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PAPER

# Design, Synthesis, and Biological Evaluation of a New Class of $MT_2$-Selective Agonists

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A novel class of chiral 2,3-dihydro-1*H*-indene derivatives were designed and synthesized as melatonergic ligands. Most of the reported $MT_2$-selective ligands behave as antagonists. By contrast, our exploration of 2,3-dihydro-1*H*-indene showed that the introduction of lipophilic group at the 2- or 3-position of this scaffold could afford highly selective $MT_2$ agonists. Among all these synthesized molecules, compounds **10b, 12a, 17a, 20a** exhibited powerful $MT_2$ agonistic activity ($EC_{50}$ < 50 nM) as well as wonderful $MT_2$ selectivity (more than 2200-fold).

## Introduction

Melatonin (5-methoxy-*N*-acetyltryptamine, MLT, Fig. **1**) is a neurohormone synthesized and secreted from the pineal gland of mammals including humans.[1] MLT plays an important role in modulation of the sleep-wake cycle and circadian rhythms in humans.[2] Other reported effects of MLT include its anti-inflammatory,[3] pain modulatory,[4] retinal,[5] vascular,[6] antitumor,[7-9] antioxidant,[10] strokeprotective [11] and neuroprotective roles.[12, 13] In mammals, MLT exerts its physiological effects mainly through the activation of the high affinity G-protein-coupled receptors $MT_1$ and $MT_2$.[14] It is known that $MT_1$ receptors are expressed in several areas such as brain, especially in the suprachiasmatic nuclei (SCN) as well as the pars tuberalis of pituitary and might be implicated in the sleep promoting effects of MLT and in mediating vasoconstriction, whereas $MT_2$ receptors are localized in the SCN and retina, and appear to play a major role in the resynchronizing activity of MLT and in mediating vasodilation.

A large number of studies regarding the treatment of circadian rhythm disorders reported that the efficacy of MLT is always limited by the unfavorable pharmacokinetic profile such as short half-life in the human body.[15] Over the last twenty years, many melatonin receptor agonists with improved properties in comparison to MLT have been published.[16, 17] Takeda Pharmaceuticals North America developed ramelteon with a longer half-life, which was approved by FDA for the treatment of insomnia in 2005.[18-20] Agomelatine, a melatonin receptor agonist ($MT_1$ and $MT_2$) and selective serotonin receptor antagonist ($5\text{-}HT_{2c}$),[21] has been approved by the European Medicines Agency in 2009 for major depressive disorder. It could relieve the symptoms of major depression and meanwhile, it enhances sleep quality in depressed patients.[22]

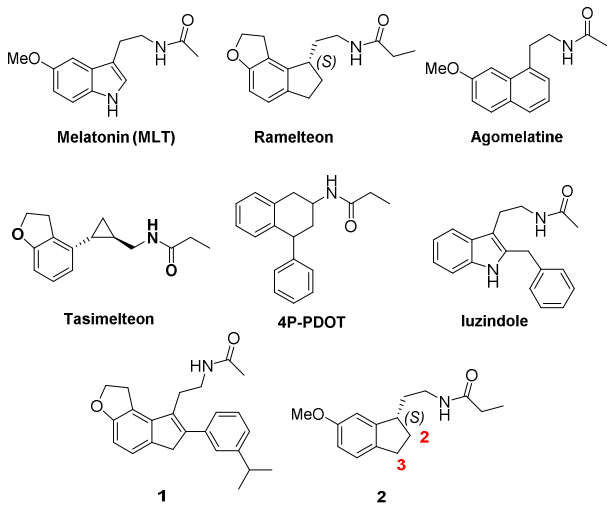
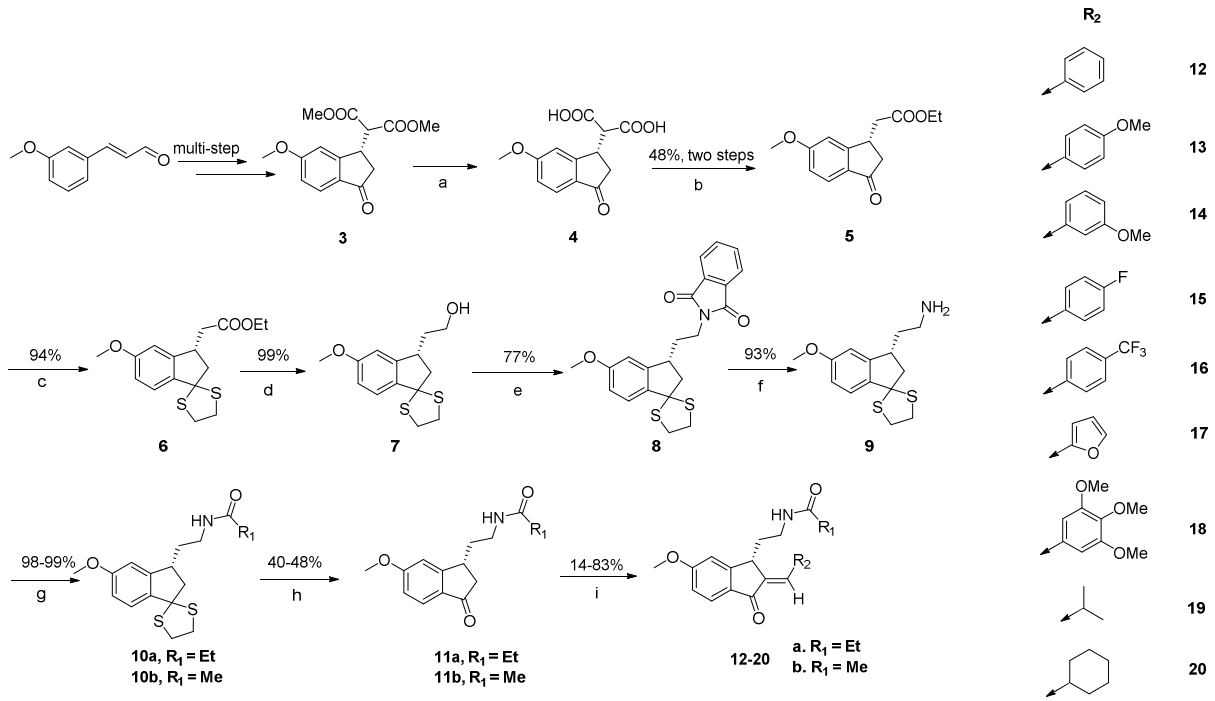



**Figure 1.** Representative melatonin receptor ligands.

Up to date, most of the melatonin receptor agonists are non-selective and only a few $MT_2$-selective ligands have been reported.[23-30] However, most of these molecules behave as antagonists, the representative compounds luzindole and 4-phenyl-2-propionamidotetralin (4P-PDOT) block the MLT-mediated phase advances of circadian rhythms in mice. Moreover, an antidepressive effect has been reported for luzindole in a mice model, being ascribed to its selective action at the $MT_2$ receptor.[31, 32] In addition, an accurate characterization of MLT receptors-mediated functions can only be obtained by using subtype-selective ligands. Koike et al reported the most efficient $MT_2$-selective agonist **1** as it shows 1200-fold higher affinity for the $MT_2$ than the $MT_1$ receptor.[33]




**Scheme 1.** Synthesis of 2,3-dihydro-1*H*-indene derivatives. Reagents and conditions: a) NaOH, EtOH, water, reflux; b) dioxane, dimethylbenzene, reflux; $SOCl_2$, EtOH; c) ethane-1,2-dithiol , $SnCl_4$, DCM; d) $LiAlH_4$, THF; e) MsCl, triethylamine, DCM; isoindoline-1,3-dione, $K_2CO_3$, $CH_3CN$, reflux; f) 85% hydrazine hydrate, EtOH, reflux; g) anhydride, triethylamine, DCM; h) $AgNO_3$, EtOH; i) aldehyde, MeONa, MeOH.

**Table 1.** Agonistic activity of compound **2** and **10-20** on human $MT_1$ and $MT_2$ receptors expressed in CHO cells.[a]

| | $EC_{50}$ $MT_1$ (nM) | $E_{max}$(%) $MT_1$ | $EC_{50}$ $MT_2$ (nM) | $E_{max}$(%) $MT_2$ | $MT_1/MT_2$ | | $EC_{50}$ $MT_1$ (nM) | $E_{max}$(%) $MT_1$ | $EC_{50}$ $MT_2$ (nM) | $E_{max}$(%) $MT_2$ | $MT_1/MT_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ramelteon** | 0.32 | 100 | 0.81 | 100 | 0.4 | **2** | 0.36 | 102 | 0.97 | 101 | 0.37 |
| **10a** | 6730 | 52 | 97.3 | 88 | 69 | **10b** | 23200 | 40 | 8.97 | 63 | 2586 |
| **11a** | 338 | 68 | 40.6 | 52 | 8 | **11b** | 3474 | 61 | 342 | 47 | 10 |
| **12a** | NA[b] | - | 20 | 46 | >5000 | **12b** | NA | - | NA | - | - |
| **13a** | NA | - | NA | - | - | **13b** | NA | - | NA | - | - |
| **14a** | NA | - | 73 | 25 | >1300 | **14b** | NA | - | NA | - | - |
| **15a** | NA | - | 92.7 | 32 | >1000 | **15b** | NA | - | NA | - | - |
| **16a** | NA | - | 222 | 40 | >450 | **16b** | NA | - | NA | - | - |
| **17a** | NA | - | 43.9 | 43 | >2200 | **17b** | NA | - | NA | - | - |
| **18a** | NA | - | NA | - | - | **18b** | NA | - | NA | - | - |
| **19a** | 23.95 | 65 | 5.08 | 71 | 4.7 | | | | | | |
| **20a** | NA | - | 18.6 | 61 | >5300 | | | | | | |

[a] Test compound potency was expressed as $EC_{50}$ (nM), while the ligand selectivity towards the two receptor subtypes was expressed as the $MT_1/MT_2$ $EC_{50}$ ratio. Data reported in the table were means of three or more experiments run at eight different concentrations in triplicates; [b] NA = no agonist effect detected at 100 μM.

Current SAR of indan derivatives disclosed that the chiral centre is essential for melatonin receptor affinity. For example, compound **2** (*S*-enantiomer) showed over 700 times higher affinity than its *R*-enantiomer ($Ki$ = 0.041 nM vs $Ki$ = 30.1 nM).[19] However,



the previous reported works are mainly focused on achiral molecules.[17, 34, 35] More recently we have reported an efficient synthesis of chiral indan derivatives.[36] By introducing lipophilic groups to 2- or 3- position of the indan core, we attempted to prepare novel melatonergic ligands with improved $MT_2$-selectivity and blood-brain barrier permeability.[33, 37]

Herein we report novel 2,3-dihydro-1*H*-indene derivatives as highly selective $MT_2$ agonists. The chiral centre of ramelteon was preserved and a variety of lipophilic groups were introduced on the non-aromatic indan cycle. The preliminary conclusions regarding structure–activity relationships are discussed.

## Result and Discussion

### Chemistry

The general route used for the synthesis of the target indan derivatives is depicted in Scheme 1. Following previously reported procedures, chiral indanone **3** can be easily prepared from (*E*)-3-(3-methoxyphenyl)acrylaldehyde.[36] Subsequently, compound **3** was subjected to hydrolysis, decarboxylation and esterification to afford chiral ester **5** with a global yield of 47%. Treatment of compound **5** with ethane-1,2-dithiol in the presence of $SnCl_4$ followed by subsequent reduction of the carbonyl group gave indanol **7**. Gabriel reaction involving the mesylated form of compound **7** followed by acylation of the corresponding amine **9** in the presence of anhydride furnished chiral amide **10**. Then the key ketone intermediate **11** was obtained in an excellent *ee* value (>99%) after dithiane deprotection with $AgNO_3$. Finally, the cyclic ketones **11** underwent Aldol condensation with the required aldehyde in the presence of MeONa to give the designed molecules **12-20**.[38]

Theoretically, the *E*- and *Z*-geometric isomers can be equally formed in the above mentioned Aldol condensation. However, only the *E*-isomers were obtained, most probably due to strong steric interaction between the aryl/alkyl and carbonyl groups. The appearance of the "diagnostic" vinyl proton signal in the appropriate range (7.70–7.50 ppm for aryl enones **12-18** and 7.00-6.90 for alkyl enones **19-20**) in the $^1H$ NMR spectra unambiguously corroborated their *E* configuration.[39] This assignment was further confirmed by key NOESY interactions (see ESI compound **18b**) and is consistent with observations made with this reaction in similar substrates.[40-45]

### Biological evaluation

The intrinsic potency of these compounds was evaluated using HTRF IP-One Terbium-based assay and the results are depicted in Table 1. Compared to full agonist ramelteon and its strict methoxy analogue **2** ($EC_{50}$ ($MT_1$) = 0.36 nM; $EC_{50}$ ($MT_2$) = 0.97 nM), the oxo derivatives **11a** (or **11b**) showed moderate potency at both subtypes and slight preference for $MT_2$ ($MT_1/MT_2$ = 8 ~ 10). It was previously reported that the relative position between the methoxy group and the amide side chain of melatonin is essential for high receptor affinity and intrinsic activity.[46] Remarkable $MT_2$ selectivity was achieved by introduction phenyl group to 2-position of indanone core. Compound **12a** showed good $MT_2$ agonist potency and wonderful $MT_2$ selectivity ($EC_{50}$ ($MT_2$) = 20 nM, $MT_1/MT_2$ > 5000). Replacement of the benzene ring with unsubstituted furan ring had little influence to the $MT_2$ agonistic activity and selectivity (**12a** vs **17a**). Substituted aromatic groups exhibited a lower potency which was probably due to the steric effect (**12a** vs **14a-16a**). Ligands with bulkier aromatic groups lost their agonistic activity at $MT_2$ subtype (**12a** vs **13a** and **18a**). On the other hand, replacement of the propionylamido group by an acetamido group caused a significant loss of agonistic activity for $MT_1$ and $MT_2$ receptors (for example, **11a** vs **11b** and **12a** vs **12b**). In addition, aliphatic substitution also led to the increase of the $MT_2$ agonistic activity (**19a** and **20a**). It's worth mentioning that the isopropyl substituted enone **19a** displayed the best agonistic activity at both subtypes and slight preference for $MT_2$. Taken together, these data suggested that the introduction of substituent group to 2-position of indanone core may have an influence on the spatial arrangement of the amide side chain. Surprisingly the dithiane analog with nanomolar to subnanomolar agonist activity showed very high selectivity ($MT_1/MT_2$ = 2586) in its acetamido form **11b**. All these effective indan derivatives behaved as partial agonists, compounds **10b, 12a, 17a, 20a** exhibited powerful $MT_2$ agonistic activity ($EC_{50}$ < 50 nM) as well as excellent $MT_2$ selectivity (more than 2200-fold).

## Conclusions

In this study, we have described the synthesis and biological evaluation of a novel class of chiral 2,3-dihydro-1*H*-indene derivatives as melatonergic ligands. Our exploration of indan core showed that the introduction of lipophilic group at the 2- or 3-position of this chiral scaffold could afford highly selective $MT_2$ partial agonists. Compounds **10b, 12a, 17a, 20a** exhibited excellent $MT_2$ selectivity (more than 2200 times). The present investigation opens up the possibility of further exploring $MT_2$-specific agonists, which might expand our knowledge about $MT_2$ receptor in the near future.

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## Notes and references

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† Electronic Supplementary Information (ESI) available: Details of experimental procedure, spectral data of all novel compounds. See DOI: 10.1039/b000000x/

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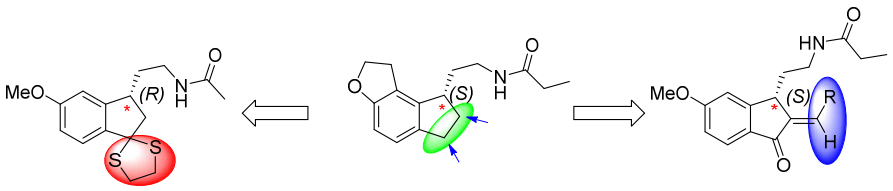
In this study, we have designed and synthesized a novel class of chiral 2,3-dihydro-1*H*-indene derivatives as highly selective $MT_2$ agonists.




**10b**
$EC_{50}$ $MT_1$ = 23200 nM
$EC_{50}$ $MT_2$ =8.97 nM
$MT_1/MT_2$ = 2586

**Ramelteon**
$EC_{50}$ $MT_1$ = 0.36 nM
$EC_{50}$ $MT_2$ = 0.81 nM
$MT_1/MT_2$ = 0.45

**12a, R = phenyl**
**17a, R = furyl**
**20a, R = cyclohexyl**
$EC_{50}$ $MT_2$ < 50 nM
$MT_1/MT_2$ > 2200