.

secretion in photoperiodic species, modulates visual function in the mouse retina [33] and induces vasoconstriction on rat caudal artery; instead, activation of MT_2 receptors induces vasodilation, produces a phase shift in circadian rhythms and inhibits dopamine release in rabbit retina (for review see Ref. [21]). Further complexity arises from the formation of MT_1 and MT_2 homo- and heterodimers in cells expressing the two MLT receptor subtypes, and the functional consequences of this association on receptor signaling are currently unknown.

Strategies to Develop Melatonin Agonists

During the last two decades a great number of MLT receptor agonists, belonging to structurally different classes have been reported in literature and in patent applications. The structure-

activity relationships (SARs) for MLT derivatives have already been extensively reviewed [34–38], and therefore they will be discussed only briefly, highlighting the general strategies used to develop these compounds. Initially, some closely structurally related MLT derivatives (essentially polysubstituted tryptamines, Figure 2) were prepared to investigate the influence of substituents nature and position on melatonergic activity and to determine the structural elements required for high binding affinity. The pharmacophore structure that can be found in almost all MLT receptor agonists is composed by an amide group connected, by a linker chain, to an aromatic nucleus (mimicking the indole of MLT) carrying a methoxy group, or a bioisostere, such as bromine. Introduction of appropriate substituents (i.e., phenyl or halogens) at the C2-indole position led to agonists with higher binding affinity (approximately 10-fold) than MLT itself, such as 2-iodomelatonin, 2-bromomelatonin [39], and 2-phenylmelatonin

[40]. Increasing the length of the *N*-acyl group, up to three carbons, as well as substitution at the 6-position of the indole ring were also well tolerated. For instance, 6-chloromelatonin demonstrated comparable affinity ($K_{iMT1} = 0.58$ nM; $K_{iMT2} = 0.32$ nM) to MLT. Combination of a 6-chlorine with a (*R*)- β -methyl group was reported in the agonist TIK-301 (Figure 1, previously known as LY156735, $K_i = 0.081$ and 0.042 nM at MT_1 and MT_2 receptors, respectively), which was advanced to clinical study for insomnia treatment [41,42]. Several potent MLT agonists have been designed by bioisosteric replacement of the indole core with other aromatic rings. Successful cases of bioisosteric replacement were the naphthalene ring system (i.e., **6**) which is present in agomelatine [43,44], and the indane (i.e., **10**), which is the scaffold of ramelteon [45]. Other scaffolds explored so far include a benzofused skeleton such as benzothiophene, benzofuran, benzimidazole (compound **1–3** [44] in Figure 2), indene **4** [46] quino-line **7** [47], azaindole **8** [48], and indoline **9** [49]. Potent MLT bioisosteres were also obtained by shifting the side chain of MLT from 3- to 1-position of the indole ring and the methoxy group from 5- to 6-position (i.e., compound **5**) [50]. Partial hydrogenation of the indole nucleus, as for the indoline derivative **9**, was also applied to naphthalene derivatives, and the SARs of the resulting tetrahydronaphthalenes [51] were similar to those observed in the indole series. Tetralins (**11**) [52] and oxygenated heterocycles (such as chromane **12** [53]) provide examples of compounds with different core scaffolds, leading to active MLT analogs when the methoxy substituent and the amide group are properly positioned. Further investigations have determined that the bicyclic aromatic nucleus is not essential for melatonergic activity. In fact, simplified analogues such as methoxyphenyl- (**13**) [54], or methoxyanilinoalkylamides (**14**) [55] were found to retain good agonist activity.

Another approach very commonly applied in the design of novel MLT agonists is the incorporation of the methoxyl group or the acylaminoethyl side chain into a conformationally restricted ring structure; this strategy was also useful in the identification of MLT active conformation at its membrane receptors [35,38]. Among the various MLT analogs that were prepared as constrained agonists (for extensive reviews see [35,37,38]), it is worth mentioning the tricyclic derivatives **16** [56,57] and **17** [58], having binding affinities similar to MLT at both receptor subtypes. The alkyl chain can also be constrained in a *trans*-cyclopropylmethyl fragment as in tasimelteon (Figure 1), or in a pyrrolidine ring as in compound **18** [59] (Figure 2). Incorporation of the critical methoxy group into a five- or six-membered ring [60], as can be seen in dihydrobenzofuran (i.e., ramelteon [45] or compound **18** [59]), in benzoxazole (**19**) [59] and benzo-1,3-dioxole (**20**) [45] derivatives, led to a series of compounds with excellent affinity for MLT receptors.

Melatonin Receptor Agonists in the Management of Insomnia and Major Depression

While synthetic MLT agonists have the potential to modulate a number of disease states, the current clinical focus is primarily

on developing treatments to manage insomnia, depression and CRSDs. These are indeed the therapeutic indications for MLT receptor agonists approved for marketing or currently under clinical evaluation. The idea that MLT might promote sleep and synchronize the internal clock stimulated some clinical trials. However, after four decades of investigation, the scientific evidence supporting the sleep-promoting effects of MLT in different experimental conditions remains controversial [61,62]. A major obstacle for the use of MLT as a clinically efficient hypnotic results from its short half-life, which is mostly in the range of 20–40 min, sometimes even less. Solutions to this problem can be sought in developing prolonged-release formulations of the natural hormone, or melatonineric agonists with longer half-life. Both possibilities were considered, resulting in the production of approved or investigative drugs, such as Circadin[®], Rozerem[®], Valdoxan[®], tasimelteon, and other subtype selective MLT receptor agonists.

Circadin[®], (Neurim Pharmaceuticals, Tel-Aviv, Israel) is a prolonged-release 2 mg MLT formulation that, when taken before bedtime, maintains effective serum concentrations of the endogenous hormone throughout the night. It was approved by the European Medicines Evaluation Agency (EU-EMA) in June 2007 for the short-term treatment of primary insomnia characterized by poor quality of sleep in patients aged 55 years and over.

Placebo controlled clinical trials highlighted, beyond the shortening of sleep latency seen with traditional hypnotics, concomitant improvements in sleep quality and next day alertness [63]. On the contrary, direct evidence for the support of sleep maintenance and total sleep time is poor. In contrast to traditional sedative hypnotics, Circadin[®] has shown no evidence of impairing cognitive and psychomotor skills, of rebound, dependence or abuse potential and no significant adverse events compared to placebo [64]. Preliminary results of a large-scale (790 patients) Phase III study for insomnia, showed that 6 months continuous administration of Circadin[®] is both safe and efficacious, thus demonstrating long term efficacy and safety [65]. Studies on treatment of jet lag and shift work disturbances reported a relatively good efficacy of 2 mg sustained-release MLT [66]. In exploratory studies Circadin[®] also improves sleep quality in patients with chronic schizophrenia [67] as well as in patients with major depressive disorders [68].

Ramelteon (Rozerem[®], TAK-375, Figure 1), an indenofuran MLT derivative produced by Takeda Pharmaceuticals Inc. (Osaka, Japan), was the first MLT agonist to be approved by the FDA (July 2005) for the treatment of insomnia characterized by difficulty in sleep onset.

Ramelteon was also filed in March 2007 in E.U. for primary insomnia. Nevertheless, EMA found the efficacy of ramelteon in improving sleep maintenance insufficient for a marketing authorization [69]. After this negative recommendation, Takeda has withdrawn its European marketing authorization application in September 2008 [70].

Ramelteon binds with high affinity to MT_1 and MT_2 receptors [45] without significant affinity for a large number of other CNS binding sites [71]. Pharmacokinetic studies revealed that ramelteon is metabolized primarily via oxidation; the major metabolite, named M-II (2-hydroxy-*N*-[2-(2,6,7,8-tetrahydro-1*H*-indeno[5,4-*b*]furan-8-yl)ethyl]propanamide), carries

a hydroxyl group at the C-2 position of the propionyl residue, retains good MLT receptors affinity (about 10-fold less than the binding affinity of the parent molecule) [72], and it is 17- to 25-fold less potent than ramelteon in *in vitro* functional assays [73]. M-II also showed a potent sleep promoting action in freely moving cats, and thus it may contribute to pharmacological effects of ramelteon.

Ramelteon has been studied in animal models of insomnia as well as of CRSDs. The agent promotes sleep in freely moving monkeys [74] and cats [75], without causing any significant adverse effects such as learning and memory deficits, impairment of motor coordination, and drug abuse [76]. In addition, it is able to enhance the rate of re-entrainment in response to a shift in the light/dark cycle in rats [77]. Beneficial effects of ramelteon on sleep have been confirmed in clinical studies, including patients with primary insomnia as well as healthy volunteers undergoing experimentally induced transient insomnia (for review of the randomized controlled trials examining the efficacy and tolerability of ramelteon see Ref. [78]).

Considering the clinical data thus far, ramelteon has consistently demonstrated significant sleep promoting effects, as evidenced by reductions in sleep latency (about 10–13 min more than placebo) and increases in total sleep time (about 12 min), among patients with chronic insomnia [79–81] and subjects with transient insomnia [82], during both short-term [79,82] and long-term treatment [80,81]. Although doses used in clinical trials ranged from 4 mg to 64 mg, the recommended dosage for the treatment of insomnia in adults is 8 mg, 30 min prior to the habitual bedtime. As also reported for the other melatonin therapeutic agent approved for the treatment of insomnia, Circadin[®], ramelteon has demonstrated no next-day residual effects, rebound insomnia, or abuse potential in either preclinical animal models or in clinical evaluation in humans, and is therefore not a controlled (scheduled) drug [83].

The naphthalenic MLT bioisostere agomelatine (Valdoxan[®], Figure 1), is a novel antidepressant with an innovative pharmacological profile which has been developed by Servier (and Novartis in US). It is a potent MT₁/MT₂ agonist and also has weak 5-HT_{2c} antagonist properties. Its antidepressant activity may be due to each of these properties, or to their combination. It does not display significant affinity for other central receptors or membrane transporters. In February 2009, Valdoxan[®] was approved by the EU-EMEA for the treatment of major depressive disorders (MDD) and is available in several European countries. Phase III clinical trials of agomelatine for depression (three short-term efficacy and safety trials and one longer-term relapse prevention trial) have also been conducted in the United States, but the results from these studies have not been released and agomelatine is not yet approved by the US FDA [84].

The antidepressant activity of agomelatine has been first extensively investigated in preclinical studies before being studied in humans [85,86]. The efficacy, tolerability and safety of agomelatine have been assessed in several double-blind, randomized, placebo- and active-controlled Phase III studies. The results of a 8-week placebo-controlled dose-ranging study involving 711 MDD patients, with paroxetine as an active control, indicate that agomelatine, at a dose of 25 mg once daily, has similar efficacy to

paroxetine [87]. Further studies versus placebo and comparators have confirmed the efficacy of Valdoxan[®] in adults of all ages, including the severely depressed and elderly depressed [88–90]. A study comparing Valdoxan[®] with venlafaxine showed comparable antidepressant efficacy of both treatments, but significantly less sexual dysfunction of Valdoxan[®] compared to the Serotonin-Norepinephrine Reuptake Inhibitors (SNRI) [91]. Furthermore, no discontinuation symptoms were observed after cessation of agomelatine treatment in contrast to that found with paroxetine [92]. Besides being an effective antidepressant, agomelatine decreased the severity of anxiety associated with depression, and, unlike other antidepressants, has a markedly positive impact on the often disrupted sleep–wake rhythm of depressed patients, without affecting daytime vigilance [93].

The combined action at MT₁, MT₂, and 5-HT_{2c} receptors, which may resynchronize disturbed circadian rhythms and abnormal sleep patterns, suggests that agomelatine might be particularly effective for the treatment of seasonal affective disorders, bipolar depression [94], as well as in other possible subtypes of MDD [95]. Preliminary open-label studies in these patient populations have suggested some benefits [96,97] and further studies are clearly warranted.

Another newly investigative synthetic melatonergic agonist is tasimelteon (Figure 1, formerly known as VEC-162 or BMS-214778). This compound, developed by Vanda Pharmaceuticals, under license from Bristol-Myers Squibb, was tested in two randomized, double-blind, placebo-controlled clinical trials for its effects on transient insomnia induced by a 5-h phase advance of the sleep–wake cycle in healthy individuals [98,99]. Compared with placebo, tasimelteon reported beneficial effects on sleep latency and maintenance at all three tested doses (20 mg, 50 mg, and 100 mg), and showed a good safety profile without significant side effects versus placebo. Moreover, the compound was effective in resetting the circadian MLT rhythm after a sleep–time shift, suggesting that it may be a good candidate for the treatment of CRSDs, especially the jet-lag and shift-work types.

Neu-P11 is a melatonin receptor agonist developed by Neurim Pharmaceuticals that recently started its evaluation in clinical trials. The chemical formula of Neu-P11 has not been disclosed, even if its sleep promoting activity and improvement in insulin sensitivity have been related to similar effects reported for a series of pyrone-indole derivatives patented by Neurim Pharmaceuticals [100]. Neu-P11 is a combined MT₁/MT₂/MT₃ and a 5-HT_{1A}/5-HT_{1B}/5-HT_{2B} agonist; it also enhances GABA activity, but it does not directly interact with GABA receptors. The sleep promoting activity of Neu-P11 has been evaluated in a double blind, randomized, placebo-controlled phase I study in which it was administered at the doses of 5 mg, 20 mg, 50 mg, and 100 mg [101]. Significant increases in the sedation assessment scores (Stanford Sleepiness Scale) at T + 2 h and T + 4 h were obtained, as well as trends of decreases in number of awakenings and wake after sleep onset (WASO) during the T + 2 h and T + 3 h EEG recording periods, with an increase in theta band activity. Neu-P11 was also evaluated in preclinical studies for its antidepressant-like properties [102]. Tests were conducted in rats at the doses of 25/100 mg/kg i.p. In the learned helplessness test rats treated with Neu-P11 at the dose of 50 mg/kg showed a significant decrease of the

escape deficit, similarly to what was observed after imipramine administration. Interestingly, MLT (25/100 mg/kg i.p.) was ineffective. Moreover, Neu-P11 decreased immobility of rats in the forced swimming test.

Melatonin Receptor Selective Agonists

The development of MLT receptor agonists with improved properties has enhanced the prospects of manipulating the MLT system to treat patients with a range of sleep disorders, or suffering from depression. Unfortunately, none of the most clinically advanced MLT agonists discriminates between MT₁ and MT₂ MLT receptor subtypes. The development of a subtype-selective MLT receptor agonist could result in a safer compound with a more favorable pharmacological profile. In the last decade, research on new MLT ligands has expanded to identify selective compounds for either the MT₁ or MT₂ receptor subtypes [37]. Few examples of MLT receptor agonists (or partial agonists) characterized by selectivity for the MT₁ or MT₂ receptor are reported in the literature. In most cases they derive from minor structural modifications of nonselective agonists, but a clear indication on the structural requirements characterizing this pharmacological behavior is still lacking.

The first high affinity ($pK_{iMT_2} = 0.05$ nM) MT₂-selective agonist to be discovered was the tetracyclic indole derivative **IIK7** [103] (Figure 3) which has a 90-fold selectivity for MT₂. This compound can be viewed as an analog of 2-phenylMLT, in which the phenyl substituent is linked to the indole nitrogen by a methylene group. Interestingly, **IIK7** administered to rats at a dose of 10 mg/kg i.p. produced a significant decrease in NREM sleep onset latency, suggesting that the MT₂ receptor is the subtype involved in the acute sleep-promoting action of MLT [104]. Similar results were previously reported in a meeting abstract [105], where the authors described the sleep-promoting properties of another recently developed MT₂-selective partial agonist (**UCM765**, Figure 3) [55] following subcutaneous administration to rats. However, rodents are active at night, when MLT concentrations are high, and the relevance of these studies has still to be fully assessed.

A more pronounced MT₂ selectivity ($K_{iMT_1/MT_2} = 117$) was displayed by the (*R*)-indanylpiperazine **21**. This conformationally restricted compound, obtained by incorporating the linking alkyl chain into a piperazine fragment, has been reported to be a full agonist possessing good affinity for the h-MT₂ receptor with little affinity for the MT₁ subtype [106].

A series of anilides [107], substituted in *para* with a 3-methoxyphenyl-ether, -thioether, -methylene or -amino moiety, has been reported to have subnanomolar MT₂ affinity and good subtype selectivity. The 4-(3-methoxyphenoxy)anilido derivative **22** demonstrated full agonism in a MT₂ adenylyl cyclase assay, and 84-fold selectivity over the MT₁ receptor. Starting from MT₁/MT₂ nonselective 3-methoxyphenylpropyl amides (i.e., **13**), Wong et al. recently discovered new potent MT₂-selective ligands by introduction of a benzyloxy substituent at the C6-position of the 3-methoxyphenyl ring (i.e., compound **23**) [108]. In binding studies using [³H]-MLT as radioligand, these compounds exhibited pM to subpM affinity toward MT₂ and over 200 nM affinity toward MT₁ receptor. Preliminary results suggest that these

compounds are potent MT₂-selective agonists as they stimulated intracellular Ca²⁺ release in CHO cells expressing the MT₂ receptor. Finally, it was possible to obtain MT₂-selective partial agonists, by introducing a phenyl substituent at the C3-position of tetrahydronaphthalene or naphthalene MLT analogs, as exemplified by compounds **24** [109] and **25** [110].

Attempts to discover MT₁-selective ligands have not yet borne good fruit. Literature only reports few examples of MT₁ selective ligands, and this lack has made it difficult to ascertain the physiological role of this receptor subtype. An interesting approach for the design of MLT receptor ligands was the preparation of symmetric dimers, by coupling two moieties deriving from known ligands [111–114]. Binding affinity, intrinsic activity and subtype selectivity of these dimers depend on the position of the junction and on the length of the linker. Among dimeric agonists, compound **26** (Figure 3), obtained by dimerization of two desmethoxy agomelatine units at the C-6 position of the aromatic ring, proved to be selective for the MT₁ receptor, displaying 50–100-fold higher binding affinity than at the MT₂ receptor [114]. Following the same “bivalent ligands” approach, a series of novel asymmetric heterodimers was recently reported [115], and some of these compounds (**27**, **28**) were described as MT₁ selective partial agonists ($K_{iMT_2/MT_1} = 70$ –90) with subnanomolar affinity.

As stated above, benzoxazole [116], benzofuran or dihydrobenzofuran are core structures able to mimic the methoxyphenyl fragment of MLT. Compounds with subnanomolar affinity and modest selectivity for the MT₁ subtype (MT₂/MT₁ selectivity ratios approximately ranging from 20 to 40) have been obtained when a 4-phenylbutyl group was introduced at the C2-position of the bicyclic scaffold, as exemplified by compounds **29** [117], **30** [118] and **31** [119] in Figure 3. By replacement of the methoxy group of MLT with a 5-hydroxyethoxy substituent interesting compounds were obtained, some of which (**32** [120], **33** [121]) have similar binding affinity for the two receptor subtypes, but functional selectivity on GTPγS binding, as they displayed MT₁ agonist and MT₂ antagonist properties. Taken together, all these results have led to the hypothesis that MT₁ selectivity is favored by replacement of the 5-methoxy group by a larger substituent.

Conclusion

MLT research is now more than 50 years old and has highlighted that this hormone is an integral part of the homeostatic mechanisms in the body. Since its discovery, several medicinal chemistry approaches have delivered many structurally diverse MLT receptor agonists, which together provided good insights into general pharmacophore requirements of MLT receptors, and have contributed to the knowledge of their putative pharmacological roles. In total, five MLT receptor agonists have advanced to clinical phases (ramelteon, agomelatine, tasimelteon, Neu-P11, and TIK-301), two of which are already in therapeutic use together with a prolonged release formulation of MLT. In particular ramelteon and prolonged release MLT, with demonstrated sleep-promoting effects in clinical trials, coupled with a favorable safety profile and lack of abuse potential or dependence, provide an important treatment option for insomnia. However, it should be noted that the improvements of sleep maintenance for ramelteon appear

Figure 3 Subtype- or functionally selective melatonin receptor agonists or partial agonists.

moderate and no sufficient to obtain marketing authorization by EMEA. The experience with agomelatine across a wide range of clinical trials suggests that this compound can be an attractive alternative for the treatment of major depression, combining efficacy with a favorable side effect profile. Although the field of receptor subtype selectivity has registered some progress in recent years, there is still a clear need for subtype selective MLT receptor agonists which may help to define the multiple functions of MLT.

Another poorly investigated field of inquiry is the possible synergistic interaction of MLT receptors with some serotonin receptors (i.e., 5-HT_{2c}) also known to be important in the management of insomnia and depression.

Conflict of Interest

The authors have no conflict of interest.

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