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Tasimelteon: A selective and unique receptor binding profile

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ABSTRACT

Hetlioz® (tasimelteon) is the first approved treatment in the United States for Non-24-Hour Sleep-Wake Disorder (Non-24). We present here data on the *in vitro* binding affinity of tasimelteon for both human melatonin receptors MT_1 and MT_2, as well as the extended screen of other receptors and enzymes. Results indicate that tasimelteon is a potent Dual Melatonin Receptor Agonist (DMRA) with 2.1–4.4 times greater affinity for the MT_2 receptor believed to mediate circadian rhythm phase-shifting (K_i = 0.0692 nM and K_i = 0.17 nM in NIH-3T3 and CHO–K1 cells, respectively), than for the MT_1 receptor (K_i = 0.304 nM and K_i = 0.35 nM, respectively). Tasimelteon was also shown to have no appreciable affinity for more than 160 other pharmacologically relevant receptors and several enzymes.

1. Introduction

Circadian rhythms are regulated by an endogenous circadian pacemaker that resides in the suprachiasmatic nuclei (SCN) in mammals. This pacemaker is the Master Body Clock that regulates the circadian rhythms of biological processes including the secretion of hormones such as melatonin and cortisol, sleep-wake cycles, alertness and performance patterns, metabolism and cardiovascular processes (Lockley et al., 2007, 2008). In human, the endogenous rhythm of the SCN is typically slightly longer than 24 h and therefore must be entrained (synchronized) to the 24-h day. The strongest zeitgeber responsible for entrainment is the daily light–dark cycle, detected primarily by the intrinsically photosensitive ganglion cells of the retina (Czeisler and Gooley, 2007; Ramsey et al., 2013).

Disruptions in a person's circadian rhythms have been recognized to play a significant role in the etiology of a number of serious and chronic disorders, including obesity (Karlsson et al., 2001), diabetes (Morikawa et al, 2005), breast cancer (Davis et al., 2001; Schernhammer et al, 2001), colorectal cancer (Schernhammer et al, 2003), cardiovascular diseases (Vyas et al, 2012; Ruger and Scheer, 2009; Knutsson, 2003), depression (Boyce and Barriball, 2010; Monteleone and Maj, 2008; Turek, 2007), and circadian-rhythm sleep-wake disorders (Sack et al, 2007) like Non-24-Hour Sleep-Wake Disorder (Non-24) (Lockley et al., 2007; Skene et al., 1999). Non-24 is a serious, debilitating, chronic disorder that occurs when individuals are unable to synchronize their endogenous circadian clock to the 24-h light-dark cycle (American Psychiatric Association, 2013; American Academy of Sleep Medicine, 2005). The majority of reported cases of Non-24 occur in blind patients with no conscious perception of light (Lockley et al., 2007; Uchiyama and Lockley, 2009). The growing understanding of the importance of circadian regulation in these complex disorders is expected to spawn the development of specifically designed circadian regulators that can address the underlying circadian component of these conditions.

Abbreviations: AMP, Adenosine monophosphate; BSA, Bovine serum albumin; cAMP, Cyclic AMP; DMRA, Dual Melatonin Receptor Agonist; EC_{50}, 50% of the maximal effect; EDTA, Ethylenediaminetetraacetic acid; E_{max}, Maximal effect; GABA, Gamma-aminobutyric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IBMX, 3-isobutyl-1-methylxanthine; IC_{50}, Concentration producing 50% of the maximal effect; K_i, Dissociation constant for the inhibitor; NMDA, N-methyl-D-aspartate; Non-24, Non-24-Hour Sleep-Wake Disorder; PKC, Protein kinase C; PMSF, phenylmethylsulfonyl fluoride; SCN, Suprachiasmatic nuclei; TRIS, Tris(hydroxymethyl)aminomethane; 4-P-PDOT, Cis-4-Phenyl-2-propionamidotetralin(.

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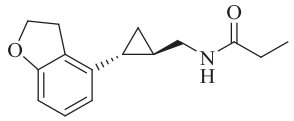

Fig. 1. Chemical structure of tasimelteon.

Tasimelteon is a novel structurally unique molecular entity, chemically designated as (1R, 2R)-N-[2- (2,3-dihydrobenzofuran-4-yl)cyclopropylmethyl]propanamide, containing two chiral centers that was developed for the treatment of Non-24. Its molecular formula is $C_{15}H_{19}NO_2$, and the molecular weight is 245.32. Tasimelteon differs structurally from melatonin and drugs with known melatonin agonist activity, in particular by its distinct aromatic group and linker (Fig. 1). Tasimelteon bears also no structural relationship to any other approved active substance.

Tasimelteon was specifically developed to treat the underlying circadian basis of Non-24, and has undergone rigorous safety evaluation (Swick et al., 2014; Sliman et al., 2014; Quera-Salva et al., 2014; Lockley et al., unpublished results). Non-24 is a circadian rhythm disorder, which primarily affects totally blind individuals, characterized by the inability to entrain the master body clock to the 24-h light–dark cycle (American Psychiatric Association, 2013; American Academy of Sleep Medicine, 2005). It is estimated that seventy percent of blind individuals with no light perception have Non-24 (Sack and Lewy, 2001). Patients with Non-24 have prolonged periods of misalignment of circadian rhythms, including the timing of melatonin and cortisol secretion and the sleep-wake cycle, which are associated with significant impairments in social and occupational functioning, and marked subjective distress (American Psychiatric Association, 2013).

Studies reported here investigated the binding characteristics of tasimelteon for a wide range of receptors and other pharmacological targets, in particular the G protein-coupled melatonin MT_1 and MT_2 receptors, which are expressed in the central nervous system and in peripheral tissues (Dubocovich and Markowska, 2005). Results point to the unique receptor binding profile of tasimelteon, which acts as a selective and potent DMRA with greater affinity for the MT_2 receptor than for the MT_1 receptor, and no appreciable affinity for more than 160 other pharmacologically relevant receptors and enzymes, including the gamma-aminobutyric acid (GABA) receptor complex and receptors that bind neuropeptides, cytokines, serotonin, noradrenaline, acetylcholine, and opiates.

Table 1
Binding affinity of tasimelteon at the human melatonin receptors.

Cell system	K_i (nM)		K_i ratio
	MT_1	MT_2	MT_1/MT_2
NIH-3T3	0.35	0.17	2.1
CHO–K1	0.304	0.0692	4.4

2. Materials and methods

The specific affinity of tasimelteon for the MT_1 and MT_2 receptors was evaluated in two independent experiments using human recombinant receptors and radioligand binding assays. The selectivity of the receptor binding was further evaluated by determining the affinity for more than 160 other receptors and several enzymes. Furthermore, inhibition of forskolin-stimulated cyclic adenosine monophosphate (cyclic AMP or cAMP) accumulation was conducted in NIH-3T3 cells stably expressing the human MT_1 and MT_2 receptors.

2.1. Binding assays at the MT_1 and MT_2 receptors

In a first experiment, two lines of NIH-3T3 cells were used; one cell line stably expressed the human MT_1 receptor while the second cell line stably expressed the human MT_2 receptor. Cells were maintained and passaged as attached monolayers in Dulbecco's Modified Eagle Medium containing D-glucose, 10 mM HEPES, 10% heat inactivated calf serum and 500 μg/mL geneticin. Cultures were incubated in vented tissue culture flasks at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were harvested and frozen as pellets at −80 °C. Membrane homogenates were prepared in assay buffer (50 mM TRIS containing 12.5 mM $MgCl_2$ and 2 mM EDTA, pH 7.4 at 37 °C with HCl) fortified with 10 μg/mL aprotinin and leupeptin and 100 μM PMSF, using a Dounce homogenizer. The resulting homogenate was centrifuged (45,000 × *g*, 10 min, 4 °C) and the pellet was resuspended in fortified assay buffer at a ratio of 0.25 mL per original flask of cells and frozen in aliquots at −80 °C until use. Binding assays were performed in duplicate in a total volume of 0.2 mL containing 0.16 mL membrane homogenate diluted appropriately, 0.02 mL 2-[^{125}I]iodomelatonin (0.1 or 0.2 nM final concentration for MT_1 or MT_2, respectively) and 0.02 mL assay buffer, melatonin or tasimelteon. Nine concentrations of tasimelteon (0.001 nM, 0.01 nM, 0.03 nM, 0.1 nM, 0.3 nM, 1 nM, 3 nM, 10 nM, and 100 nM) and 5 concentrations of melatonin (0.01 nM, 0.1 nM, 1 nM, 10 nM, and 100 nM) were used for each MT_1 or MT_2 binding assay. Non-specific binding was determined in the presence of 10 μM melatonin. Assays were initiated by the addition of membrane

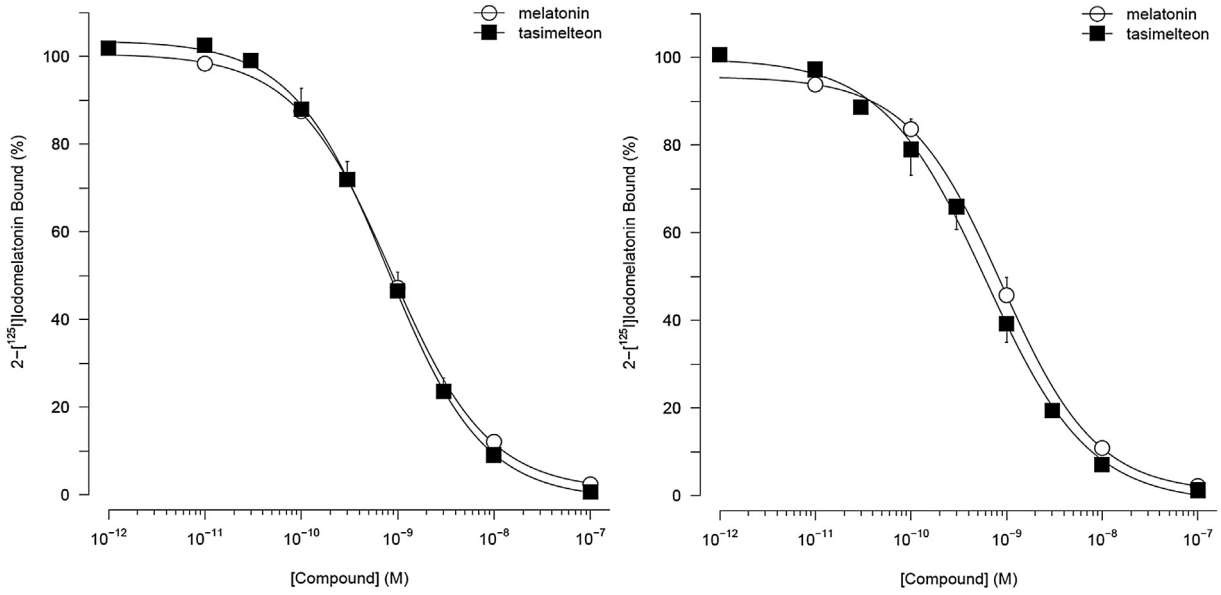

Fig. 2. Affinity of tasimelteon at the human melatonin receptors MT_1 and MT_2 in NIH-3T3 cells. Data used for the non-linear regression analysis are shown as the mean ± standard error of the mean for the MT_1 receptor (left graph) and for the MT_2 receptor (right graph) stably expressed in NIH-3T3 cells.