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Highly Potent and Selective MT2 Melatonin Receptor Full Agonists from Conformational Analysis of 1-Benzyl-2-acylaminomethyl-tetrahydroquinolines

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Highly potent and selective MT2 melatonin receptor full agonists from conformational analysis of 1-benzyl-2-acylaminomethyl-tetrahydroquinolines.

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3 **analysis of 1-benzyl-2-acylaminomethyl-tetrahydroquinolines.**
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Abstract

Molecular superposition models guided the design of novel melatonin receptor ligands characterized by a 2-acylaminomethyltetrahydroquinoline scaffold. Starting from the structure of *N*-anilinoethylamide ligands, the flexible chain was conformationally constrained to reproduce the bioactive conformation of melatonin. Structure-activity relationships were investigated, focusing on the substituent at the nitrogen atom, the position of the methoxy group and the replacement of the amide side chain by urea and thiourea groups.

The compounds were tested for binding affinity and intrinsic activity at human MT₁ and MT₂ receptors. Structural optimization resulted in *N*-[(1-benzyl-1,2,3,4-tetrahydro-5-methoxyquinolin-2-yl)methyl]propionamide (UCM1014), with picomolar MT₂ binding affinity ($K_i = 0.001$ nM), more than 10,000-fold selectivity over the MT₁ receptor and a full agonist profile (GTP γ S test), being the most potent MT₂-selective full agonist reported to date.

Molecular dynamics simulations provided a rationale for high binding affinity, stereoselectivity and agonist behavior of these novel melatonin receptor ligands, based on superposition models and conformational preference.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, **1** in Figure 1) is a neurohormone primarily secreted by the pineal gland following a circadian rhythm, with peak concentrations at night. Melatonin exerts an important role in the control of circadian rhythms and in sleep regulation. Additionally, it has been shown to influence a variety of physiological functions, such as the activity of the immune system and of reproductive organs, the homeostasis of the cardiovascular system and pain perception.¹ Melatonin administration has shown beneficial effects in animal models of different diseases,^{2,3} such as stroke, cancer, neurodegenerative diseases and a number of clinical trials have been set up to evaluate its potential in different human pathological conditions.⁴ Melatonin has a pleiotropic mechanism of action as it displays antioxidant effects, activates membrane receptors and interacts with intracellular constituents, such as calmodulin and the MT₃ binding site.⁵ In mammals, melatonin activates the MT₁ and MT₂ G protein-coupled receptors which are mainly expressed in the central nervous system, but are also present in peripheral organs and tissues.^{6,7}

Melatonin is classified as a dietary supplement in many countries and it is widely used as a sleep inducer or to promote the re-synchronization of disrupted circadian rhythms, such as in case of jet lag. A prolonged-release melatonin formulation has been approved as a drug for insomnia therapy.⁸

Besides the natural ligand, MT₁/MT₂ nonselective melatonin receptor agonists have been developed and are available for the treatment of different pathologies. Ramelteon is used to treat insomnia, tasimelteon has been granted marketing authorization for non-24-hour sleep-wake disorder and agomelatine, also endowed with 5HT_{2c} antagonism, is approved for major depression.^{9,10} Medicinal chemistry research has also led to the discovery of MT₁ or MT₂ subtype-selective ligands as well as of compounds with different intrinsic activities, with partial agonists, antagonists or inverse agonists reported in the literature.^{11,12} The availability of pharmacological tools and of receptor knockout animals has allowed the functional characterization of MT₁ and MT₂ receptors, that, even if far from being complete, has highlighted some differential roles for the two subtypes. Indeed, the MT₁ receptor is mainly involved in inhibition of neuronal firing in mice suprachiasmatic nucleus

(SCN), inhibition of prolactin secretion in photoperiodic species, modulation of visual function and vasoconstriction in rat caudal arteries. On the other hand, studies on animal models showed that MT₂ receptor activation mediates the phase-shifting effect of melatonin in the SCN, is involved in promotion of non-REM sleep in mice, and mediates arterial vasodilation.¹³

During our studies on melatonin receptor ligands, we identified *N*-anilinoethylamides (Figure 1) as a class of compounds that could be easily modulated to provide different receptor subtype selectivity and intrinsic activity profiles.¹⁴ Indeed, substituents with limited size on the aniline nitrogen (e.g., a methyl group) gave potent MT₁/MT₂ nonselective agonists, while bulkier substituents led to selectivity for the MT₂ receptor and to limited intrinsic activity, moving toward a partial agonist or an antagonist behavior. Selectivity for the MT₁ receptor could be obtained replacing the methoxy group with a lipophilic arylalkyloxy chain (e.g., a phenylbutyloxy substituent).¹⁵

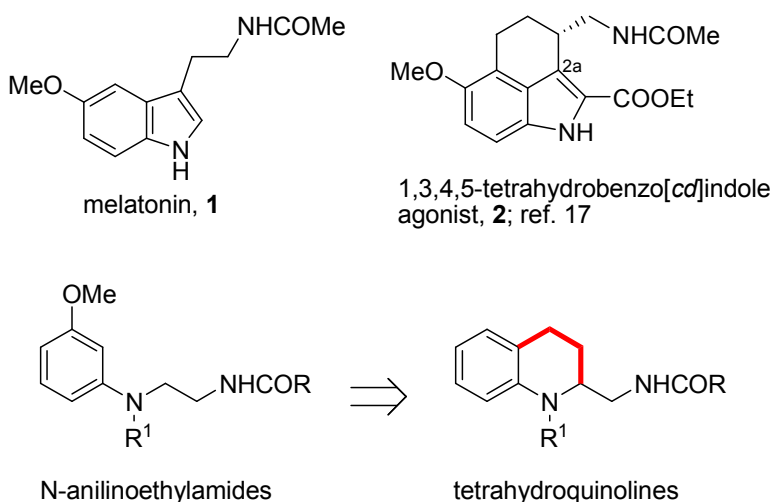


Figure 1. Structures of melatonin (**1**) and tetrahydrobenzo[*cd*]indole agonist **2**; general formulae of *N*-anilinoethylamides and of conformationally constrained tetrahydroquinoline compounds.

Starting from the structure of *N*-anilinoethylamides, characterized by high conformational freedom, we designed a novel series of conformationally-constrained MT₂-selective agonists. Among the different possible ring closures, inclusion of the carbon atom bound to the aniline nitrogen into a

1 six-membered ring gives tetrahydroquinoline derivatives carrying an acylaminomethyl side chain in
2 position 2 (Figure 1, bottom). Indeed, preliminary molecular modeling studies suggested that 2-
3 acylaminomethyltetrahydroquinolines could reproduce the putative bioactive conformation of
4 melatonin, which had been previously identified and validated by means of pharmacophore models
5 and synthesis of potent conformationally-constrained compounds.^{16,17} In particular, superposition of
6 melatonin and the tricyclic 1,3,4,5-tetrahydrobenzo[*cd*]indole agonist **2** (Figure 1) pointed out that
7 the two fused six-membered rings could represent a suitable scaffold, in which carbon 2a of
8 compound **2** can be replaced with the tetrahydroquinoline nitrogen (Figure 2). Insertion of suitable
9 substituents on the tetrahydroquinoline scaffold could in principle allow the modulation of binding
10 affinity, receptor subtype selectivity and intrinsic activity toward the desired profile by fulfilling the
11 structural requirements outlined by structure-activity relationships (SARs) and 3D-QSAR studies.¹⁸
12 It is well recognized that the presence of the methoxy group in position 5 of the indole ring of
13 melatonin increases MT₁ and MT₂ binding affinity and intrinsic activity, and insertion of a
14 substituent in position 2 (e.g., a phenyl ring, a iodine atom) increases binding affinity. On the other
15 hand, a substituent in position 1 or 2 occupying a region of space located outside the plane defined
16 by the indole ring leads to MT₂-selectivity and to an antagonist behavior.

17 Herein we report the synthesis and evaluation of the binding affinity and intrinsic activity at human
18 MT₁ and MT₂ receptors of 2-acylaminomethyltetrahydroquinoline derivatives. We analyzed their
19 SARs focusing the attention on the role of substituents on the nitrogen atom, of the methoxy group
20 and on the importance of side chain length and terminal acyl group. Moreover, stereoselectivity is
21 also discussed in light of the ability of enantiomers to reproduce the active conformation of
22 melatonin.
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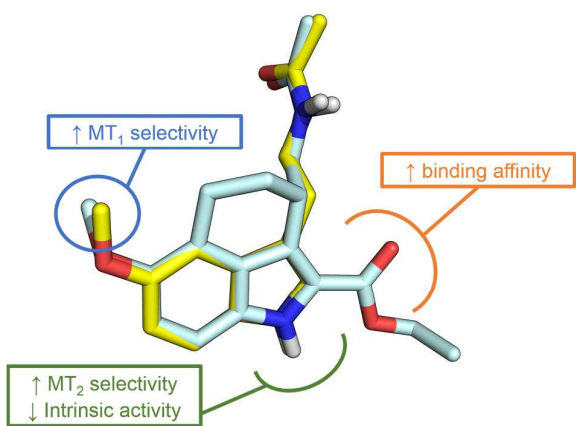
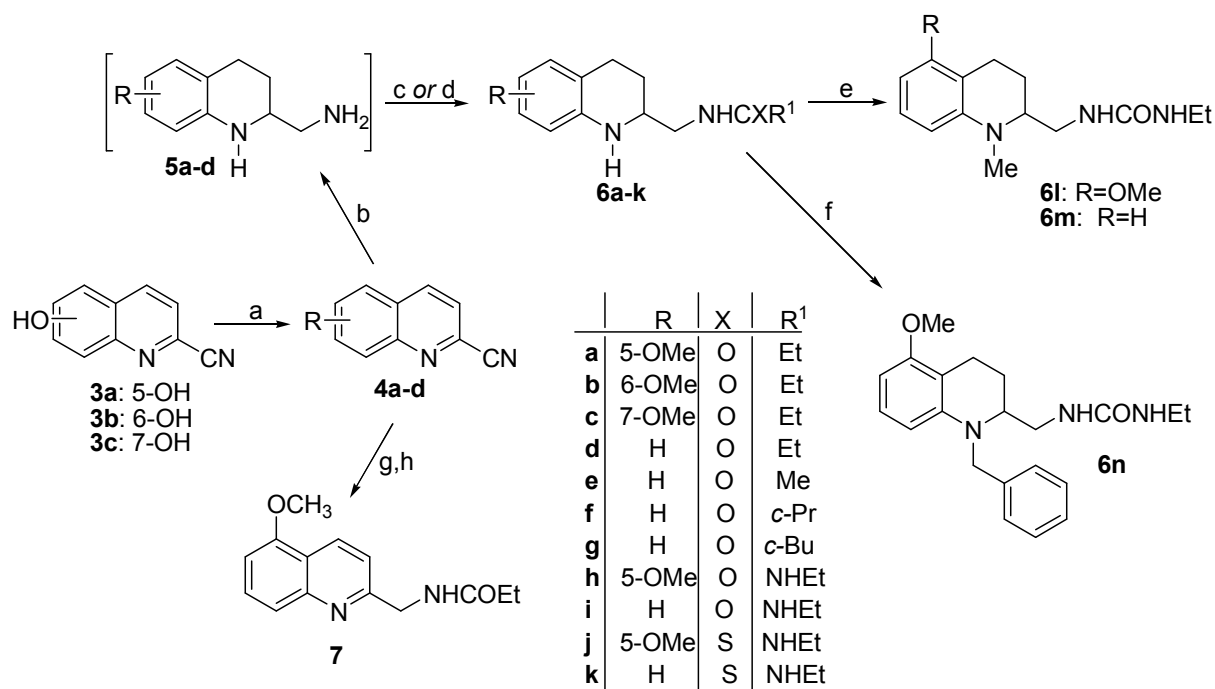


Figure 2. Superposition of compound **2** (white carbons) to the putative bioactive conformation of melatonin (**1**, yellow carbons) and effect of substituents on MT₁ and MT₂ binding affinity and intrinsic activity.

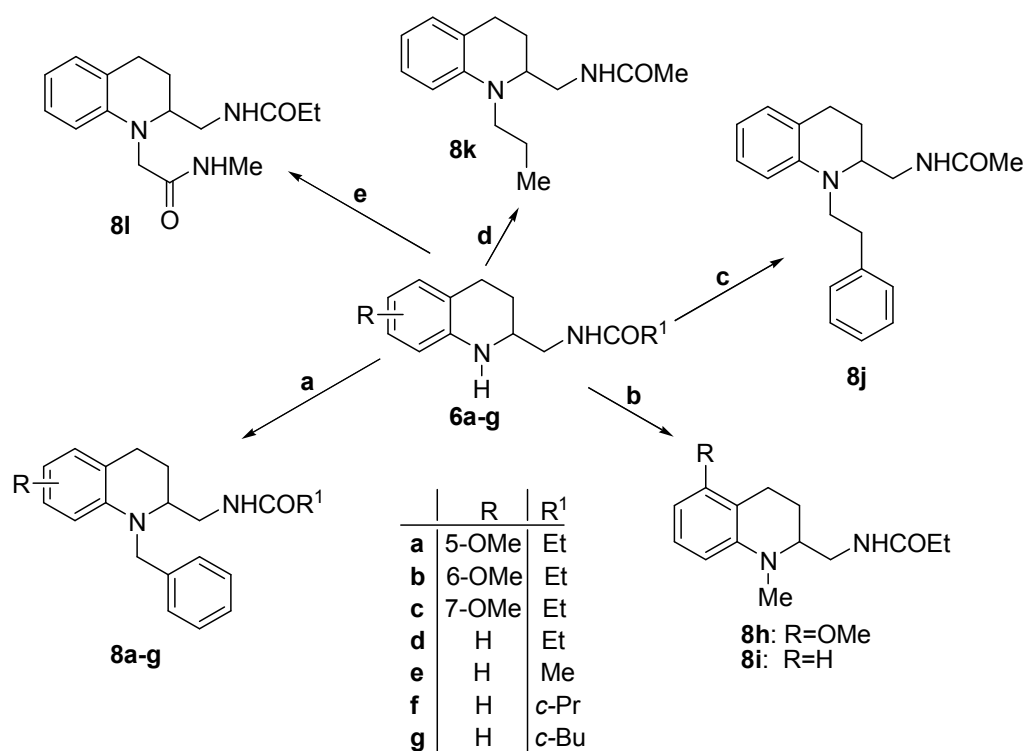
Chemistry

2-Methanamidotetrahydroquinolines **6a-g** (Scheme 1) were prepared by hydrogenation (4 atm, 60 °C) of the corresponding 2-cyanoquinoline **4a-d** in the presence of Raney-Ni and ammonia (a contemporary reduction of the cyano group and of the heterocycle ring occurs), followed by *N*-acylation with the suitable acylating reagent (acetic or propionic anhydride, cyclopropanecarbonyl or cyclobutanecarbonyl chloride). 2-Quinolinecarbinitrile (**4d**) is commercially available, whereas the methoxy-2-cyanoquinolines **4a-c** were prepared by *O*-methylation of the corresponding hydroxy-2-cyanoquinolines **3a**,¹⁹ **3b**,²⁰ and **3c**²¹ with methyl iodide. *N*-[(5-Methoxyquinolin-2-yl)methyl]propionamide (**7**) was obtained by Raney-Ni catalyzed hydrogenation (3.5 atm, rt) of the cyanoquinoline **4a** and subsequent *N*-acylation of the primary amine with propionic anhydride (Scheme 1). The (tetrahydroquinolin-2-yl)ureido derivatives **6h-k** were prepared by reacting the suitable crude (1,2,3,4-tetrahydroquinolin-2-yl)methanamine (**5a** or **5d**) with the appropriate ethyl isocyanate. The *N*¹-methyl- or *N*¹-benzyl-(tetrahydroquinolin-2-yl)ureido derivatives (**6l-n**) were prepared by *N*¹-alkylation of **6h** or **6i** with methyl iodide or benzyl bromide (Scheme 1). The *N*¹-alkylated tetrahydroquinolines **8a-l** were obtained by *N*¹-alkylation of the suitable

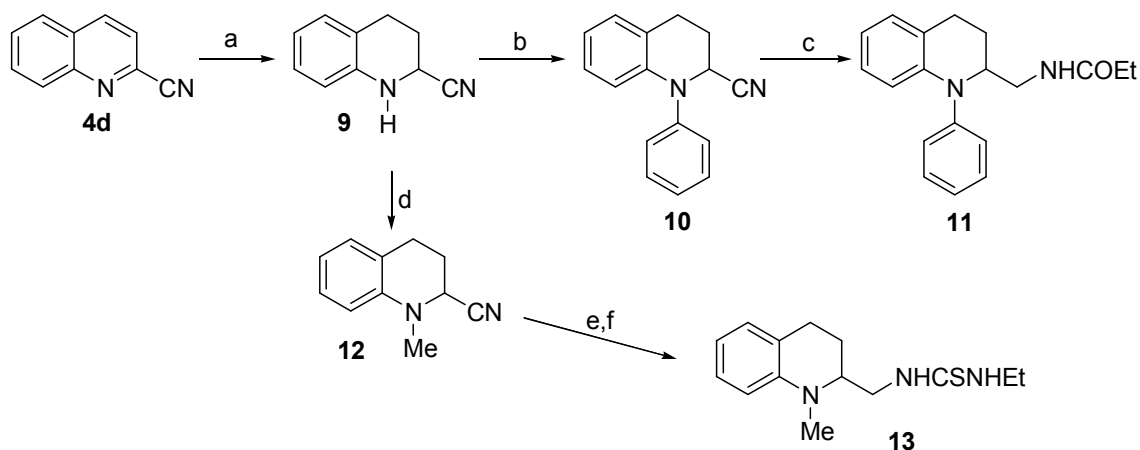
(alkanamidomethyl)tetrahydroquinoline **6a-g** with benzyl bromide (**8a-g**), methyl iodide (**8h-i**), phenylethylbromide (**8j**), 1-iodopropane (**8k**) or 2-chloro-*N*-methylacetamide (**8l**) in the presence of a base (Scheme 2). The *N*¹-phenyl-tetrahydroquinoline derivative **11** was prepared by *N*-arylation of 2-cyanotetrahydroquinoline²² **9** with benzyne (generated by reaction of 2-(trimethylsilyl)phenyl triflate with cesium fluoride), under very mild reaction conditions. Hydrogenation of the cyano group of compound **10** and concomitant acylation of the intermediate primary amine completed the synthesis of **11** (Scheme 3). To prepare the thioureido derivative **13**, 1-methyl-1,2,3,4-tetrahydroquinoline-2-carbonitrile²³ **12** was submitted to hydrogenation (Raney-Ni, 4 atm) and the corresponding free amine was treated with ethyl isothiocyanate (Scheme 3). Finally, the higher homologue **15** was obtained by reduction of (1,2,3,4-tetrahydroquinolin-2-yl)acetonitrile²⁴ followed by *N*-acylation of the intermediate amine with propionic anhydride and final *N*¹-benzylation of the tetrahydroquinoline skeleton of intermediate **14** (Scheme 4).

Scheme 1^a

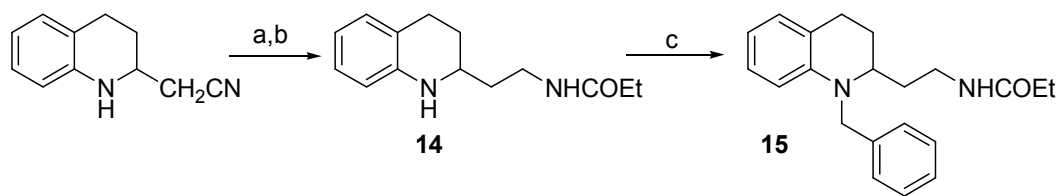
^aReagents and conditions: (a) CH₃I, K₂CO₃, acetone, rt, 15 h, yield 74-95%; (b) H₂ (4 atm), Raney-Ni, 2M NH₃ in EtOH, THF, 60 °C, 16 h; (c) anhydride or acyl chloride, Et₃N, THF, rt, 1 h, two steps (b and c) yield 34-73%; (d) ethyl isocyanate or ethyl isothiocyanate, CH₂Cl₂, rt, 30 min, two steps (b and d) yield 49-65%; (e) ethyl isocyanate or ethyl isothiocyanate, CH₂Cl₂, rt, 30 min, two steps (b and d) yield 49-65%; (f) CH₃I, NaHCO₃, MeOH, 50 °C, 24 h, yield 77%; (g) benzyl bromide, Et₃N, toluene, reflux, 2 h, yield 88%; (h) H₂ (3.5 atm), Raney-Ni, 2N NH₃ in MeOH, rt, 30 min; (i) propionic anhydride, Et₃N, THF, rt, 1 h, two steps yield 48%.

Scheme 2^a

^aReagents and conditions: (a) benzyl bromide, Et₃N, toluene, reflux, 2 h, yield 54-92%; (b) CH₃I, NaHCO₃, MeOH, 50 °C, 24 h, yield 87-95%; (c) phenylethyl bromide, Et₃N, toluene/DMF, 90 °C, 18 h, yield 68%; (d) 1-iodopropane, Et₃N, toluene/DMF, 90 °C, 18 h, yield 43%; (e) 2-chloro-N-methylacetamide, Et₃N, DMF, 100 °C, 16 h, yield 26%.

Scheme 3^a

^aReagents and conditions: (a) NaCNBH₃, AcOH, 40 °C, 16 h, yield 48%; (b) 2-(trimethylsilyl)phenyl triflate, CsF, CH₃CN, rt, 16 h, yield 55%; (c) H₂ (4 atm), Raney-Ni, propionic anhydride, THF, 60 °C, 5 h, yield 69%; (d) NaCNBH₃, 37% HCHO, MeOH/AcOH, rt, 16 h; (e) H₂ (4 atm), Raney-Ni, 2M NH₃ in EtOH, THF, rt, 24 h; (f) ethyl isothiocyanate, CH₂Cl₂, rt, 16 h, two steps yield 52%.

Scheme 4^a

^aReagents and conditions: (a) H₂ (4 atm), Raney-Ni, 2M NH₃ in EtOH, THF, 60 °C, 6 h; (b) propionic anhydride, Et₃N, THF, rt, 1 h, two steps yield 57%; (c) benzyl bromide, Et₃N, toluene, reflux, 2 h, yield 70%.

Results and discussion

The newly synthesized tetrahydroquinoline derivatives were evaluated for their binding affinity and intrinsic activity at human MT₁ and MT₂ receptors stably transfected in Chinese Hamster Ovarian (CHO) cells using 2-[¹²⁵I]iodomelatonin as radioligand, and the results are reported in Table 1.

Table 1. Binding Affinity and Intrinsic Activity of Newly Synthesized Tetrahydroquinoline Derivatives for Human MT₁ and MT₂ Melatonin Receptors.^a

				MT ₁			MT ₂		
Compd. ^b	R ¹	R ²	R	K _i (nM)	E _{max} (%)	EC ₅₀ (nM)	K _i (nM)	E _{max} (%)	EC ₅₀ (nM)
1^c melatonin				0.23 [0.21;0.26]	123 ± 13	1.7 [1.1;2.5]	0.52 [0.4;0.58]	76 ± 6	0.4 [0.3;0.6]
6a	Et	H	5-O-Me	57 [51;62]	70±5	906 [567;1450]	21 [20;25]	98±9	165 [81;333]
6d	Et	H	H	13 [9;16]	37± 1	242 [168;349]	2.2 [2;2.3]	86±2	27 [22;35]
6h	NHEt	H	5-O-Me	480 [423;502]	nd	nd	114 [98;132]	nd	nd
6i	NHEt	H	H	115 [98;123]	30±3	2420 [1978;2736]	17 [16;18]	80±0	356 [258;492]
6j		H	5-O-Me	>1000	nd	nd	>1000	nd	
6k		H	H	305 [274;382]	35±2	2660 [1270;5580]	90 [67;111]	72±2	1430 [1350;1530]
6l	NHEt	Me	5-O-Me	5 [4;5.5]	92±12	160 [81;317]	1.4 [1.3;1.5]	105±3	10 [9;10]
6m	NHEt	Me	H	5.3 [4.8;5.7]	69±8	110 [52;235]	3.4 [2.8;3.7]	97±1	6.2 [4.7;8.2]
6n	NHEt	Bn	5-O-Me	132 [127;154]	44±4	393 [375;412]	2.9 [2.5;3.1]	107±1	13 [11;15]
7				>10000	nd	nd	4450 [2480;7990]	nd	nd
8a UCM1014	Et	Bn	5-O-Me	17 [15;86]	79±7.5	177 [92;340]	0.001 [0.0002;0.005]	111±3.5	0.9 [0.7;1]
8b	Et	Bn	6-O-Me	186 [106;324]	nd	nd	1.5 [0.8;2.9]	75±7	4.6 [3.4;6.4]
8c	Et	Bn	7-O-Me	214 [111;414]	nd	nd	26 [10;66]	42±5	25 [22;29]
8d	Et	Bn	H	37 [19;72]	42±10	36 [35;37]	0.09 [0.04;0.2]	73±2	0.4 [0.4;0.5]
8e	Me	Bn	H	45 [40;50]	35±2	31 [20;49]	0.04 [0.02;0.1]	60±2	0.4 [0.3;0.6]
(-)-8e	Me	Bn	H	45 [18;110]	52±4	192 [106;348]	0.1 [0.04;0.3]	80±8	1.9 [1.5;2.4]

(+)- 8e	Me	Bn	H	122 [112;132]	nd	nd	6.2 [5.2;7.2]	60±15	19 [8.7;40]
8f	cPr	Bn	H	291 [168;505]	nd	nd	0.2 [0.2;0.3]	38±4	0.3 [0.1;0.8]
8g	cBu	Bn	H	633 [201;2000]	nd	nd	6.2 [5.6;6.8]	<15	nd
8h	Et	Me	5-OMe	0.03 [0.01;0.05]	112±12	1.7 [1.4;2]	0.5 [0.3;0.9]	122±9	0.5 [0.5;0.6]
8i	Et	Me	H	0.7 [0.6;0.8]	69 ± 3	0.9 [0.7;1.2]	0.2 [0.2;0.3]	122±11	0.6 [0.5;0.8]
8j	Me	CH ₂ -CH ₂ -Ph	H	171 [81;361]	nd	nd	3[1.5;4.7]	<15	nd
8k	Me	nPr	H	5.8 [4.1;8.2]	39±5	2 [1;6]	0.1 [0.06;0.2]	58±0.5	0.2 [0.07;0.4]
8l	Et	CH ₂ CONHMe	H	>10000	nd	nd	385 [312;423]	nd	nd
11	Et	Ph	H	1.6 [0.6;4.3]	69±12	6.2 [6.1;6.2]	0.06 [0.03;0.1]	90±0.5	0.5 [0.3;0.7]
13		Me	H	22 [20;25]	76±4	809 [623;1050]	15 [11;18]	73±1	66 [51;85]
15				326 [190;554]	nd	nd	1.6 [0.7;3.4]	59±4	4.7 [3.2;6.9]

^a K_i (nM) values are geometric mean values (with 95% confidence limits shown in brackets) of at least 2 separate experiments performed in duplicate. Emax values are arithmetic mean ± S.E.M. nd: non determined.

^b Compounds were tested as racemates, with the exception of compounds **7**, (+)-**8e**, and (-)-**8e**.

^c Data for melatonin were taken from Landagaray E. et al. *Bioorg. Med. Chem.* **2014**, *22*, 986-996.

Compound **8i**, having a methyl group on the tetrahydroquinoline nitrogen, showed MT₁ and MT₂ binding affinities in the same range as melatonin, with an MT₂ full agonist behavior, while at the MT₁ receptor it behaved as a partial agonist. These data confirm tetrahydroquinoline as an efficient conformationally constrained analog of anilinoethylamide and a valuable bioisoster for the indole scaffold of melatonin. Removal of the methyl group on the nitrogen atom (**6d**) caused a 10-fold reduction of MT₁ and MT₂ binding affinity and a decreased ability to stimulate the receptors, with lower Emax and EC₅₀ values. This finding is in line with what observed in the *N*-anilinoethylamide series, in which removal of the *N*-methyl group reduced both binding affinity and intrinsic activity.¹⁴ Bulkier substituents on the nitrogen atom, such as a phenyl ring (**11**) and especially a benzyl group (**8d**), were detrimental for MT₁ binding affinity. On the contrary, these substituents

1 led to an increase in MT₂ binding affinity compared to the methyl derivative **8i**, with limited
2 reduction of intrinsic activity (E_{max}). The benzyl derivative **8d** has more than 400-fold selectivity
3 for the MT₂ receptor and acts as a potent MT₂ partial agonist. These data further support that the
4 behavior of tetrahydroquinolines is consistent with SARs obtained from classical melatonin
5 receptor ligands, given the MT₂-selectivity and reduced intrinsic activity provided by the benzyl
6 substituent.^{18,25} Elongation of the side chain in position 2, with insertion of an ethylene spacer
7 between the amide group and the tetrahydroquinoline ring (**15**), hampered the proper
8 accommodation of the compound at the receptor binding site leading to reduced potency. Insertion
9 of a hydrophilic acetamide substituent on the nitrogen atom (**8l**) was even more detrimental,
10 abolishing any binding to the MT₁ receptor and severely reducing the affinity for the MT₂ receptor.
11

12 To evaluate the conformational equilibria of the most interesting *N*-benzyltetrahydroquinoline
13 derivatives, molecular dynamics (MD) simulations were set up for compound **8e**, the analog of
14 compound **8d** having a shorter acetyl side chain instead of a propionyl, and similar binding affinity
15 and intrinsic activity. A 50 ns long well-tempered metadynamics simulation of compound **8e**
16 solvated into a water box was performed, setting as collective variables the dihedral angles τ_1 and
17 τ_2 (Figure 3A), which describe the arrangement of the amide side chain and of the benzyl
18 substituent, respectively. Compound **8e** has a stereogenic center and the free-energy profiles here
19 presented refer to the *R* enantiomer (the same results, with opposite values of dihedral angles, can
20 be obtained for the *S* enantiomer). The well-tempered metadynamics simulation reached
21 convergence after 20 ns (see Experimental Section for details). According to the free-energy surface
22 (FES) depicted in Figure 3B, the amide side chain (τ_1) can assume an axial ($\sim -50^\circ$) or an equatorial
23 arrangement ($\sim 50^\circ$), with lower energies associated to the former one. The benzyl substituent has a
24 different conformational freedom depending on the arrangement of the amide side chain. With the
25 amide side chain in equatorial arrangement, the benzyl substituent adopts two prevalent
26 conformations, above ($\tau_2 \sim -50^\circ$) or beneath ($\sim 100^\circ$) the plane defined by the tetrahydroquinoline
27 ring. On the contrary, it has higher conformational freedom with the amide side chain in axial
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arrangement. Indeed, the benzyl group explores almost all the possible conformations with similar free-energy contents, with the exception of $\tau_2 \sim 0^\circ$, a saddle point in the FES corresponding to an eclipsed arrangement of the benzyl substituent and the tetrahydroquinoline ring. In fact, the minimum free-energy conformation of compound **8e** has an axial amide side chain with the benzyl substituent oriented toward the opposite side of the tetrahydroquinoline ring (Figure 3C). Consistent results in terms of conformational equilibrium were obtained with a more time-consuming plain MD simulation. When the trajectory of solvated compound **8e** was simulated for 1 μ s, the amide side chain almost exclusively adopted an axial arrangement ($\tau_1 \sim -50^\circ$), with only about 4.5% of equatorial conformations. The benzyl substituent spent most of the time perpendicular to the plane of the tetrahydroquinoline ring ($\tau_2 \approx -70^\circ$, 55% of sampled conformations), with higher conformational freedom associated to the axial side chain arrangement than to the equatorial one (Supplementary Figure 1). Thus, a 50 ns-long metadynamics simulation gave the same result as a 1 μ s simulation of plain MD, which proposes the first as a convenient approach to explore conformational equilibria of bioactive compounds.

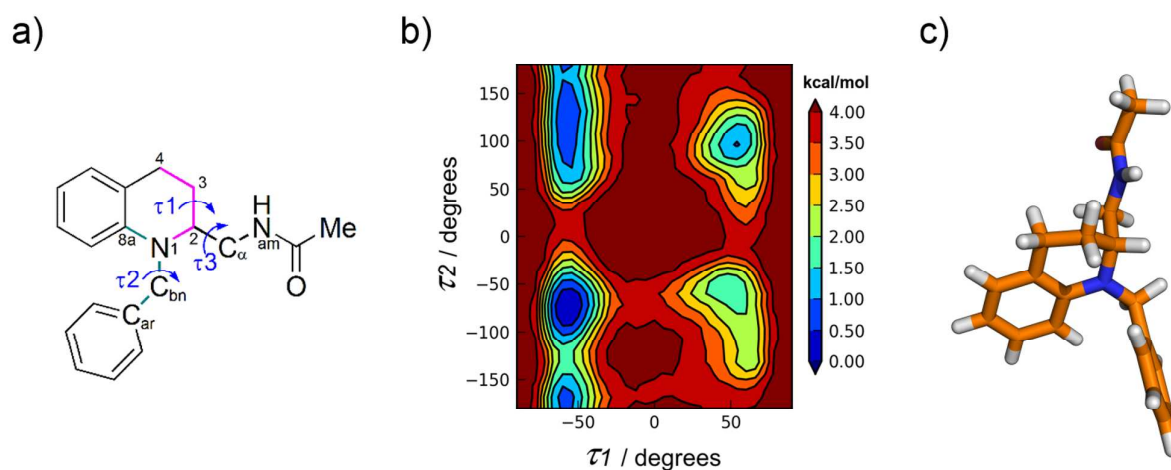


Figure 3. a) Representation of τ_1 (N₁-C₂-C₃-C₄, pink bonds), τ_2 (C_{8a}-N₁-C_{bn}-C_{ar}, green bonds) and τ_3 (N₁-C₂-C_α-N_{am}) dihedral angles. b) Free-energy surface for compound **8e** obtained after 20 ns of

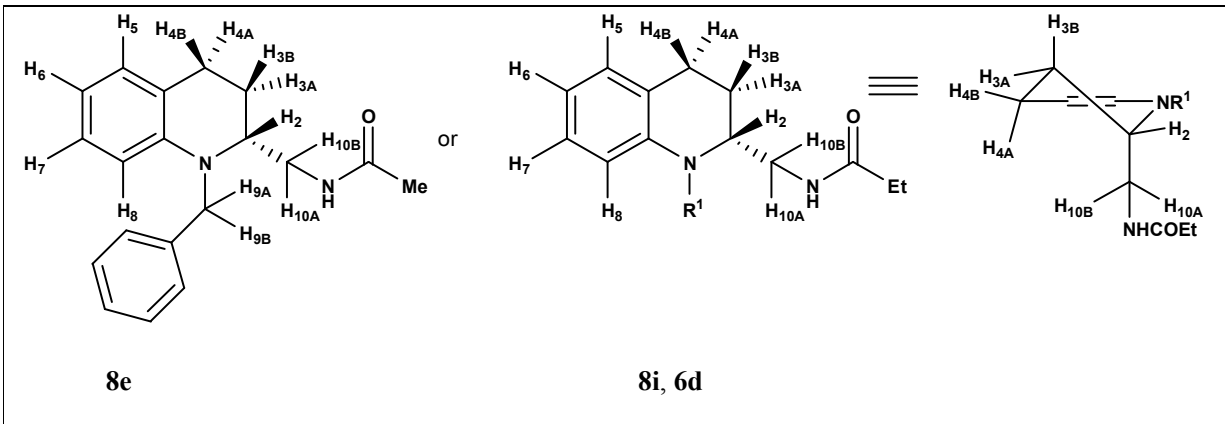
1 well-tempered metadynamics simulation. c) Representation of one of the conformations of
2 compound **8e** corresponding to the free-energy minimum ($\tau_1 = -57.7^\circ$, $\tau_2 = -71.1^\circ$).
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9 To evaluate the reliability of the metadynamics simulation, we also performed a well-tempered
10 metadynamics of compound **8e** in methanol and we compared the results with the information
11 obtained from NMR experiments in CD₃OD. The FES obtained after 20 ns of metadynamics
12 simulation in methanol is qualitatively the same as that obtained with water solvation
13 (Supplementary Figure S2). Analysis of 1D and 2D NMR data for compound **8e** in CD₃OD
14 revealed a high prevalence of half-chair conformation for the tetrahydroquinoline ring wherein the
15 C2 amide side chain is axially arranged (Table 2 and Supplementary Figure 3; detailed description
16 of ¹H spectrum is reported in the Supplementary Material). ¹H-¹H Vicinal coupling constant
17 analysis showed *J* values = 3.0 and 4.8 for H₂ with H_{3A} and H_{3B}, respectively, indicative of a gauche
18 arrangement of H₂ with both H₃ protons and consistent with the ring conformation depicted in
19 Figure 3C. Additionally, *J* values for protons H₃ and H₄ are consistent with an antiperiplanar
20 arrangement of H_{4A} and H_{3B} (*J*_{3B,4A} = 13.2, Table 2). 2D NOESY data of compound **8e**
21 (Supplementary Figure 4) are also consistent with the prevalence of conformations with axial amide
22 side chain. In fact, strong NOE contacts of the same intensities were recorded for H_{3A} and H_{3B} with
23 both H₂ and H_{4B}: similar distances among these couples of protons are only consistent with
24 equatorial arrangements of H₂ and H_{4B}. NMR data also supported the conformational equilibrium
25 simulated for the benzyl group and the amide side chain. The presence of NOE contacts for the two
26 methylene hydrogens H₉ with protons H₂, H₈ and H_{10A} suggests that the benzyl substituent is rather
27 free to rotate and can assume different conformations. NOE signals observed for H₉ protons with
28 H_{10A}, but not with H_{10B}, as well as strong NOE contacts between H_{4A} and H_{10B}, but not with H_{10A},
29 reveal limited conformational freedom of the amide side chain. This is further supported by the two
30 different values of *J* recorded for H₂ with H_{10A} and H_{10B}, with H_{10A} spending most of the time in
31 gauche to H₂, closer to the benzyl protons H₉, while H_{10B} was principally anti to H₂, pointing
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toward H_{4a}. This preferred arrangement of the side chain, corresponding to $\tau_3 \sim 180^\circ$ was captured by the plain MD simulation (Supplementary Figure 5).

The reliability of the results obtained from MD simulations, tested on these tetrahydroquinolines as well as on tetralin²⁶ derivatives, supports the application of this technique to estimate the conformational abundance of compounds in solution, to drive the design and synthesis of new compounds. Moreover, metadynamics simulations gave an additional advantage over plain MD in terms of computational speed.

Table 2. Selected Vicinal Coupling Constants Values Observed by NMR Spectroscopy (600 or 400 MHz, CD₃OD, 25 °C) for Compounds **6d**, **8e** and **8i** (*R* enantiomers depicted).



The figure shows three chemical structures. Structure **8e** is a tetrahydroquinoline derivative with a phenyl group at C2, a methyl amide group at C3, and protons labeled H₅ through H_{10B}. Structure **8i** is similar but with an ethyl amide group and an R¹ group at C2. Structure **6d** is a bicyclic system with an NHCOEt group. The table below summarizes the vicinal coupling constants (³J) for these compounds.

Protons ^a	8e <i>J</i> (Hz)	8i (R ¹ = Me) <i>J</i> (Hz)	6d (R ¹ = H) <i>J</i> (Hz)
³ <i>J</i> _{2,3A}	3.0 ^b	3.2	9.2
³ <i>J</i> _{2,3B}	4.8 ^b	4.4	3.2
³ <i>J</i> _{3A,4B}	3.6	3.2	9.2
³ <i>J</i> _{3B,4B}	3.6	4.8	5.4
³ <i>J</i> _{3B,4A}	13.2	12.8	5.6
³ <i>J</i> _{3A,4A}	5.4	5.7	6.0
³ <i>J</i> _{2,10A}	4.8 ^b	5.0	5.2
³ <i>J</i> _{2,10B}	9.0 ^b	9.0	6.4

^a Letter A denotes protons under the plane defined by the tetrahydroquinoline ring in the orientation depicted in the Table, letter B above the plane. ^b Reported *J* values are those obtained from NMR signals of protons H_{3A}, H_{3B}, H_{10A} and H_{10B}, as the signal of proton H₂ is not well resolved.

When the amide side chain is in axial arrangement, only one of the two enantiomers of **8e** can fit the pharmacophore model for melatonergic ligands and reproduce the putative bioactive conformation of melatonin.¹⁷ Thus, the conformational equilibrium described above implies that this compound would show significant stereoselectivity, which was confirmed by separation of the enantiomers on a chiral chromatographic column and by individual testing at the MT₁ and MT₂ receptors. The enantiomer (-)-**8e** showed about 60-fold higher MT₂ binding affinity than (+)-**8e** and greater potency in activating the MT₂ receptor. As shown in Figure 4, the enantiomer (*R*)-**8e** perfectly fits the bioactive conformation of melatonin, which suggests it should correspond to the enantiomer (-)-**8e**.

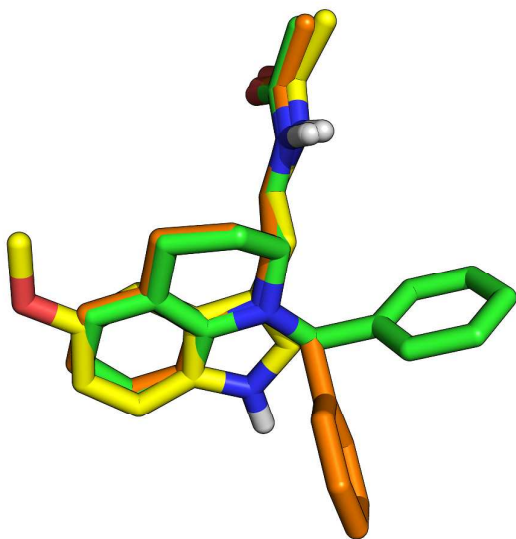


Figure 4. Superposition of melatonin (yellow carbons) and two representative conformations of compound **8e** with the benzyl group in an out-of-plane arrangement ($\tau_2 = -71.1^\circ$, orange carbons) and in an in-plane arrangement ($\tau_2 = -172.7^\circ$, green carbons).

The substituent at the tetrahydroquinoline nitrogen affects the conformational equilibrium of these compounds, as it promotes the axial arrangement of the amide side chain, with important

1 consequences on the binding profile and SARs. This is supported by 1D and 2D NMR experiments
2 performed on the *N*-methyl (**8i**) and the *N*-unsubstituted (**6d**) derivatives (Table 2 and
3 Supplementary Figures 6-8). Vicinal coupling constants as well as NOE contacts recorded for
4 compound **8i** highlighted the same preferential axial arrangement of the amide side chain seen for
5 the benzyl derivative **8e**. On the other hand, in the case of **6d** experimental *J* values can be
6 interpreted by assuming an equilibrium between two half chair conformations of the
7 tetrahydroquinoline ring, one with equatorial amide side chain and the other with axial amide,
8 inverting rapidly on the NMR time scale. In particular the J_{2-3A} value ≈ 9 Hz must be considered as
9 the average *J* value of both arrangements. A well-tempered metadynamics simulation performed on
10 compound **6d** provided results consistent with these NMR data and estimated almost equal free-
11 energy content for the axial and the equatorial arrangements of the amide side chain
12 (Supplementary Figure 9). Therefore, while both the *N*-methyl and the *N*-benzyl substituent
13 promote the conformations which fit the pharmacophore model, the lower potency observed for the
14 *N*-unsubstituted derivative **6d** might be due, at least in part, to the presence of the equatorial species
15 in solution. According to MD simulations and NMR experiments, the benzyl group of compound **8e**
16 can alternatively be arranged perpendicular to the tetrahydroquinoline ring, with an out-of-plane
17 hindrance typical of MT₂-selective antagonists, or coplanar with the tetrahydroquinoline, occupying
18 a region of space where substituents of melatonin receptor agonists are usually present, like the
19 phenyl ring of 2-phenylmelatonin (Figure 4). We speculate that this conformational flexibility could
20 be responsible for the MT₂ selectivity of compound **8e**, and also for the residual intrinsic activity.
21 Indeed, selectivity for the MT₂ receptor should be guaranteed by the bulky benzyl group that could
22 hardly fit the MT₁ binding site, which is supposed to have reduced tolerance toward out-of-plane
23 space occupancy. On the other hand, at the MT₂ receptor compound **8e** could also assume an in-
24 plane conformation of the benzyl group, leading to receptor activation. Of course, this behavior is
25 likely to involve some conformational flexibility of the receptor.

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The effect of substituents on the nitrogen atom was further analyzed. Lengthening of the benzyl substituent into a phenethyl one (**8j**) led to a significant drop of binding affinity at both receptor subtypes, suggestive of some steric hindrance at the receptor binding site. The smaller N-propyl derivative **8k** showed significantly higher MT₁ and MT₂ binding affinity.

A well-known SAR feature of melatonin receptor ligands is related to the methoxy group, which increases both binding affinity and intrinsic activity when it can mimic the same group of melatonin. According to the superposition model represented in Figure 4, the best position for the methoxy group should be position 5 of the tetrahydroquinoline scaffold. To test this hypothesis, we synthesized three isomers having the methoxy group in position 5 (**8a**), 6 (**8b**) or 7 (**8c**), maintaining the benzyl substituent at the nitrogen atom. For these compounds, we selected the propionyl group at side chain due to the slightly higher intrinsic activity at MT₂ receptor for **8d**, compared to the acetyl derivative **8e**. While 6- and 7-methoxy groups were detrimental for receptor recognition, the 5-methoxy group led to increased MT₁ and MT₂ binding affinities. The positive effect of the 5-methoxy group was particularly relevant at the MT₂ receptor, leading to compound **8a** with picomolar binding affinity, more than 10,000-fold MT₂-selectivity and a full agonist behavior at the MT₂ receptor. This compound is the most selective MT₂ full agonist reported to date.²⁷ We further tested the efficacy of the 5-methoxy group on the tetrahydroquinoline scaffold by modifying the substituent at the nitrogen atom. Compound **8h**, having 5-methoxy and N-methyl substituent has higher MT₁ binding affinity than the desmethoxy analogue **8i**, behaving as a full agonist at both receptor subtypes. Unexpectedly, insertion of the 5-methoxy group on the N-unsubstituted tetrahydroquinoline **6d** led to compound **6a** with decreased binding affinity and intrinsic activity. This peculiar behavior might be related to the presence of the polar hydrogen which might favor a different accommodation at the receptor binding site. SARs typical of melatonin receptor ligands were further confirmed by compounds with different acylating groups. Replacement of the ethyl group on the amide side chain by a cyclopropyl (**8f**) or a cyclobutyl (**8g**) ring led to the expected drop in binding affinity and intrinsic activity. As a confirmation of the

1 importance of the tetrahydroquinoline scaffold to reproduce the active conformation of melatonin,
2 we synthesized the fully-unsaturated quinoline **7**, which was devoid of binding affinity.
3

4 We also explored different side chains which could represent an alternative to the amide one,
5 focusing on ethylurea and thioethylurea derivatives. The ethylurea side chain, with either a methyl
6 (**6m**) or a hydrogen atom (**6i**) at the tetrahydroquinoline nitrogen, led to significant decreases of
7 binding affinity, as it had been observed for agomelatine²⁸ and 1,6-dihydro-2*H*-indeno[5,4-*b*]furan
8 derivatives.²⁹ Insertion of a 5-methoxy group on the tetrahydroquinoline scaffold (**6l**, **6h** and **6n**) did
9 not produce the expected increase of binding affinity. The three thioethylurea derivatives **13**, **6k** and
10 **6j** were even less potent than the corresponding urea derivatives **6m**, **6i** and **6l**, respectively.
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26 Conclusions

27 Starting from molecular superpositions on previously developed pharmacophore models, the
28 scaffold of *N*-anilinoethylamide melatonin receptor ligands, which had provided potent and MT₂-
29 selective antagonists or partial agonists, was partially constrained to a 2-acylaminomethyl-
30 tetrahydroquinoline nucleus. This nucleus proved to be an excellent new scaffold to develop new
31 melatonergic selective agonists and the benzyl substituent on the tetrahydroquinoline nitrogen
32 favored the proper arrangement of the amide side chain, mimicking the active conformation of
33 melatonin.
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44 Superposition models, supported by extended conformational analysis by MD simulations and
45 NMR experiments, allowed to predict the suitable position for a methoxy group on the
46 tetrahydroquinoline ring, leading to the benzyl derivative **8a** (UCM1014), an extremely potent and
47 selective full agonist at MT₂ receptor.
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52 As a final remark, metadynamics simulations proved to give reliable results on the free-energy
53 profile and conformational preferences of these compounds, at much lower computational cost than
54 classical plain molecular dynamics.
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Experimental section

General procedures. Melting points were determined on a Buchi B-540 capillary melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 200 or 400 instrument, or on a Varian INOVA 600 MHz spectrometer, using CDCl_3 as solvent unless stated otherwise. Chemical shifts (δ scale) are reported in parts per million (ppm) relative to the central peak of the solvent. Coupling constants (J) are given in hertz (Hz). EI MS spectra (70 eV) were taken on a Fisons Trio 1000 instrument; only molecular ions (M^+) and base peaks are given. ESI MS spectra were taken on a Waters Micromass ZQ instrument; only molecular ions ($\text{M}+1$) $^+$ are given. The purity of tested compounds, determined by high pressure liquid chromatography (HPLC), was greater than 95% (Table S1). These analyses were performed on a Waters HPLC/UV/MS system (separation module Alliance HT2795, Photo Diode Array Detector 2996, mass detector Micromass ZQ; software: MassLynx 4.1).

Column chromatography purifications were performed under “flash” conditions using Merck 230–400 mesh silica gel. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates. 2-Quinolinecarbonitrile (**4d**) was purchased from commercial suppliers and was used without further purification.

Synthesis of 5-, 6-, and 7-methoxyquinoline-2-carbonitriles (4a-c). CH_3I (0.15 mL, 2.4 mmol) and K_2CO_3 (0.412 g, 3 mmol) were added to a solution of the suitable hydroxy-2-cyanoquinoline derivative **3a-c** (0.17 g, 1 mmol) in acetone (8 mL) and the resulting mixture was stirred at room temperature for 15 h. The solvent was evaporated, the residue was taken up with water and extracted (3x) with EtOAc. The organic phases were combined, dried (Na_2SO_4) and evaporated to afford a crude product which was purified by flash chromatography over silica gel (cyclohexane/EtOAc 7:3 as eluent) and crystallization.

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2 **5-Methoxyquinoline-2-carbonitrile (4a)**. White solid, mp 113-4 °C (EtOAc-petroleum ether);
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4 95% yield. ¹H NMR (200 MHz, CDCl₃) δ 4.05 (s, 3H), 7.00 (dd, 1H, *J*₁=*J*₂=4.5), 7.69 (d, 1H,
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6 *J*=8.5), 7.74-7.77 (m, 2H), 8.73 (d, 1H, *J*=8.5 Hz). ESI MS (*m/z*): 185 (M+H)⁺.
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10 **6-Methoxyquinoline-2-carbonitrile (4b)**. White solid, mp 176-7 °C (EtOH-H₂O); 88% yield.
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12 Physicochemical data were in agreement to those previously reported.³⁰
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17 **7-Methoxyquinoline-2-carbonitrile (4c)**. White solid, mp 187-8 °C (EtOAc-petroleum ether);
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19 74% yield. ¹H NMR (200 MHz, CDCl₃) δ 3.99 (s, 3H), 7.36 (dd, 1H, *J*=2.5 and 9.0 Hz), 7.46 (d,
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21 1H, *J*=2.5 Hz), 7.60 (d, 1H, *J*=8.5 Hz), 7.78 (d, 1H, *J*=9.0 Hz), 8.22 (d, 1H, *J*=8.5 Hz). ESI MS
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23 (*m/z*): 185 (M+H)⁺.
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28 **Synthesis of *N*-[(1,2,3,4-tetrahydroquinolin-2-yl)methyl]alkanamides (6a-g)**. A solution of the
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30 suitable quinoline-2-carbonitrile **4a-d** (1 mmol) in THF (5 mL) and 2M NH₃ in EtOH (0.6 mL) was
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32 hydrogenated over Raney nickel (4 atm) for 16 h at 60 °C. The catalyst was filtered on Celite, the
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34 filtrate was concentrated *in vacuo*, and the residue partitioned between EtOAc and water. The
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36 filtrate was concentrated *in vacuo*, and the residue partitioned between EtOAc and water. The
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38 organic phase was washed with brine, dried (Na₂SO₄) and evaporated under reduced pressure to
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40 give the corresponding crude oily methanamine (**5a-d**) which was used without any further
41
42 purification.
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44 Et₃N (0.14 mL) and the suitable anhydride or acyl chloride (1 mmol) were added to a cold solution
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46 of the appropriate above crude methanamine **5a-d** in THF (6 mL) and the resulting mixture was
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48 stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, the residue
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50 was taken up in EtOAc, washed with a saturated aqueous solution of NaHCO₃ and with brine. After
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52 drying over Na₂SO₄, the solvent was removed by distillation *in vacuo* to give a crude product that
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54 was purified by flash chromatography.
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2 ***N*-[(5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (6a).** Flash
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4 chromatography: silica gel, EtOAc as eluent. White solid, mp 108-9 °C (Et₂O-petroleum ether);
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6 54% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.17 (t, 3H, *J*=7.5 Hz), 1.61-1.75 (m, 1H), 1.87-1.96 (m,
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8 1H), 2.25 (q, 2H, *J*=7.5 Hz), 2.48-2.65 (m, 1H), 2.75-2.88 (m, 1H), 3.37-3.45 (m, 3H), 3.79 (s, 3H),
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10 4.01 (br s, 1H), 5.76 (br s, 1H), 6.20-6.24 (m, 2H), 6.96 (dd, 1H, *J*₁=*J*₂=8.0 Hz). ¹³C NMR (50
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12 MHz, CDCl₃) δ 174.6, 157.8, 144.5, 126.9, 109.8, 108.1, 99.9, 55.3, 50.9, 44.1, 29.7, 25.0, 19.7,
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14 9.9. ESI MS (*m/z*): 249 (M+H)⁺.
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20 ***N*-[(6-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (6b).** Flash
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22 chromatography: silica gel, EtOAc as eluent. Oil; 50% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.18 (t,
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24 3H, *J*=7.5 Hz), 1.65-1.76 (m, 1H), 1.84-1.95 (m, 1H), 2.25 (q, 2H, *J*=7.5 Hz), 2.73-2.86 (m, 2H),
25
26 3.38-3.49 (m, 3H), 3.74 (s, 3H), 5.79 (brs, 1H), 6.48-6.65 (m, 3H). EI MS (*m/z*): 248 (M⁺), 162
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28 (100).
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33 ***N*-[(7-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (6c).** Flash
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35 chromatography: silica gel, EtOAc/cyclohexane 9:1 as eluent. Amorphous solid; 47% yield. ¹H
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37 NMR (200 MHz, CDCl₃) δ 1.17 (t, 3H, *J*=7.5 Hz), 1.66-1.77 (m, 1H), 1.83-1.95 (m, 1H), 2.26 (q,
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39 2H, *J*=7.5 Hz), 2.72-2.79 (m, 2H), 3.42-3.49 (m, 3H), 3.74 (s, 3H), 5.91 (br s, 1H), 6.17 (d, 1H,
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41 *J*=2.5 Hz), 6.28 (dd, 1H, *J*=2.5 and 8.0 Hz), 6.89 (d, 1H, *J*=8.0 Hz). ESI MS (*m/z*): 249 (M+H)⁺.
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46 ***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]propionamide (6d).** Flash chromatography: silica
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48 gel, EtOAc/cyclohexane 7:3 as eluent. Beige solid, mp 67-8 °C (Et₂O-petroleum ether); 63% yield.
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50 ¹H NMR (400 MHz, CD₃OD) δ 1.16 (t, 3H, *J*=7.5 Hz), 1.65 (dddd, 1H, *J*₁=6.0, *J*₂≈*J*₃=9.0 and
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52 *J*₄=13.0 Hz, H_{3B}), 1.94 (dddd, 1H, *J*₁=3.0, *J*₂≈*J*₃=5.5 and *J*₄=13.5, H_{3A}), 2.25 (q, 2H, *J*=7.5 Hz), 2.73
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54 (ddd, 1H, *J*=5.5, 6.0 and 16.0 Hz, H_{4B}), 2.79 (ddd, 1H, *J*=5.5, 9.0 and 16.0 Hz, H_{4A}), 3.27 (dd, 1H,
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56 *J*=6.5 and 13.0 Hz, H_{10B}), 3.31 (dd, 1H, *J*=5.0 and 13.0 Hz, H_{10A}), 3.37 (dddd, 1H, *J*₁=3.0, *J*₂=5.0,
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2 $J_3=6.5$ and $J_4=9.0$ Hz, H₂), 6.52-6.56 (m, 2H, arom), 6.88-6.90 (m, 2H, arom). ¹³C NMR (50 MHz,
3 CDCl₃) δ 174.7, 144.0, 129.2, 126.9, 121.1, 117.5, 114.5, 51.2, 44.4, 29.7, 25.9, 25.4, 9.9. ESI MS
4 (*m/z*): 219 (M+H)⁺.
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8 ¹H NMR spectrum of compound **6d** is depicted in Supplementary Figure S8.
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12 ***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]acetamide (6e)**. Flash chromatography: silica gel,
13 EtOAc/MeOH 96:4 as eluent. Oil; 73% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.58-1.77 (m, 1H),
14 1.84-1.97 (m, 1H), 2.01 (s, 3H), 2.68-2.86 (m, 2H), 3.38-3.50 (m, 3H), 6.49 (d, 1H, $J=8.0$ Hz), 6.60
15 (dd, 1H, $J_1=J_2=7.5$ Hz), 6.80 (br t, 1H), 6.93-7.04 (m, 2H). EI MS (*m/z*): 204 (M⁺), 132 (100).
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22 ***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]cyclopropanecarboxamide (6f)**. Flash
23 chromatography: silica gel, EtOAc/cyclohexane 8:2 as eluent. Oil; 73% yield. ¹H NMR (200 MHz,
24 CDCl₃) δ 0.70-0.80 (m, 2H), 0.95-1.02 (m, 2H), 1.30-1.42 (m, 1H), 1.63-1.97 (m, 2H), 2.65-2.85
25 (m, 2H), 3.38-3.54 (m, 3H), 5.97 (br s, 1H), 6.52 (d, 1H, $J=8.5$ Hz), 6.61 (dd, 1H, $J_1=J_2=7.5$ Hz),
26 6.94-7.02 (m, 2H). ESI MS (*m/z*): 231 (M+H)⁺.
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37 ***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]cyclobutanecarboxamide (6g)**. Flash
38 chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent. Oil; 34% yield. ¹H NMR (200 MHz,
39 CDCl₃) δ 1.60-2.38 (m, 8H), 2.66-2.90 (m, 2H), 2.93-3.10 (m, 1H), 3.37-3.53 (m, 3H), 5.68 (br s,
40 1H), 6.52 (d, 1H, $J=8.0$ Hz), 6.63 (dd, 1H, $J_1=J_2=7.5$ Hz), 6.94-7.02 (m, 2H). ESI MS (*m/z*): 245
41 (M+H)⁺.
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51 **General procedure for the synthesis of (1,2,3,4-tetrahydroquinolin-2-yl)ureido derivatives (6h-**
52 **k)**. The suitable ethyl isocyanate (1 mmol) was added to a cold solution of the appropriate above
53 crude (1,2,3,4-tetrahydroquinolin-2-yl)methanamine (**5a** or **5d**) (from 1 mmol) in CH₂Cl₂ (6 mL)
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1 and the resulting mixture was stirred at room temperature for 30 min. The solvent was evaporated
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4 under reduced pressure, and the residue was purified by flash chromatography.
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8 **1-Ethyl-3-[(5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]urea (6h).** Flash
9 chromatography: silica gel, EtOAc as eluent. White solid, mp 131-2 °C (EtOAc-petroleum ether);
10 60% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.14 (t, 3H, *J*=7.2 Hz), 1.58-1.74 (m, 1H), 1.85-1.97 (m,
11 1H), 2.47-2.64 (m, 1H), 2.74-2.86 (m, 1H), 3.15-3.43 (m, 5H), 3.79 (s, 3H), 4.15 (br s, 1H), 4.37 (br
12 s, 1H), 4.63 (br s, 1H), 6.22 (app t, 2H), 6.95 (dd, 1H, *J*₁=*J*₂=8.0 Hz). ¹³C NMR (50 MHz, CDCl₃) δ
13 159.0, 157.8, 144.9, 126.9, 109.7, 108.0, 99.6, 55.2, 51.3, 45.4, 35.3, 25.1, 19.7, 15.4. EI MS (*m/z*):
14 263 (M⁺), 162 (100).
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24 **1-Ethyl-3-[(1,2,3,4-tetrahydroquinolin-2-yl)methyl]urea (6i).** Flash chromatography: silica gel,
25 EtOAc as eluent. White solid, mp 86-7 °C (Et₂O); 49% yield. ¹H NMR (CDCl₃, 200 MHz) δ 1.15 (t,
26 3H, *J*=7.2 Hz), 1.60-1.80 (m, 1H), 1.85-1.95 (m, 1H), 2.72-2.86 (m, 2H), 3.16-3.36 (m, 4H), 3.41-
27 3.48 (m, 1H), 4.13 (br s, 1H), 4.30 (br s, 1H), 4.57 (br s, 1H), 6.52 (d, 1H, *J*=8.0 Hz), 6.63 (dd, 1H,
28 *J*₁=*J*₂=7.0 Hz), 6.95-7.02 (m, 2H). ¹³C NMR (50 MHz, CDCl₃) δ 158.9, 143.8, 129.2, 126.8, 121.4,
29 117.6, 114.6, 51.8, 45.6, 35.3, 25.9, 25.4, 15.4. ESI MS (*m/z*): 234 (M+H)⁺.
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41 **1-Ethyl-3-[(5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]thiourea (6j).** Flash
42 chromatography: silica gel, cyclohexane-EtOAc 8:2 as eluent. White solid, mp 115-6 °C (EtOAc-
43 petroleum ether); 63% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.22 (t, 3H, *J*=7.2 Hz), 1.63-1.81 (m,
44 1H), 1.86-1.98 (m, 1H), 2.58-2.85 (m, 2H), 3.32-3.45 (m, 2H), 3.54-3.70 (m, 3H), 3.80 (s, 3H), 4.04
45 (br s, 1H), 6.01 (br s, 2H), 6.22 (d, 1H, *J*=8.0 Hz), 6.28 (d, 1H, *J*=8.0 Hz), 6.98 (dd, 1H, *J*₁=*J*₂=8.0
46 Hz). ¹³C NMR (50 MHz, CDCl₃) δ 182.3, 157.8, 144.1, 127.0, 110.1, 108.4, 100.3, 55.3, 50.4, 49.2,
47 39.1, 24.9, 19.3, 14.1. EI MS (*m/z*): 279 (M⁺), 162 (100).
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1-Ethyl-3-[(1,2,3,4-tetrahydroquinolin-2-yl)methyl]thiourea (6k). Flash chromatography: silica gel, cyclohexane/EtOAc 7:3 as eluent. White solid, mp 107-8 °C (Et₂O-petroleum ether); 65% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.23 (t, 3H, *J*=7.2 Hz), 1.66-1.86 (m, 1H), 1.91-2.01 (m, 1H), 2.70-2.93 (m, 2H), 3.28-3.51 (m, 2H), 3.52-3.69 (m, 3H), 4.07 (br s, 1H), 6.10 (br s, 2H), 6.56 (d, 1H, *J*=8.0 Hz), 6.69 (dd, 1H, *J*₁=*J*₂=8.0 Hz), 6.98-7.05 (m, 2H). ¹³C NMR (50 MHz, CDCl₃) δ 182.4, 143.3, 129.3, 127.0, 121.5, 118.2, 115.1, 50.8, 49.5, 39.0, 25.6, 25.3, 14.1. ESI MS (*m/z*): 250 (M+H)⁺.

1-Ethyl-3-[(5-methoxy-1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]urea (6l). A suspension of **6i** (1 mmol), NaHCO₃ (84 mg, 1 mmol) and methyl iodide (0.4 mL, 6.5 mmol) in dry methanol (11 mL) was heated at 50 °C for 24 h. After removing the solvent by distillation *in vacuo*, the residue was poured into water, extracted with EtOAc (4x), and the combined organic phases were washed with brine and dried (Na₂SO₄). The solvent was removed by distillation under reduced pressure and the residue was purified by silica gel flash chromatography (cyclohexane/EtOAc 3:7 as eluent). White solid, mp 172-3 °C (EtOAc-petroleum ether); 77% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.12 (t, 3H, *J*=7.3 Hz), 1.78-2.00 (m, 2H), 2.38-2.56 (m, 1H), 2.77-2.90 (m, 1H), 3.03 (s, 3H), 3.08-3.47 (m, 5H), 3.80 (s, 3H), 4.25 (br s, 1H), 4.42 (br s, 1H), 6.28 (d, 1H, *J*=8.0 Hz), 6.30 (d, 1H, *J*=8.0 Hz), 7.07 (dd, 1H, *J*₁=*J*₂=8.0 Hz). ¹³C NMR (50 MHz, CDCl₃) δ 158.7, 157.1, 145.8, 127.0, 109.5, 104.7, 98.7, 57.9, 55.3, 41.4, 39.1, 35.2, 22.5, 17.0, 15.5. ESI MS (*m/z*): 278 (M+H)⁺.

1-Ethyl-3-[(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]urea (6m). This product was obtained starting from **6d** and using the above *N*¹-methylation procedure described for compound **6l**. Flash chromatography: silica gel, EtOAc as eluent. White solid, mp 116-7 °C (EtOAc-petroleum ether); 77% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.13 (t, 3H, *J*=7.2 Hz), 1.83-1.95 (m, 2H), 2.71-2.79 (m, 2H), 3.02 (s, 3H), 3.12-3.27 (m, 3H), 3.34-3.49 (m, 2H), 4.24 (br s, 1H), 4.41 (br s, 1H), 6.58-6.66 (m, 2H), 6.99 (d, 1H, *J*=7.0 Hz), 7.11 (dd, 1H, *J*=7.0 and 8.0 Hz). ¹³C NMR (50 MHz,

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CDCl₃) δ 158.3, 145.1, 128.6, 127.2, 122.2, 116.0, 111.1, 58.3, 42.1, 38.3, 35.3, 23.9, 23.5, 15.4. EI MS (*m/z*): 247 (M⁺), 146 (100).

1-[(1-Benzyl-5-methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]-3-ethylurea (6n). A solution of **6h** (0.263 g, 1 mmol), Et₃N (0.27 mL) and benzyl bromide (0.19 mL, 1.6 mmol) in dry toluene (3 mL) was heated at reflux for 2 h. After cooling to room temperature the reaction mixture was poured into water and extracted with EtOAc (3x); the combined organic phases were washed with brine and dried (Na₂SO₄). After removing the solvent by distillation under reduced pressure, the residue was purified by silica gel flash chromatography (EtOAc as eluent) and crystallization. White solid, mp 147-8 °C (acetone-petroleum ether); 88% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.06 (t, 3H, *J*=7.2 Hz), 1.83-2.01 (m, 2H), 2.44-2.62 (m, 1H), 2.82-3.13 (m, 3H), 3.22-3.28 (m, 2H), 3.45-3.57 (m, 1H), 3.82 (s, 3H), 3.96 (br s, 1H), 4.30 (br s, 1H), 4.53 (d, 1H, *J*=17.0 Hz), 4.65 (d, 1H, *J*=17.0 Hz), 6.28 (d, 1H, *J*=8.0 Hz), 6.29 (d, 1H, *J*=8.0 Hz), 6.99 (dd, 1H, *J*₁=*J*₂=8.0 Hz), 7.24-7.37 (m, 5H). ¹³C NMR (50 MHz, CDCl₃) δ 158.3, 157.3, 145.1, 139.6, 128.6, 126.9, 126.8, 126.7, 109.8, 106.3, 98.8, 57.4, 55.9, 55.3, 42.0, 35.2, 22.5, 17.1, 15.4. ESI MS (*m/z*): 354 (M+H)⁺.

N-[(5-Methoxyquinolin-2-yl)methyl]propionamide (7). A solution of **4a** (1 mmol) in 2N NH₃ in MeOH (8 mL) was hydrogenated over Raney nickel (3.5 atm) for 0.5 h at room temperature. The catalyst was filtered on Celite, the filtrate was concentrated *in vacuo*, and the residue partitioned between EtOAc and water. The organic phase was washed with brine, dried (Na₂SO₄) and evaporated under reduced pressure to give crude (5-methoxyquinolin-2-yl)methanamine which was used without any further purification.

Et₃N (0.14 mL) and propionic anhydride (1 mmol) were added to a cold solution of above (5-methoxyquinolin-2-yl)methanamine in THF (6 mL) and the resulting mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, the residue was taken up in EtOAc, washed with a saturated aqueous solution of NaHCO₃ and with brine. After drying over

1 Na₂SO₄, the solvent was removed by distillation *in vacuo*, and the residue was purified by silica gel
2 flash chromatography (EtOAc as eluent) and crystallization; Beige solid, mp 117-8 °C (CH₂Cl₂-
3 petroleum ether); 48% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.26 (t, 3H, *J*=7.5 Hz), 2.40 (q, 2H,
4 *J*=7.5 Hz), 4.01 (s, 3H), 4.73 (d, 2H, *J*=4.5 Hz), 6.84-6.89 (m, 1H), 7.20 (br s, 1H), 7.31 (d, 1H,
5 *J*=8.5 Hz), 7.61-7.65 (m, 2H), 8.54 (d, 1H, *J*=8.5 Hz). ¹³C NMR (50 MHz, CDCl₃) δ 173.9, 156.4,
6 155.2, 148.0, 131.7, 129.8, 120.7, 119.7, 119.1, 104.3, 55.7, 44.7, 29.7, 9.9. ESI MS (*m/z*): 245
7 (M+H)⁺.
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20 ***N*-(1-Benzyl-5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methylpropionamide (8a).** This
21 product was obtained starting from **6a** and using the above *N*¹-benzylation procedure described for
22 compound **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent and
23 crystallization. White solid, mp 154-5 °C (CH₂Cl₂-petroleum ether); 89% yield. ¹H NMR (200
24 MHz, CDCl₃) δ 1.04 (t, 3H, *J*=7.5 Hz), 1.85-2.05 (m, 4H), 2.44-2.62 (m, 1H), 2.80-2.96 (m, 1H),
25 3.26-3.41 (m, 2H), 3.43-3.55 (m, 1H), 3.81 (s, 3H), 4.48 (d, 1H *J*=17.0 Hz), 4.64 (d, 1H *J*=17.0 Hz),
26 5.45 (br s, 1H), 6.28 (d, 1H *J*=8.0 Hz), 6.32 (d, 1H *J*=8.0 Hz), 6.99 (dd, 1H, *J*₁=*J*₂=8.0 Hz), 7.23-
27 7.37 (m, 5H). ¹³C NMR (50 MHz, CDCl₃) δ 173.9, 157.3, 145.2, 139.7, 128.4, 126.8, 126.7, 126.5,
28 109.5, 106.2, 98.6, 56.8, 56.5, 55.4, 41.1, 29.5, 23.9, 23.3, 9.7. ESI MS (*m/z*): 339 (M+H)⁺.
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42 ***N*-(1-Benzyl-6-methoxy-1,2,3,4-tetrahydroquinolin-2-yl-methyl)propionamide (8b).** This
43 product was obtained starting from **6b** and using the above *N*¹-benzylation procedure described for
44 compound **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 1:1 as eluent and
45 crystallization. White solid, mp 147-8 °C (EtOAc-hexane); 78% yield. ¹H NMR (200 MHz, CDCl₃)
46 δ 1.02 (t, 3H *J*=7.5 Hz), 1.90-2.05 (m, 4H), 2.72-2.85 (m, 2H), 3.14-3.47 (m, 3H), 3.74 (s, 3H), 4.39
47 (d, 1H, *J*=16.5 Hz), 4.54 (d, 1H, *J*=16.5 Hz), 5.45 (br s, 1H), 6.61-6.64 (m, 3H), 7.23-7.39 (m, 5H).
48 ¹³C NMR (50 MHz, CDCl₃) δ 173.8, 151.6, 139.8, 139.1, 128.7, 127.1, 127.0, 124.0, 115.5, 114.5,
49 112.9, 57.3, 57.0, 55.7, 41.2, 29.6, 24.2, 23.0, 9.7. EI MS (*m/z*): 338 (M⁺), 91 (100).
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4 ***N*-[**(1-Benzyl-7-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl**]propionamide (8c).** This
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6 product was obtained starting from **6c** and using the above *N*¹-benzylation procedure described for
7
8 compound **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent and
9
10 crystallization. White solid, mp 129-30 °C (EtOAc-petroleum ether); 90% yield. ¹H NMR (200
11
12 MHz, CDCl₃) δ 1.05 (t, 3H *J*=7.5 Hz), 1.90-2.10 (m, 4H), 2.67-2.91 (m, 2H), 3.35 (t, 1H, *J*=6.2
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14 Hz), 3.51-3.57 (m, 1H), 3.67 (s, 3H), 4.52 (d, 1H *J*=17.2 Hz), 4.64 (d, 1H *J*=17.2 Hz), 5.47 (brs,
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16 1H), 6.15 (d, 1H *J*=2.0 Hz), 6.23 (dd, 1H *J*=8.2 Hz), 6.94 (d, 1H *J*=8.2 Hz), 7.20-7.37 (m, 5H). ¹³C
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18 NMR (50 MHz, CDCl₃) δ 174.0, 159.2, 145.4, 139.1, 129.5, 128.7, 127.0, 126.5, 114.6, 101.2, 99.2,
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20 57.3, 55.4, 55.1, 41.4, 29.5, 23.6, 23.0, 9.7. ESI MS (*m/z*): 339 (M+H)⁺.
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26 ***N*-[**(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl**]propionamide (8d).** This product was
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28 obtained starting from **6d** and using the above *N*¹-benzylation procedure described for compound
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30 **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent and crystallization. White
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32 solid, mp 108-9° C (Et₂O-petroleum ether); 92% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.05 (t, 3H
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34 *J*=7.5 Hz), 1.92-2.08 (m, 4H), 2.73-2.93 (m, 2H), 3.32-3.40 (m, 2H), 3.43-3.59 (m, 1H), 4.53 (d, 1H
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36 *J*=17.0 Hz), 4.66 (d, 1H *J*=17.0 Hz), 5.47 (br s, 1H), 6.56-6.69 (m, 2H), 6.98-7.05 (m, 2H), 7.21-
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38 7.38 (m, 5H). ¹³C NMR (50 MHz, CDCl₃) δ 173.9, 144.6, 139.3, 129.1, 128.7, 127.3, 127.0, 126.6,
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40 122.1, 116.7, 113.0, 57.5, 55.6, 41.5, 29.6, 23.9, 23.3, 9.7. ESI MS (*m/z*): 309 (M+H)⁺.
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46 ***N*-[**(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl**]acetamide (8e).** This product was obtained
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48 starting from **6e** and using the above *N*¹-benzylation procedure described for compound **6n**. Flash
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50 chromatography: silica gel, EtOAc as eluent and crystallization. Greyish solid, mp 120-1 °C (Et₂O-
51
52 petroleum ether); 59% yield. ¹H NMR (600 MHz, CD₃OD) δ 1.84 (tt, 1H *J*₁ = *J*₂ = 4.8, *J*₃ = *J*₄
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54 =13.2, H_{3B}), 1.85 (s, 3H, CH₃), 1.96 (ddt, 1H *J*₁ = *J*₂ = 3.0, *J*₃ = 5.4, *J*₄ = 13.2 Hz H_{3A}), 2.65 (ddd,
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56 1H, *J*₁ = *J*₂ = 3.6, *J*₃ = 16.2 Hz, H_{4B}), 2.87 (ddd, 1H, *J* = 5.4, 13.2, 16.2 Hz, H_{4A}), 3.18 (dd, 1H, *J*=
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9.0, 13.2 Hz, H_{10B}), 3.32 (dd, 1H, $J = 4.8, 13.2$ Hz, H_{10A}), 3.49 (dq, 1H, $J_1 = J_2 = J_3 = 4.2, J_4 = 7.8$ Hz, H₂), 4.52 (½ABq, 1H, $J = 17.4$ Hz, H_{9B}), 4.61 (½ABq, 1H $J = 17.4$ Hz, 1H, H_{9A}), 6.37 (d, $J = 7.8$ Hz, 1H, H₈), 6.46 (td, 1H, $J_1 = J_2 = 7.2, J_3 = 0.6$ Hz, H₆), 6.81 (bt, 1H, $J_1 = J_2 = 7.8$ Hz, H₇), 6.89 (d, 1H, $J = 7.2$ Hz, H₅), 7.12-7.15 (m, 1H, Ph), 7.18-7.24 (m, 4H, Ph). ¹³C NMR (50 MHz, CDCl₃) δ 170.2, 144.6, 139.3, 129.1, 128.7, 127.2, 127.0, 126.7, 122.0, 116.7, 113.1, 57.3, 55.7, 41.6, 23.8, 23.3, 23.1. EI MS (m/z) 294 (M⁺), 91 (100).

¹H NMR and NOESY spectra of compound **8e** are depicted in Supplementary Figures S3 and S4. A description of the ¹H NMR spectrum can be found at page S6 of the Supplementary Material.

Milligram-scale optical resolution of (±)-**8e** by MPLC, using triacetylcellulose (TAC) as chiral stationary phase,³¹ afforded optical isomers (+)-**8e** and (-)-**8e**.

Compound (+)-8e: [α]²⁵ = +74.3 at $\lambda = 365$ nm (Hg lamp), (c 0.152 in abs EtOH). $t_R = 17.09$ min; [97% enantiomeric purity, as determined by analytical HPLC analysis on a Chiralcel (Chiralpak) AD-H column, using hexane/*i*-PrOH 9:1 as eluent, at 262 nm and a flow rate = 1.0 mL/min].

Compound (-)-8e: [α]²⁵ = -74.8 at $\lambda = 365$ nm (Hg lamp), (c 0.096 in abs EtOH). $t_R = 19.5$ min; [98% enantiomeric purity; Chiralcel (Chiralpak) AD-H column, hexane/*i*-PrOH 9:1 as eluent, at 262 nm and a flow rate = 1.0 mL/min].

***N*-(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]cyclopropanecarboxamide (8f).** This product was obtained starting from **6f** and using the above *N*¹-benzylation procedure described for compound **6n**. Flash chromatography: silica gel, cyclohexane/EtOAc 7:3 as eluent and crystallization. White solid, mp 158-9 °C (CH₂Cl₂-petroleum ether); 58% yield. ¹H NMR (200 MHz, CDCl₃) δ 0.62-0.71 (m, 2H), 0.83-0.93 (m, 2H), 1.02-1.15 (m, 1H), 1.92-2.01 (m, 2H), 2.72-2.97 (m, 2H), 3.33-3.39 (m, 2H), 3.49-3.56 (m, 1H), 4.54 (d, 1H $J = 17.3$ Hz), 4.66 (d, 1H $J = 17.3$ Hz), 5.62 (br s, 1H), 6.56 (d, 1H $J = 8.0$ Hz), 6.63 (ddd, 1H $J_1 = 1, J_2 = J_3 = 7.5$ Hz), 6.96-7.04 (m, 2H), 7.22-7.36 (m, 5H). ¹³C NMR (50 MHz, CDCl₃) δ 173.8, 144.5, 139.3, 129.1, 128.7, 127.2, 126.9, 126.6, 122.0, 116.5, 112.7, 57.5, 55.4, 41.7, 23.9, 23.3, 14.6, 7.1. ESI MS (m/z): 321 (M+H)⁺.

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4 ***N*-[(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]cyclobutanecarboxamide (8g).** This
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6 product was obtained starting from **6g** and using the above *N*^l-benzylation procedure described for
7
8 compound **6n**. Flash chromatography: silica gel, cyclohexane/EtOAc 7:3 as eluent and
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10 crystallization. Amorphous solid; 54% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.69-2.23 (m, 8H),
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12 2.70-2.92 (m, 3H), 3.32-3.38 (m, 2H), 3.48-3.55 (m, 1H), 4.53 (d, 1H *J*=17.2 Hz), 4.64 (d, 1H
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14 *J*=17.2 Hz), 5.38 (br s, 1H), 6.54 (d, 1H *J*=8.2 Hz), 6.96-7.04 (m, 2H), 7.23-7.36 (m, 5H arom). ¹³C
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16 NMR (50 MHz, CDCl₃) δ 175.1, 144.6, 139.2, 129.0, 128.7, 127.2, 126.9, 126.5, 122.0, 116.5,
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18 112.7, 57.5, 55.4, 41.5, 39.8, 25.2, 23.9, 23.4, 18.0. ESI MS (*m/z*): 335 (M+H)⁺.

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24 ***N*-[(1-Methyl-5-methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (8h).** This
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26 product was obtained starting from **6a** and using the above *N*^l-methylation procedure described for
27
28 compound **6l**. Flash chromatography: silica gel, EtOAc as eluent and crystallization. White solid,
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30 mp 139-40 °C (CH₂Cl₂-petroleum ether); 87% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.15 (t, 3H
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32 *J*=7.5 Hz), 1.84-1.89 (m, 2H), 2.21 (q, 2H *J*=7.5 Hz), 2.40-2.58 (m, 1H), 2.77-2.90 (m, 1H), 3.02 (s,
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34 3H), 3.20-3.50 (m, 3H), 3.80 (s, 3H), 5.63 (br s, 1H), 6.27-6.33 (m, 2H), 7.08 (dd, 1H *J*₁=*J*₂=8.0
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36 Hz). ¹³C NMR (50 MHz, CDCl₃) δ 174.1, 157.1, 145.8, 127.0, 109.5, 104.7, 98.8, 57.2, 55.4, 40.6,
37
38 39.1, 29.7, 22.7, 17.0, 9.8. ESI MS (*m/z*): 263 (M+H)⁺.

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44 ***N*-[(1-Methyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (8i).** This product was
45
46 obtained starting from **6d** and using the above *N*^l-methylation procedure described for compound
47
48 **6l**. Flash chromatography: silica gel, CH₂Cl₂/acetone 9:1 as eluent and crystallization. White solid,
49
50 mp 63-4 °C (Et₂O-petroleum ether); 95% yield. ¹H NMR (400 MHz, CD₃OD) δ 1.15 (t, 3H, *J*=7.5),
51
52 1.81 (dddd, 1H, *J*₁=4.5, *J*₂=5.0, *J*₃=*J*₄=13.0, H_{3B}), 1.96 (dddd, 1H, *J*₁=*J*₂=3.0, *J*₃=5.5, *J*₄=13.0, H_{3A}),
53
54 2.23 (q, 2H, *J*=7.5), 2.65 (ddd, 1H, *J*₁=3.0, *J*₂=5.0, *J*₃=16.5 Hz, H_{4B}), 2.86 (ddd, 1H, *J*=5.5, 13.0,
55
56 16.5 Hz, H_{4A}), 3.01 (s, 3H, N-CH₃), 3.19 (m, 1H, H_{10B}), 3.36-3.41 (m, 1H, H_{10A}), 3.43 (dddd, 1H,
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2 $J=3.0, 4.5, 5.0, 9$ Hz, H_2), 6.55 (ddd, 1H $J=1.0, 7.0, 7.5$ Hz, ArH), 6.56 (d, 1H, $J=8.5$, ArH), 6.92 (d,
3
4 1H, $J=7.0$, ArH), 7.02 (ddd, 1H, $J=1.0, 7.5, 8.5$, ArH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.1, 145.1,
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6 128.7, 127.3, 122.0, 116.0, 111.0, 57.6, 41.1, 38.3, 29.7, 23.9, 23.5, 9.8. EI MS (m/z): 232 (M^+), 146
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8 (100).
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10 1H NMR and NOESY spectra of compound **8i** are depicted in Supplementary Figures S6 and S7.
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15 ***N*-[(1-Phenethyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]acetamide (8j)**. A solution of **6e** (0.204
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17 g, 1 mmol), Et_3N (0.27 mL) and phenylethyl bromide (0.54 mL, 4 mmol) in dry toluene (5 mL) and
18
19 dry DMF (1 mL) was heated at 90 °C for 18 h. After cooling to room temperature the reaction
20
21 mixture was poured into water and extracted with EtOAc (3x); the combined organic phases were
22
23 washed with brine and dried (Na_2SO_4). After removing the solvent by distillation under reduced
24
25 pressure, the residue was purified by silica gel flash chromatography (EtOAc as eluent).
26
27 Amorphous solid; 68% yield. 1H NMR (200 MHz, $CDCl_3$) δ 1.63-1.85 (m, 2H), 1.96 (s, 3H), 2.63-
28
29 2.92 (m, 4H), 3.26-3.50 (m, 4H), 3.68-3.83 (m, 1H), 5.56 (br s, 1H), 6.65 (dd, 1H $J=7.0, 7.5$ Hz),
30
31 6.76 (d, 1H $J=8.0$ Hz), 7.01 (d, 1H $J=7.5$ Hz), 7.09-7.36 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$) δ
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33 170.3, 143.4, 139.4, 129.4, 128.8, 128.5, 127.3, 126.3, 122.1, 116.1, 111.8, 56.3, 52.7, 41.3, 33.3,
34
35 23.7, 23.3, 23.0. EI MS (m/z): 308 (M^+), 236 (100).
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42 ***N*-[(1-Propyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]acetamide (8k)**. This product was prepared
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44 according the above described procedure for **8j** starting from **6e** and using 1-iodopropane instead of
45
46 phenylethyl bromide as alkylating reagent. Flash chromatography: silica gel, EtOAc as eluent and
47
48 crystallization. White solid, mp 106-7 °C (CH_2Cl_2 -petroleum ether); 43% yield. 1H NMR (200
49
50 MHz, $CDCl_3$) δ 0.92 (t, 3H $J=7.5$ Hz), 1.54-1.66 (m, 2H), 1.75-1.91 (m, 2H), 1.99 (s, 3H), 2.69-
51
52 2.79 (m, 2H), 3.03-3.51 (m, 5H) 5.66 (br s, 1H), 6.56-6.64 (m, 2H), 6.98 (d, 1H $J=9.0$ Hz), 7.07
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54 (ddd, 1H $J=1.5, 8.5, 9.0$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.4, 144.0, 129.2, 127.1, 121.7,
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56 115.8, 112.0, 56.2, 53.0, 41.2, 23.6, 23.3, 22.9, 20.4, 11.4. EI MS (m/z): 246 (M^+), 174 (100).
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***N*-{[1-(2-Methylamino)-2-oxoethyl]-1,2,3,4-tetrahydroquinolin-2-yl}methyl}propionamide (8l).**

2-Chloro-*N*-methylacetamide³² (0.162 g, 1.5 mmol) was added to a solution of **6d** (0.218 g, 1 mmol) and Et₃N (0.21 mL, 1.5 mmol) in dry DMF (2 mL) and the resulting mixture was stirred under nitrogen at 100 °C for 16 h. The reaction mixture was poured into water and extracted with EtOAc. The organic phases were combined, washed with brine, dried (Na₂SO₄) and the solvent evaporated under reduced pressure to give a crude product which was purified by flash chromatography (cyclohexane/acetone 1:1 as eluent) and crystallization. Beige solid, mp 168-70 °C (EtOAc-petroleum ether); 26% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.14 (t, 3H *J*=7.5 Hz), 1.59-2.02 (m, 2H), 2.21 (q, 2H *J*=7.5 Hz), 2.78-2.90 (m, 5H), 3.35-3.48 (m, 3H), 3.89 (d, 1H *J*=17.0 Hz), 3.92 (d, 1H *J*=17.0 Hz), 6.21 (br s, 1H), 6.32 (br s, 1H), 6.45 (d, 1H (*J*=8.0 Hz)), 6.74 (dd, (d, 1H *J*₁≈*J*₂=7.0 Hz), 7.02-7.12 (m, 2H). ESI MS (*m/z*): 290 (M+H)⁺.

1,2,3,4-Tetrahydroquinoline-2-carbonitrile²² (9). NaCNBH₃ (0.283 g, 4.5 mmol) was added portionwise under nitrogen and during 5 min to a solution of quinolin-2-carbonitrile (1 mmol) in AcOH (6 mL) and the resulting mixture was stirred at 40 °C for 16 h. The reaction mixture was quenched with water, adjusted to pH 11 by adding 30% NaOH, and the aqueous phase was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄) and evaporated under reduced pressure to afford a crude residue which was purified by silica gel flash chromatography (cyclohexane/EtOAc 8:2 as eluent). Oil; 48% yield. ¹H NMR (200 MHz, CDCl₃) δ 2.17-2.29 (m, 2H), 2.77-2.88 (m, 1H), 3.03-3.20 (m, 1H), 4.16 (br s, 1H), 4.42-4.46 (m, 1H), 6.56 (dd, 1H *J*=1.0 and 8.0 Hz), 6.77 (ddd, 1H *J*=1.0, 7.0 and 7.5 Hz), 7.02-7.09 (m, 2H). EI MS (*m/z*): 158 (M⁺) 130 (100).

1-Phenyl-1,2,3,4-tetrahydroquinoline-2-carbonitrile (10). Cesium fluoride (0.22 g, 1.44 mmol) was added to a solution of **9** (0.16 g, 1 mmol) and 2-(trimethylsilyl)phenyl trifluoromethanesulfonate

1 (0.125 mL, 0.84 mmol) in CH₃CN (13 mL) and the resulting mixture was stirred at room
2 temperature for 16 h. The reaction mixture was quenched with water and then extracted with EtOAc
3 (3x). The combined organic phases were dried over Na₂SO₄ and evaporated under reduced pressure
4 to give a crude oily residue which was purified by silica gel flash chromatography
5 (cyclohexane/EtOAc 9:1 as eluent). Oil; 55% yield. ¹H NMR (200 MHz, CDCl₃) δ 2.21-2.38 (m,
6 2H), 2.77-2.82 (m, 1H), 3.07-3.31 (m, 1H), 4.54 (m, 1H), 6.38 (d, 1H *J*=8.0 Hz), 6.69 (*J*₁≈*J*₂=7.5
7 Hz), 6.81-7.39 (m, 7H). ESI MS (*m/z*): 235 (M+H)⁺.
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20 ***N*-[(1-Phenyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (11)**. A solution of **10**
21 (0.234 g, 1 mmol) in THF (5 mL) and propionic anhydride (1.2 mL, 9 mmol) was hydrogenated (4
22 atm) over Raney nickel for 5 h at 60 °C. The catalyst was filtered on Celite, the filtrate was
23 concentrated *in vacuo*, and the residue partitioned between EtOAc and water. The organic phase
24 was washed with brine, dried (Na₂SO₄) and evaporated under reduced pressure to give the crude
25 desired product which was purified by crystallization. Beige solid, mp 86-7 °C (Et₂O-petroleum
26 ether); 69% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.14 (t, 3H, *J*=7.5 Hz), 1.93-2.17 (m, 2H), 2.25 (q,
27 2H, *J*=7.5 Hz), 2.82-2.90 (m, 2H), 3.19-3.32 (m, 1H), 3.47-3.61 (m, 1H), 3.92-4.03 (m, 1H), 5.66
28 (br s, 1H), 6.71-6.83 (m, 2H), 6.93-7.02 (m, 1H), 7.06-7.37 (m, 6H). ¹³C NMR (50 MHz, CDCl₃) δ
29 173.9, 148.6, 142.8, 129.6, 129.5, 126.5, 124.9, 124.7, 123.9, 119.5, 118.8, 58.5, 41.2, 29.7, 23.7,
30 23.5, 9.8. EI MS (*m/z*) 294 (M⁺), 208 (100).
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46 **1-Methyl-1,2,3,4-tetrahydroquinoline-2-carbonitrile**²³ (**12**). Sodium cyanoborohydride (0.08 g,
47 1.3 mmol) and a 37% aqueous solution of HCHO (0.5 mL) were added to a solution of **9** (0.5
48 mmol), in MeOH (3 mL) and AcOH (to pH = 5), and the resulting mixture was stirred at room
49 temperature for 16 h. An aqueous solution of 30% NaOH was added, and the aqueous phase was
50 extracted with EtOAc. After drying over Na₂SO₄, the combined organic layers were concentrated
51 by distillation under reduced pressure to give a crude residue which was purified by filtration on
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1 silica gel (cyclohexane/EtOAc 8:2 as eluent). Oil; 58% yield. ^1H NMR (200 MHz, CDCl_3) δ 2.25-
2 2.35 (m, 2H), 2.79-2.89 (m, 1H), 3.01 (s, 3H), 3.07-3.25 (m, 1H), 4.29-4.33 (m, 1H), 6.70-6.83 (m,
3 2H), 6.71 (d, 1H, $J=7.5$ Hz) 7.16 (dd, 1H, $J=7.5$ and 8.0 Hz). EI MS (m/z): 172 (M^+ , 100).
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10 **1-Ethyl-3-[(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]thiourea (13)**. A solution of **12**²³ (1
11 mmol) in THF (5 mL) and 2M NH_3 in EtOH (0.6 mL) was hydrogenated over Raney nickel (4 atm)
12 for 2 h at room temperature. The catalyst was filtered on Celite, the filtrate was concentrated *in*
13 *vacuo*, and the residue partitioned between EtOAc and water. The organic phase was washed with
14 brine, dried (Na_2SO_4) and evaporated under reduced pressure to give the corresponding crude (1-
15 methyl-1,2,3,4-tetrahydroquinolin-2-yl)methanamine which was used without any further
16 purification. Ethyl isothiocyanate (1 mmol) was added to a cold solution of the crude (1-methyl-
17 1,2,3,4-tetrahydroquinolin-2-yl)methanamine in CH_2Cl_2 (6 mL) and the resulting mixture was
18 stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure, and the
19 residue was purified by silica gel flash chromatography (cyclohexane/EtOAc 7:3 as eluent) and
20 crystallization. White solid, mp 94-5 °C (Et_2O -petroleum ether); 52% yield. ^1H NMR (200 MHz,
21 CDCl_3) δ 1.17 (t, 3H $J=7.5$ Hz), 1.90-1.95 (m, 2H), 2.73-2.79 (m, 2H), 3.04 (s, 3H), 3.18-3.37 (m,
22 2H), 3.51-3.68 (m, 3H), 5.84 (br s, 1H), 5.98 (br s, 1H), 6.63-6.71 (m, 2H), 7.01 (d, 1H $J=7.0$ Hz),
23 7.13 (dd, 1H $J_2=7.5$ and 8.0 Hz). ^{13}C NMR (50 MHz, CDCl_3) δ 181.8, 144.9, 128.9, 127.4, 122.3,
24 116.7, 111.9, 57.5, 46.5, 38.8, 36.3, 24.0, 23.7, 14.0. ESI MS (m/z): 264 ($\text{M}+\text{H}^+$).
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46 ***N*-[2-(1,2,3,4-Tetrahydroquinolin-2-yl)ethyl]propionamide (14)**. A solution of (1,2,3,4-
47 tetrahydroquinolin-2-yl)acetonitrile²⁴ (1 mmol) in THF (5 mL) and 2M NH_3 in EtOH (0.6 mL) was
48 hydrogenated over Raney nickel (4 atm) for 6 h at 60 °C. The catalyst was filtered on Celite, the
49 filtrate was concentrated *in vacuo*, and the residue partitioned between EtOAc and water. The
50 organic phase was washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to
51 give the corresponding crude oily amine which was used without any further purification.
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1 Et₃N (0.14 mL) and propionic anhydride (1 mmol) were added to a cold solution of the above crude
2 amine in THF (6 mL) and the resulting mixture was stirred at room temperature for 1 h. The solvent
3 was evaporated under reduced pressure, the residue was taken up in EtOAc, washed with a
4 saturated aqueous solution of NaHCO₃ and with brine. After drying over Na₂SO₄, the solvent was
5 removed by distillation *in vacuo* to give a crude product that was purified by flash chromatography:
6 EtOAc as eluent. Oil; 57% yield. ¹H NMR (200 MHz, CDCl₃) δ 1.20 (t, 3H *J*=7.5 Hz), 1.59-1.78
7 (m, 3H), 1.92-2.01 (m, 1H), 2.22 (q, 2H *J*=7.5 Hz), 2.68-2.82 (m, 2H), 3.20-3.39 (m, 2H), 3.52-3.69
8 (m, 1H), 5.72 (br s, 1H), 6.53-6.66 (m, 2H), 6.94-7.02 (m, 2H). EI MS (*m/z*): 232 (M⁺), 132 (100).

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22 ***N*-[2-(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)ethyl]propionamide (15)**. This product was
23 obtained starting from **14** and using the previous *N*¹-benzylation procedure described for compound
24 **6n**. The crude product was purified by silica gel flash chromatography: cyclohexane/EtOAc 3:7 as
25 eluent. Beige solid, mp 66-7 °C (Et₂O-petroleum ether); 70% yield. ¹H NMR (200 MHz, CDCl₃) δ
26 1.10 (t, 3H *J*=7.5 Hz), 1.61-2.02 (m, 4H), 2.09 (q, 2H *J*=7.5 Hz), 2.71-2.98 (m, 2H), 3.20-3.42 (m,
27 3H), 4.42 (d, 1H, *J*=16.5 Hz), 4.53 (d, 1H, *J*=16.5 Hz), 5.21 (br s, 1H), 6.55-6.68 (m, 2H), 6.98-7.05
28 (m, 2H), 7.23-7.39 (m, 5H). ¹³C NMR (50 MHz, CDCl₃) δ 173.8, 144.6, 139.2, 129.2, 128.6, 127.1,
29 127.0, 127.0, 122.2, 116.6, 113.4, 55.4, 54.8, 36.5, 31.8, 29.6, 24.1, 23.5, 9.9. EI MS (*m/z*): 322
30 (M⁺), 91 (100).

31 32 33 34 35 36 37 38 39 40 41 42 43 44 **Optical resolution of compound (±)-8e**

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46 Racemic (±)-**8e** was separated into its constituent enantiomers using a semi-preparative column
47 having triacetylcellulose (TAC) as a chiral stationary phase. A Büchi (Switzerland) borosilicate
48 column (length: 40 cm; internal diameter: 3.0 cm) was filled with a slurry of 100 g of TAC in
49 EtOH/H₂O 7:3 and packed following a previously described procedure.³¹ Solvent delivery system:
50 Gilson 307 pump; mobile phase: EtOH/H₂O 7:3; flow rate: 1 mL/min; sample concentration: 1.7%
51 (w/v in EtOH). Ultraviolet detection was achieved at 262 nm using a Waters Lamba-Max Model
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2 480 tunable absorbance detector. Fractions were collected using a Gilson FC 203B fraction
3 collector (12 min/tube). The chromatograms were registered with a Linseis recorder (05.80 L)
4 (range AUFS 2.0 x 20 mV).
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8 The enantiomeric purity of chromatographic fractions was determined by analytical HPLC analysis
9 on a Chiralcel (Chiralpak) AD-H column, using hexane/*i*-PrOH 9:1 as eluent, at 262 nm and a flow
10 rate = 1.0 mL/min. The chromatographic fractions showing a single enantiomer ($\lambda = 262$ nm: $t_{R1} =$
11 17.09 min; $t_{R2} = 19.5$ min) were collected, the others recombined and recycled through the TAC
12 column. Repeating the procedure just described several times, it was possible to obtain an enriched
13 fraction (ee >97%) for both enantiomers. The enantiomer enriched fractions were collected,
14 evaporated under reduced pressure, and further purified by flash chromatography (silica gel, EtOAc
15 as eluent) and crystallization (Et₂O-petroleum ether).
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19 Optical rotation analysis was performed using a Perkin-Elmer 241 polarimeter using a Hg lamp
20 ($\lambda = 365$ nm). α values were determined at 25 °C and are reported in 10⁻¹ deg cm² g⁻¹; concentration
21 (*c*) is in g per 100 mL. Enantiomeric purity was determined by HPLC on the following apparatus:
22 Shimadzu LC-10AT (liquid chromatograph), Shimadzu SPD-10A (UV detector), Shimadzu C-R6A
23 Chromatopac, using Chiralcel AD-H as column.
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39 **Pharmacology**

40 **Reagents and Chemicals**

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42 2-[¹²⁵I]-Iodomelatonin (2200 Ci/mmol) was purchased from NEN (Boston, MA). Other drugs and
43 chemicals were purchased from Sigma-Aldrich (Saint Quentin, France).
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50 **Cell Culture**

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52 CHO cell lines stably expressing the human melatonin MT₁ or MT₂ receptors were grown in
53 DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/mL penicillin
54 and 100 µg/ml streptomycin. Grown at confluence at 37 °C (95% O₂/5% CO₂), they were harvested
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1 in PBS containing EDTA 2 mM and centrifuged at 1000 g for 5 min (4 °C). The resulting pellet was
2
3 suspended in TRIS 5 mM (pH 7.5), containing EDTA 2 mM and homogenized using a Kinematica
4
5 polytron. The homogenate was then centrifuged (95000 g, 30 min, 4 °C) and the resulting pellet
6
7 suspended in 75 mM TRIS (pH 7.5), 12.5 mM MgCl₂ and 2 mM EDTA. Aliquots of membrane
8
9 preparations were stored at -80 °C until use.
10
11

12 13 14 15 **Binding Assays**

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17 2-[¹²⁵I]iodomelatonin binding assay conditions were essentially as previously described.³³ Briefly,
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19 binding was initiated by addition of membrane preparations from stable transfected CHO cells
20
21 diluted in binding buffer (50 mM Tris-HCl buffer, pH 7.4 containing 5 mM MgCl₂) to 2-[¹²⁵I]-
22
23 iodomelatonin (20 pM for MT₁ and MT₂ receptors) and the tested drug. Nonspecific binding was
24
25 defined in the presence of 1 μM melatonin. After 120 min incubation at 37 °C, reaction was stopped
26
27 by rapid filtration through GF/B filters presoaked in 0.5% (v/v) polyethylenimine. Filters were
28
29 washed three times with 1 mL of ice-cold 50 mM Tris-HCl buffer, pH 7.4.
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33 Data from the dose-response curves (7 concentrations in duplicate) were analyzed using the
34
35 program PRISM (Graph Pad Software Inc., San Diego, CA) to yield IC₅₀ (inhibitory concentration
36
37 50). Results are expressed as $K_i = IC_{50} / (1 + ([L]/K_D))$, where [L] is the concentration of radioligand
38
39 used in the assay and K_D, the dissociation constant of the radioligand characterizing the membrane
40
41 preparation.³⁴
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44
45

46 **Functional Assays:**

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48 [³⁵S]GTPγS binding assay was performed according to published methodology.³³ Briefly,
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50 membranes from transfected CHO cells expressing MT₁ or MT₂ receptor subtype and compounds
51
52 were diluted in binding buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 3 μM GDP, 3 mM MgCl₂,
53
54 and 20 μg/mL saponin). Incubation was started by the addition of 0.2 nM [³⁵S]GTPγS to
55
56 membranes (20 μg/mL) and drugs, and further followed for 1 h at room temperature. Nonspecific
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1 binding was defined using cold GTP γ S (10 μ M). Reaction was stopped by rapid filtration through
2 GF/B filters followed by three successive washes with ice cold buffer.
3

4 Usual levels of [35 S]GTP γ S binding (expressed in dpm) were for CHO-MT $_1$ or MT $_2$ membranes:
5
6 2000 for basal activity, 8000 in the presence of melatonin 1 μ M, and 180 in the presence of GTP γ S
7
8 10 μ M which defined the nonspecific binding. Data from the dose-response curves (7
9
10 concentrations in duplicate) were analyzed by using the program PRISM (Graph Pad Software Inc.,
11
12 San Diego, CA) to yield EC $_{50}$ (Effective concentration 50 %) and Emax (maximal effect) for
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14 agonists.
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20 21 22 **Molecular modeling**

23
24 Compounds **8e** and **6d** were built in Maestro 9.6³⁵ and minimized with Macromodel 10.2³⁶ applying
25
26 the OPLS2005 force field³⁷ and the GB/SA continuum solvation model for water³⁸ to an energy
27
28 gradient of 0.05 kJ mol $^{-1}$ \AA^{-1} . The minimized structures were solvated with explicit TIP3P water or
29
30 methanol molecules with Desmond 3.6,³⁹ placing simulation box boundaries 10 \AA far from ligand
31
32 atoms on each side. The resulting systems were first equilibrated applying the default relaxation
33
34 protocol implemented in Desmond 3.6 and then submitted to standard plain molecular dynamics
35
36 (MD) or well-tempered metadynamics simulations applying the Langevin coupling scheme⁴⁰ and
37
38 setting temperature and pressure at 298 K and 1 atm, respectively. Plain MD simulation was carried
39
40 out for 1 μ s, collecting one snapshot every 10 ps. For metadynamics simulations, dihedral angles τ_1
41
42 and τ_2 (τ_1 : N1-C2-C3-C4; τ_2 : C8a-N1-Cbn-Car, see legend of Figure 3) were chosen as the
43
44 collective variables for compound **8e**, while for compound **6d** only τ_1 was considered. The initial
45
46 height and width of the Gaussian potentials were set to 0.03 kcal mol $^{-1}$ and 2 degrees, respectively,
47
48 while the time interval between subsequent potentials was set to 0.09 ps.
49
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52 The convergence of well-tempered metadynamics simulations was assessed by evaluating the time
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54 evolution of the relative free-energy levels of the free-energy minima (see Supplementary Figures
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56 S9b and S10b). To this aim, well-tempered metadynamics were run for 50 ns, free-energy profiles
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1 were reconstructed after every ns and the simulation was considered to be converged when free-
2 energy differences among global and local minima became approximately constant. For both
3 energy differences among global and local minima became approximately constant. For both
4 energy differences among global and local minima became approximately constant. For both
5 energy differences among global and local minima became approximately constant. For both
6 compounds **8e** and **6d** metadynamics simulations converged approximately after 20 ns. Therefore,
7 only the free-energy profiles obtained at 20 ns for compounds **8e** and **6d** are reported and discussed.
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9 The maximum free-energy values were truncated at 4 kcal/mol.
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17 **Supporting Information**

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19 ¹H NMR spectra of compounds **8e**, **8i** and **6d**, NOESY spectra of compounds **8e** and **8i**, free-energy
20 surfaces obtained from plain molecular dynamics and metadynamics simulations of compounds **8e**
21 and **6d**, purity of target compounds.
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55 **Abbreviations used**

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2 DMEM, Dulbecco's modified eagle medium; Emax, maximum activation of receptor; GTP γ S,
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4 guanosine 5'-O-(3-thiotriphosphate); MD, molecular dynamics; MPLC, medium-pressure liquid
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6 chromatography; MT₁, melatonin receptor subtype 1; MT₂, melatonin receptor subtype 2; NOE,
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8 nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; PBS, phosphate-
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10 buffered saline; TAC, triacetylcellulose; THF, tetrahydrofuran.
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