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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1199–1202

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Indanyl Piperazines as Melatonergic MT_2 Selective Agents

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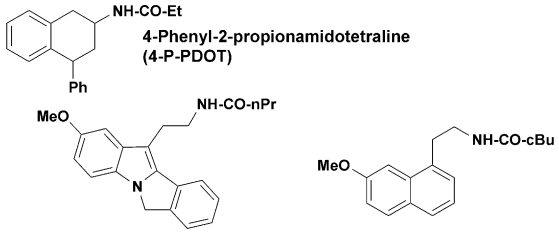
Received 11 September 2002; accepted 18 November 2002

Abstract—Optimization of a benzyl piperazine pharmacophore produced *N*-acyl-4-indanyl-piperazines that bind with high affinity to melatonergic MT_2 receptors. (*R*)-4-(2,3-dihydro-6-methoxy-1*H*-inden-1-yl)-*N*-ethyl-1-piperazine-carboxamide fumarate (**13**) is a water soluble, selective MT_2 agonist, which produces advances in circadian phase in rats at doses of 1–56 mg/kg that are no different from those of melatonin at 1 mg/kg. Unlike melatonin, **13** produced only weak contractile effects in rat tail artery.

The secretion of melatonin in the pineal gland plays a major role in the regulation of circadian and seasonal behavior in mammals.[1] The MT_1 (formerly Mel_{1A}) and the MT_2 (formerly Mel_{1B}) melatonin receptors were cloned[2] from human hypothalamus in 1994–1995. While the MT_1 melatonin receptor was previously thought to mediate the entrainment of circadian rhythms, melatonin shows phase shifting activity in knock-out mice lacking the MT_1 receptor,[3] and the MT_2 selective antagonist, 4-P-PDOT,[4] blocks the phase shifting activity of melatonin.[5] This evidence supports the involvement of MT_2 melatonin receptors in the entrainment of circadian rhythms.

In previous work, the 4-benzyl piperazine moiety was identified as a new 5-HT_{1A} pharmacophore.[6] Further optimization of this pharmacophore identified the 2-fluoro-5-methoxy substituent pattern as potentiating binding and giving a compound with sub-nanomolar affinity for the 5-HT_{1A} receptor.[7] Since melatonin is structurally related to serotonin, we chose to investigate whether this serotonergic pharmacophore could be modified into a melatonergic pharmacophore by simple acylation. We now report that while the N-acyl-4-benzylpiperazines possess modest melatonergic affinity, adding an element of structural rigidity by ring fusion gives N-acyl-4-indanylpiperazines[8] that bind with high affinity to MT_2 melatonergic receptors. Two other MT2 selective agonists have been described in the literature: N-butanoyl-2-(2-methoxy-6*H*-isoindolo[2,1-*a*]indol-11-yl)ethyl amine,[9] and N-[2-(7-methoxy-1-naphthyl)-ethyl]cyclobutylcarboxamide.[10]



4-(2-Fluoro-5-methoxybenzyl)piperazines, **1**-**3**, were prepared by the method shown in Scheme 1. 4-Fluoroanisole was lithiated[11] and reacted with DMF to give the benzaldehyde. Reductive amination with piperazine[12] and subsequent acylation gave the benzyl piperazines **1**–**3**. Compounds **1**–**3** were weakly active at MT_2 receptors, and we sought to improve their affinities by conformational constraint. While (2,3-dihydro-6-methoxy-1*H*-inden-1-yl) piperazine was previously prepared by alkylation of piperazine with 1-chloro-6-methoxyindane,[13] this method could not be reproduced in our hands and gave only dehydrochlorination. A titanium(IV) isopropoxide reductive amination procedure[14]

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doi:10.1016/S0960-894X(03)00090-8

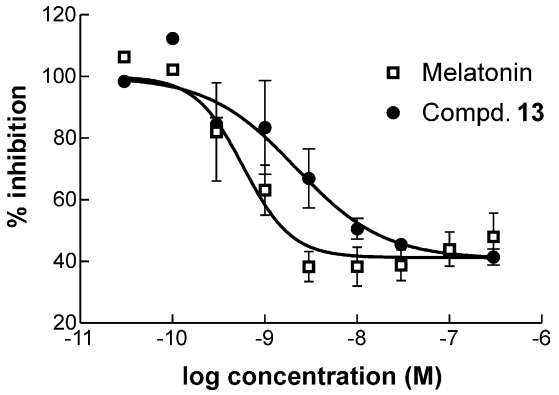
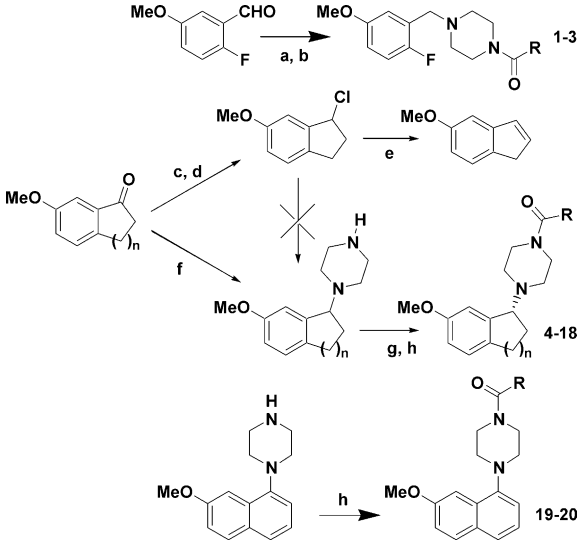


Figure 1. Agonist effects of melatonin and **13** on forskolin-induced cAMP production in NIH3T3 cells expressing the nhuman MT2 receptor.



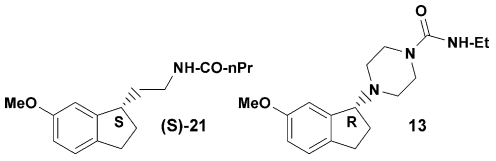
Scheme 1. (a) Piperazine, $NaBH_3CN$; (b) acylation; (c) $NaBH_4$; (d) $SOCl_2$; (e) piperazine; (f) piperazine, $Ti(OiPr)_4$, $NaBH_4$, EtOH; (g) resolution; (h) acylation with either an acid chloride, anhydride, or isocyanate.

was modified[15] as shown in Scheme 1 to give the desired 6-methoxyindanyl piperazine (80% yield), which was resolved[16] using camphorsulfonic acid and absolute stereochemistry was determined by X-ray crystallography of (−)-(1′*R*)-(2,3-dihydro-6-methoxy-1*H*-inden-1-yl)piperazine, (1*S*)-10-camphor-sulfonic acid salt.[17] The tetralin homologue was prepared in a similar manner from 7-methoxy-1-tetralone, and 7-methoxy-naphthyl-piperazine was prepared by literature methods.[18] The substituted piperazines were acylated using acid chlorides or isocyanates to give the desired amide and urea products (**1**–**20**).[19]

Compounds **1**–**20** were evaluated for MT_1 and MT_2 binding using published assay methods (Table 1).[2] The initial group of benzyl piperazine amides failed to demonstrate potent affinity for melatonergic receptors. Acetamide, **1**, lacked affinity for either melatonergic receptor, while the cyclopropylcarboxamide (**2**), and isobutyramide, (**3**), had only modest affinity for the MT_2 receptor and no affinity for the MT_1 receptor. In contrast, the conformationally restricted indanyl piperazines (**4**–**10**), had good to excellent affinity for the MT_2 receptor with little affinity for the MT_1 receptor. MT_2 binding was optimal with butyramide **6**, cyclopropane carboxamide **8**, isobutyramide **9**, and ethyl urea **10**. MT_2 affinity was attenuated with smaller amides **4** and **5**, or with larger amides, e.g., **7**. MT_2 and MT_1 affinities were found only in the R-enantiomers (**11**–**13**), while the *S*-enantiomers (**14**–**16**), are inactive at both receptors. The larger tetralin homologues (**17**–**18**), showed a reduced level of MT_2 affinity, while in the naphthyl analogues (**19**–**20**), MT_2 affinity was even further reduced.

Table 1. Melatonin MT_1 and MT_2 receptor binding[22] of Melatonin (Mel) and Compounds **1**–**20**

Compd	R	*n*	Chirality	MT_1 IC_{50} (nM)	MT_2 IC_{50} (nM)
Mel	—	—		0.6	0.3
1	Me	—		>1000	>1000
2	*c*Pr	—		>1000	270
3	*i*Pr	—		>1000	224
4	Me	1	±	>1000	44
5	Et	1	±	>1000	32
6	*n*Pr	1	±	>1000	2.3
7	*n*Bu	1	±	>1000	103
8	*c*Pr	1	±	156	3.0
9	*i*Pr	1	±	160	1.5
10	NH-Et	1	±	>1000	3.1
11	*c*Pr	1	R	44.5	1.6
12	*i*Pr	1	R	116	1.5
13	NH-Et	1	R	200	1.7
14	*c*Pr	1	S	>1000	>1000
15	*i*Pr	1	S	>1000	>1000
16	NH-Et	1	S	>1000	>1000
17	*c*Pr	2	±	>1000	75
18	NH-Et	2	±	>1000	94
19	*c*Pr	—		>1000	90
20	NH-Et	—		>1000	>1000



A structurally similar series of amides, for example, **21**, has been described in the literature[20] which have the amide side chain in the same orientation as the indanyl piperazines (**11**–**13**). Unlike the piperazine amides, **11**–**13**, which are MT_2 selective, compound **21** binds with high affinity to both MT_1 and MT_2 receptors.[21]

These binding studies identified **13** as a potent and selective ligand for the MT_2 receptor. Both MT_1 and MT_2 are G-protein-coupled receptors and are negatively linked to adenylyl cyclase by pertussis toxin sensitive G-proteins.[23] Compound **13** was further tested for functional activity in a MT_2 adenylyl cyclase assay[24] (Fig. 1) and found to be a full agonist (EC_{50}: 2.4±1.6 nM; intrinsic activity: 0.98±0.12).

In vascular tissue, melatonin potentiates the contractile responses to serotonin.[25] It has been proposed that MT_2 receptors mediate relaxation and that the contractile effects of melatonin may be mediated via MT_1 receptors.[26] Supporting this hypothesis, the MT_2 agonist, **13**,

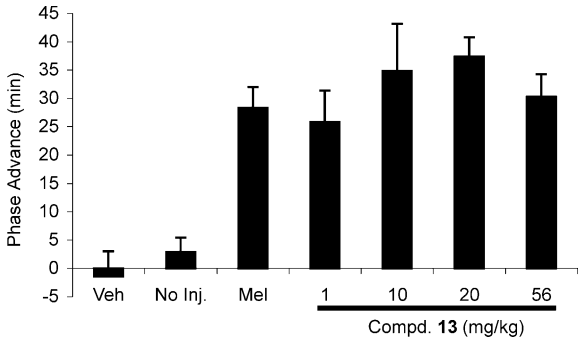


Figure 2. Acute effects of vehicle (5% DMSO + 60% PEG-400 + 35% saline), no injection, melatonin (Mel, 1 mg/kg), and **13** (1, 10, 20, and 56 mg/kg) on circadian phase advance.

produced only weak contractile effects in rat tail artery (0.24 relative to melatonin).[27]

The effects of the selective MT_2 agonist, **13**, on circadian phase advance[28,29] were investigated (Fig. 2). Both vehicle injection and no injection gave no significant phase advance, while melatonin (1 mg/kg) gave a significant phase advance of 28 min. Compound **13** at doses from 1 to 56 mg/kg produced phase advances that were not significantly different from that produced by melatonin. This data further supports the involvement of the MT_2 receptor in the entrainment of circadian rhythms.

The present studies have identified the water-soluble[30] compound, **13**, as a potent MT_2 agonist that produces advances in circadian phase similar to those produced by melatonin. Unlike melatonin, **13** produces only weak contractile effects in vitro in rat tail artery. These studies provide further support for the hypothesis that MT_2 receptors mediate the entrainment of circadian rhythms and not vasoconstriction.

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12. A solution of 2-fluoro-5-methoxybenzaldehyde (5.0 g, 33 mmol), piperazine (26 g, 0.3 mol), and $NaBH_3CN$ (3.1 g, 50 mmol) in ethanol (400 mL) was heated to reflux for 18 h. The reaction was concentrated in vacuo. The residue dissolved in water and extracted with CH_2Cl_2. The extracts were concentrated and the residue was dissolved in 1 N HCl. The acidic solution was washed with CH_2Cl_2 and then made basic with NaOH. The product was extracted from the basic aqueous solution with CH_2Cl_2. Concentrating the extracts gave the product as a light yellow oil (3.63 g, 50%).
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15. **(2,3-Dihydro-6-methoxy-1*H*-inden-1-yl) piperazine.** An intimate mixture of 6-methoxy-1-indanone (10 g, 62 mmol), piperazine (53 g, 0.62 mol) and titanium(IV) isopropoxide (17.6 g, 12.4 mmol) was heated on a steam bath for 10 min. The IR spectrum of the mixture showed no carbonyl absorption. The material was dissolved in ethanol and sodium borohydride (2.5 g, 62 mmol) added. After stirring for 1 h, the solution was heated to reflux and when a solution was achieved, 15% sodium hydroxide (50 mL) was added. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in ether and washed with water and 1N HCl. The acid washes were made basic and the mixture was extracted with CH_2Cl_2 to give the product [80%, mp: 150–152 °C (HCl salt)].
16. **Resolution of (2,3-dihydro-6-methoxy-1*H*-inden-1-yl)-piperazine.** (−)-(1′*R*)-(2,3-dihydro-6-methoxy-1*H*-inden-1-yl)piperazine (1*S*)-10-camphorsulfonic acid salt was recrystallized from ethanol-water to a constant melting point (mp: 234–235 °C; $[\alpha]_D^{25}$ −36.3°, *c* 2.51, MeOH). calcd for $C_{14}H_{20}N_2O_2 \cdot C_{10}H_{10}O_4S$: C, 62.04; H, 7.81; N, 6.03. Found: C, 62.01; H, 7.96; N, 5.97.

A sample of the above salt was converted to the fumarate salt and recrystallized from ethanol-water (mp: 170–172 °C; $[\alpha]_D^{25}$ −72.3°, *c* 2.66, MeOH). calcd for $C_{14}H_{20}N_2O_2 \cdot C_4H_4O_4$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.08; H, 6.61; N, 8.26. (1′*S*)-(2,3-dihydro-6-methoxy-1*H*-inden-1-yl)-piperazine (1*R*)-(−)-10-camphor-sulfonic acid salt salt was recrystallized from ethanol–water to a constant melting point (mp: 234–235 °C; $[\alpha]_D^{25}$ +35.8°, *c* 2.51, MeOH). calcd for $C_{14}H_{20}N_2O_2 \cdot C_{10}H_{10}O_4S$: C, 62.04; H, 7.81; N, 6.03. Found: C, 62.06; H, 7.73; N, 6.01.

A sample of the above salt was converted to the fumarate salt and recrystallized from ethanol-water (mp: 165–166 °C; $[\alpha]_D^{25}$ +71.3°, *c* 2.66, MeOH). calcd for $C_{14}H_{20}N_2O_2 \cdot C_4H_4O_4$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.08; H, 6.61; N, 8.26.
17. Crystallographic data (excluding structure factors) has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 197571. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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19. ***N*-Ethyl-4-(2,3-dihydro-6-methoxy-1*H*-inden-1-yl)-1-piperazinecarboxamide, 10.** Ethyl isocyanate (0.27 mL, 3.4 mmol) was added to a solution of (6-methoxy-1-indanyl)-1-piperazine (0.8 g, 3.4 mmol) in CH_2Cl_2. After stirring for 2 h, the solution was washed with 1N HCl. The acid washes were made basic

with sodium hydroxide solution and the mixture was extracted with CH_2Cl_2. The extracts were dried and concentrated. The fumarate salt was prepared in methanol. (0.88 g, 85%, mp: 167–168 °C). calcd for $C_4H_4O_4 \cdot 0.1H_2O$: C, 59.87; H, 6.99; N, 9.97. Found: C, 59.65; H, 6.91; N, 9.81.

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21. (*R*)-**21**, MT_1 $IC_{50} = 100$ nM, MT_2 $IC_{50} = 16$ nM; (*S*)-**21**, MT_1 $IC_{50} = 0.3$ nM, MT_2 $IC_{50} = 0.2$ nM.

22. IC_{50} values are the mean of at least 3 determinations run at five different concentrations with the radioligand at the K_d concentration. Standard errors were typically ±20% of the mean value.

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24. **MT_2 Adenylyl Cyclase Functional Assay.** Cyclic AMP assays were performed using NIH-3T3 cells stably expressing human MT_2 receptors. Cells were incubated in culture media containing 1 mM IBMX and 10 μM forskolin for 10 min at 37 °C in the presence of increasing concentrations of melatonin or **13**. Assays were terminated by the addition of 0.1 N HCl. Following centrifugation, supernatants were collected and stored at −20 °C until assayed. Cyclic AMP levels were measured by radioimmunoassay (Amersham). Radioactivity was quantitated by gamma emission spectrometry. Data were analyzed by a 4-parameter logistic, non-linear least squares regression to yield EC_{50} and E_{max} values.

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27. **Rat caudal artery.** Rings of rat caudal artery (4 mm long) were maintained in 30 mL organ baths containing PSS (conc. in mM: NaCl, 115; KCl, 4.7; $NaHCO_3$, 22; $CaCl_2$, 1.6; KH_2PO_4, 1.2; $MgCl_2$, 1.2; and glucose, 11; aerated with 95% O_2/ 5% CO_2, pH 7.4) at 37 °C. The rings were equilibrated for 1 h under resting tension of 0.7 g. The tension was measured with a Grass FT.03 force development transducer and recorded on a Sensormedic Dynograph R612 recorder. The rings were precontracted with 2 μM PGF_{2A} for 5 min, then melatonin or **13** (100 μM) was added after the contractile response had stabilized. Data were expressed as grams of agonist induced contraction ($N = 6$): melatonin, 0.76 g ± 0.14 g; **13**, 0.18 ± 0.13 g, 0.24 relative to melatonin.

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29. **Circadian Running Wheel Method.** Male hooded Long-Evans rats were kept under a 12:12 h light-dark cycle schedule until a reliable entrainment was established between the lighting conditions and the onset of running-wheel activity (~two weeks). After entrainment, the rats were maintained in constant darkness until the end of the experiment. Under constant darkness, rats show an endogenous rhythm of running-wheel activity characterized by a predictable daily onset of activity. This endogenously controlled rhythm under constant environmental conditions is called a 'free-running' rhythm. After 2 wk in constant darkness, each rat was injected approximately 2 h before the expected onset of running-wheel activity for that individual animal. Seven groups of rats (9–11 rats each) were used: Group 1: no injection; Group 2: vehicle (5% DMSO, 60% PEG 400, 35% saline); Group 3: melatonin (1.0 mg/kg); Groups 4–7: **13** (1, 10, 20, & 56 mg/kg). All injections were subcutaneous at CT 10. The onset of activity was predicted using the wheel-running activity record (actogram) and the rhythm period. **Data Measurement and Analyses.** Phase shift was calculated for each animal from the difference between the expected onset of running-wheel activity and the real value obtained from the actogram for post treatment recording. The data was analyzed for significance using ANOVA followed by Bonferroni/Dunn post hoc analysis when appropriate.

30. The fumarate salt of **13** (10 mg) freely dissolved in water (1 mL).