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Highly potent and selective MT_2 melatonin receptor full agonists from conformational analysis of 1-benzyl-2-acylaminomethyl-tetrahydroquinolines.

Gilberto Spadoni,[a] Annalida Bedini,[a] Simone Lucarini,[a] Michele Mari,[a] Daniel-Henri Caignard,[b] Jean A. Boutin,[b] Philippe Delagrange,[b] Valeria Lucini,[c] Francesco Scaglione,[c] Alessio Lodola,[d] Franca Zanardi,[d] Daniele Pala,[d] Marco Mor,[d] Silvia Rivara[d,*]

[a] Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino "Carlo Bo", Piazza Rinascimento 6, I-61029 Urbino, Italy

[b] Institut de Recherches Servier, 125 Chemin de Ronde, F-78290 Croissy sur Seine, France

[c] Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Via Vanvitelli 32, I-20129 Milano, Italy

[d] Dipartimento di Farmacia, Università degli Studi di Parma, Parco Area delle Scienze 27/A, I-43124 Parma, Italy

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 MT_1 and MT_2 receptors. Structural optimization resulted in *N*-[(1-benzyl-1,2,3,4-tetrahydro-5-methoxyquinolin-2-yl)methyl]propionamide (UCM1014), with picomolar MT_2 binding affinity (K_i = 0.001 nM), more than 10,000-fold selectivity over the MT_1 receptor and a full agonist profile (GTPγS test), being the most potent MT_2-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 MT_3 binding site.[5] In mammals, melatonin activates the MT_1 and MT_2 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, MT_1/MT_2 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 MT_1 or MT_2 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 MT_1 and MT_2 receptors, that, even if far from being complete, has highlighted some differential roles for the two subtypes. Indeed, the MT_1 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 MT_2 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 MT_1/MT_2 nonselective agonists, while bulkier substituents led to selectivity for the MT_2 receptor and to limited intrinsic activity, moving toward a partial agonist or an antagonist behavior. Selectivity for the MT_1 receptor could be obtained replacing the methoxy group with a lipophilic arylalkyloxy chain (e.g., a phenylbutyloxy substituent).[15]

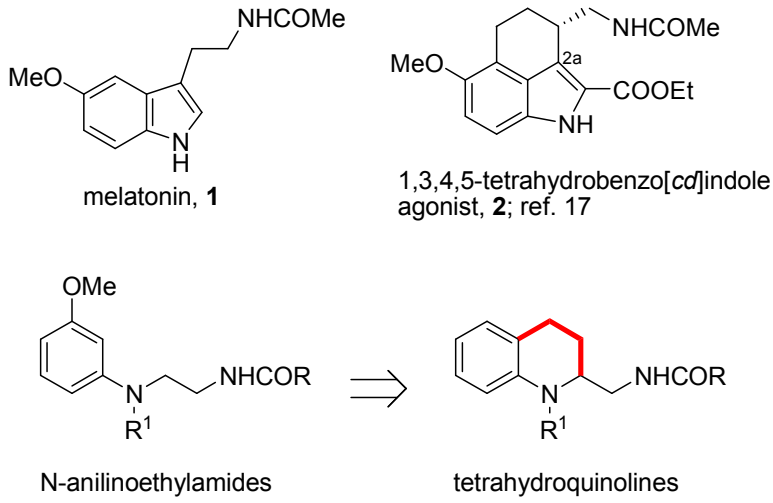


Figure 1. Structures of melatonin (**1**) and tetrahydrobenzo[*cd*]indole agonist **2**; general formulae of *N*-anilinoethylamides and of conformationally constrained tetrahydroquinoline compounds.

Starting from the structure of *N*-anilinoethylamides, characterized by high conformational freedom, we designed a novel series of conformationally-constrained MT_2-selective agonists. Among the different possible ring closures, inclusion of the carbon atom bound to the aniline nitrogen into a

six-membered ring gives tetrahydroquinoline derivatives carrying an acylaminomethyl side chain in position 2 (Figure 1, bottom). Indeed, preliminary molecular modeling studies suggested that 2-acylaminomethyltetrahydroquinolines could reproduce the putative bioactive conformation of melatonin, which had been previously identified and validated by means of pharmacophore models and synthesis of potent conformationally-constrained compounds.[16,17] In particular, superposition of melatonin and the tricyclic 1,3,4,5-tetrahydrobenzo[*cd*]indole agonist **2** (Figure 1) pointed out that the two fused six-membered rings could represent a suitable scaffold, in which carbon 2a of compound **2** can be replaced with the tetrahydroquinoline nitrogen (Figure 2). Insertion of suitable substituents on the tetrahydroquinoline scaffold could in principle allow the modulation of binding affinity, receptor subtype selectivity and intrinsic activity toward the desired profile by fulfilling the structural requirements outlined by structure-activity relationships (SARs) and 3D-QSAR studies.[18] It is well recognized that the presence of the methoxy group in position 5 of the indole ring of melatonin increases MT_1 and MT_2 binding affinity and intrinsic activity, and insertion of a substituent in position 2 (e.g., a phenyl ring, a iodine atom) increases binding affinity. On the other hand, a substituent in position 1 or 2 occupying a region of space located outside the plane defined by the indole ring leads to MT_2-selectivity and to an antagonist behavior.

Herein we report the synthesis and evaluation of the binding affinity and intrinsic activity at human MT_1 and MT_2 receptors of 2-acylaminomethyltetrahydroquinoline derivatives. We analyzed their SARs focusing the attention on the role of substituents on the nitrogen atom, of the methoxy group and on the importance of side chain length and terminal acyl group. Moreover, stereoselectivity is also discussed in light of the ability of enantiomers to reproduce the active conformation of melatonin.

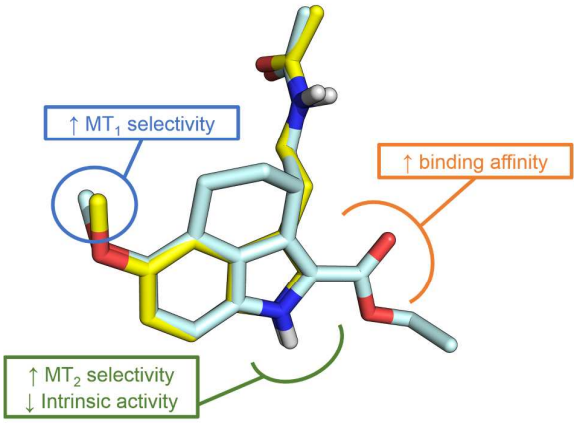


Figure 2. Superposition of compound **2** (white carbons) to the putative bioactive conformation of melatonin (**1**, yellow carbons) and effect of substituents on MT_1 and MT_2 binding affinity and intrinsic activity.

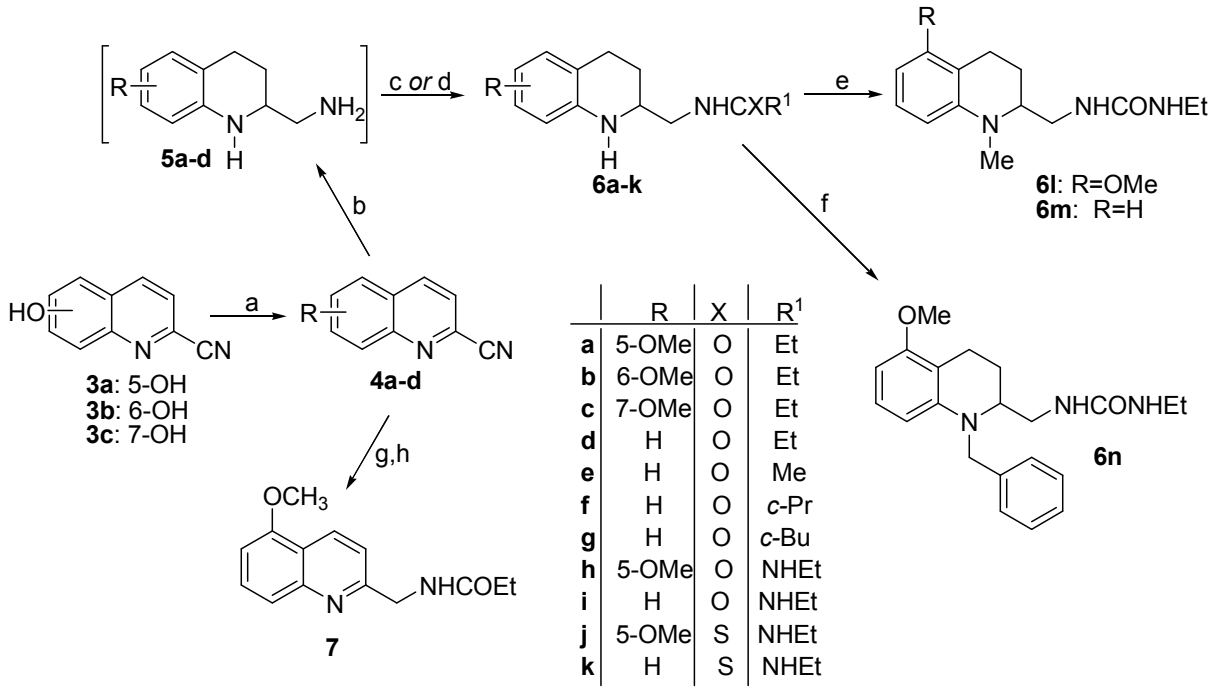
Chemistry

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-cyanotetrahydroquinoline[22] **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-carbonitrile[23] **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)acetonitrile[24] 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).

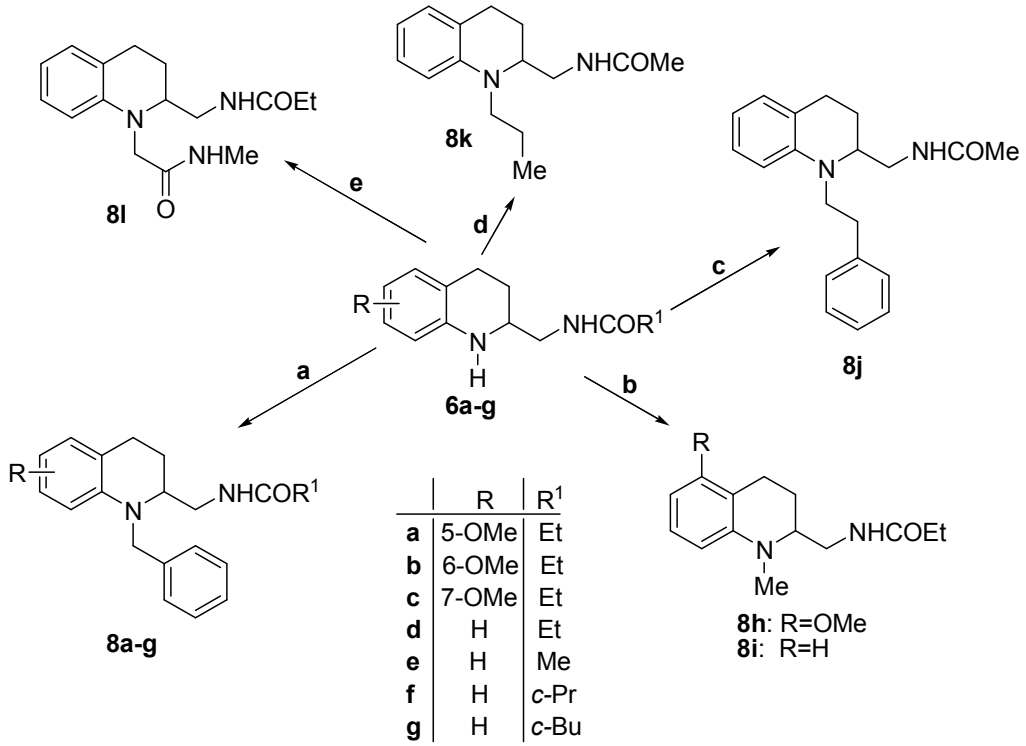
Scheme 1[a]



	R	X	R^1
a	5-OMe	O	Et
b	6-OMe	O	Et
c	7-OMe	O	Et
d	H	O	Et
e	H	O	Me
f	H	O	*c*-Pr
g	H	O	*c*-Bu
h	5-OMe	O	NHEt
i	H	O	NHEt
j	5-OMe	S	NHEt
k	H	S	NHEt

[a]*Reagents and conditions*: (a) CH_3I, K_2CO_3, acetone, rt, 15 h, yield 74-95%; (b) H_2 (4 atm), Raney-Ni, 2M NH_3 in EtOH, THF, 60 °C, 16 h; (c) anhydride or acyl chloride, Et_3N, THF, rt, 1 h, two steps (b and c) yield 34-73%; (d) ethyl isocyanate or ethyl isothiocyanate, CH_2Cl_2, rt, 30 min, two steps (b and d) yield 49-65%; (e) CH_3I, $NaHCO_3$, MeOH, 50 °C, 24 h, yield 77%; (f) benzyl bromide, Et_3N, toluene, reflux, 2 h, yield 88%; (g) H_2 (3.5 atm), Raney-Ni, 2N NH_3 in MeOH, rt, 30 min; (h) propionic anhydride, Et_3N, THF, rt, 1 h, two steps yield 48%.

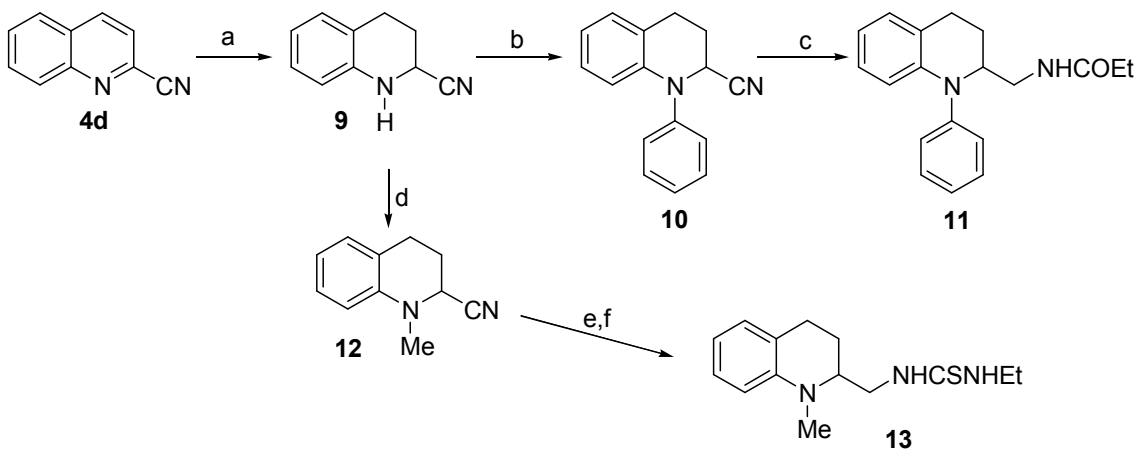
Scheme 2[a]



	R	R^1
a	5-OMe	Et
b	6-OMe	Et
c	7-OMe	Et
d	H	Et
e	H	Me
f	H	*c*-Pr
g	H	*c*-Bu

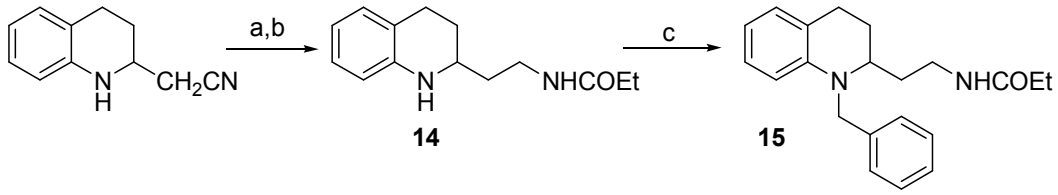
[a]*Reagents and conditions*: (a) benzyl bromide, Et_3N , toluene, reflux, 2 h, yield 54-92%; (b) CH_3I, $NaHCO_3$, MeOH, 50 °C, 24 h, yield 87-95%; (c) phenylethyl bromide, Et_3N, toluene/DMF, 90 °C, 18 h, yield 68%; (d) 1-iodopropane, Et_3N, toluene/DMF, 90 °C, 18 h, yield 43%; (e) 2-chloro-*N*-methylacetamide, Et_3N, DMF, 100 °C, 16 h, yield 26%.

Scheme 3[a]



[a]*Reagents and conditions*: (a) $NaCNBH_3$, AcOH, 40 °C, 16 h, yield 48%; (b) 2-(trimethylsilyl)phenyl triflate, CsF, CH_3CN, rt, 16 h, yield 55%; (c) H_2 (4 atm), Raney-Ni, propionic anhydride, THF, 60 °C, 5 h, yield 69%; (d) $NaCNBH_3$, 37% HCHO, MeOH/AcOH, rt, 16 h; (e) H_2 (4 atm), Raney-Ni, 2M NH_3 in EtOH, THF, rt, 24 h; (f) ethyl isothiocyanate, CH_2Cl_2, rt, 16 h, two steps yield 52%.

Scheme 4[a]

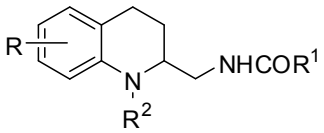


[a]*Reagents and conditions*: (a) H_2 (4 atm), Raney-Ni, 2M NH_3 in EtOH, THF, 60 °C, 6 h; (b) propionic anhydride, Et_3N, THF, rt, 1 h, two steps yield 57%; (c) benzyl bromide, Et_3N, toluene, reflux, 2 h, yield 70%.

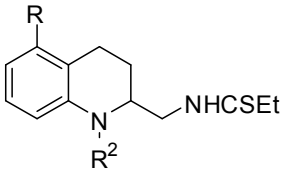
Results and discussion

The newly synthesized tetrahydroquinoline derivatives were evaluated for their binding affinity and intrinsic activity at human MT_1 and MT_2 receptors stably transfected in Chinese Hamster Ovarian (CHO) cells using 2-[^{125}I]iodomelatonin as radioligand, and the results are reported in Table 1.

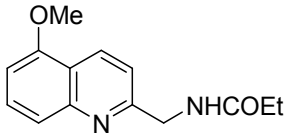
Table 1. Binding Affinity and Intrinsic Activity of Newly Synthesized Tetrahydroquinoline Derivatives for Human MT_1 and MT_2 Melatonin Receptors.[a]



6a, **6d**, **6h-n**, **8a-l**, **11**



6j-k, **13**



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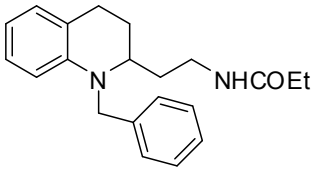


15

				MT_1			MT_2		
Compd.[b]	**R**[1]	**R**[2]	**R**	K_i **(nM)**	**Emax (%)**	EC_{50} **(nM)**	K_i **(nM)**	**Emax (%)**	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-OMe	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-OMe	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-OMe	>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-OMe	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-OMe	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-OMe	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-OMe	186 [106;324]	nd	nd	1.5 [0.8;2.9]	75±7	4.6 [3.4;6.4]
8c	Et	Bn	7-OMe	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	*c*Pr	Bn	H	291 [168;505]	nd	nd	0. 2 [0.2;0.3]	38±4	0.3 [0.1;0.8]
8g	*c*Bu	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	$CH_2CONHMe$	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 MT_1 and MT_2 binding affinities in the same range as melatonin, with an MT_2 full agonist behavior, while at the MT_1 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 MT_1 and MT_2 binding affinity and a decreased ability to stimulate the receptors, with lower Emax and EC_{50} 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 MT_1 binding affinity. On the contrary, these substituents

led to an increase in MT_2 binding affinity compared to the methyl derivative **8i**, with limited reduction of intrinsic activity (Emax). The benzyl derivative **8d** has more than 400-fold selectivity for the MT_2 receptor and acts as a potent MT_2 partial agonist. These data further support that the behavior of tetrahydroquinolines is consistent with SARs obtained from classical melatonin receptor ligands, given the MT_2-selectivity and reduced intrinsic activity provided by the benzyl substituent.[18,25] Elongation of the side chain in position 2, with insertion of an ethylene spacer between the amide group and the tetrahydroquinoline ring (**15**), hampered the proper accommodation of the compound at the receptor binding site leading to reduced potency. Insertion of a hydrophilic acetamide substituent on the nitrogen atom (**8l**) was even more detrimental, abolishing any binding to the MT_1 receptor and severely reducing the affinity for the MT_2 receptor.

To evaluate the conformational equilibria of the most interesting *N*-benzyltetrahydroquinoline derivatives, molecular dynamics (MD) simulations were set up for compound **8e**, the analog of compound **8d** having a shorter acetyl side chain instead of a propionyl, and similar binding affinity and intrinsic activity. A 50 ns long well-tempered metadynamics simulation of compound **8e** solvated into a water box was performed, setting as collective variables the dihedral angles τ1 and τ2 (Figure 3A), which describe the arrangement of the amide side chain and of the benzyl substituent, respectively. Compound **8e** has a stereogenic center and the free-energy profiles here presented refer to the *R* enantiomer (the same results, with opposite values of dihedral angles, can be obtained for the *S* enantiomer). The well-tempered metadynamics simulation reached convergence after 20 ns (see Experimental Section for details). According to the free-energy surface (FES) depicted in Figure 3B, the amide side chain (τ1) can assume an axial (~ -50°) or an equatorial arrangement (~ 50°), with lower energies associated to the former one. The benzyl substituent has a different conformational freedom depending on the arrangement of the amide side chain. With the amide side chain in equatorial arrangement, the benzyl substituent adopts two prevalent conformations, above (τ2 ~ -50°) or beneath (~100°) the plane defined by the tetrahydroquinoline ring. On the contrary, it has higher conformational freedom with the amide side chain in axial

arrangement. Indeed, the benzyl group explores almost all the possible conformations with similar free-energy contents, with the exception of τ2 ~ 0°, 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 μs, the amide side chain almost exclusively adopted an axial arrangement (τ1 ~ -50°), with only about 4.5% of equatorial conformations. The benzyl substituent spent most of the time perpendicular to the plane of the tetrahydroquinoline ring (τ2 ≈ -70°, 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 μs simulation of plain MD, which proposes the first as a convenient approach to explore conformational equilibria of bioactive compounds.

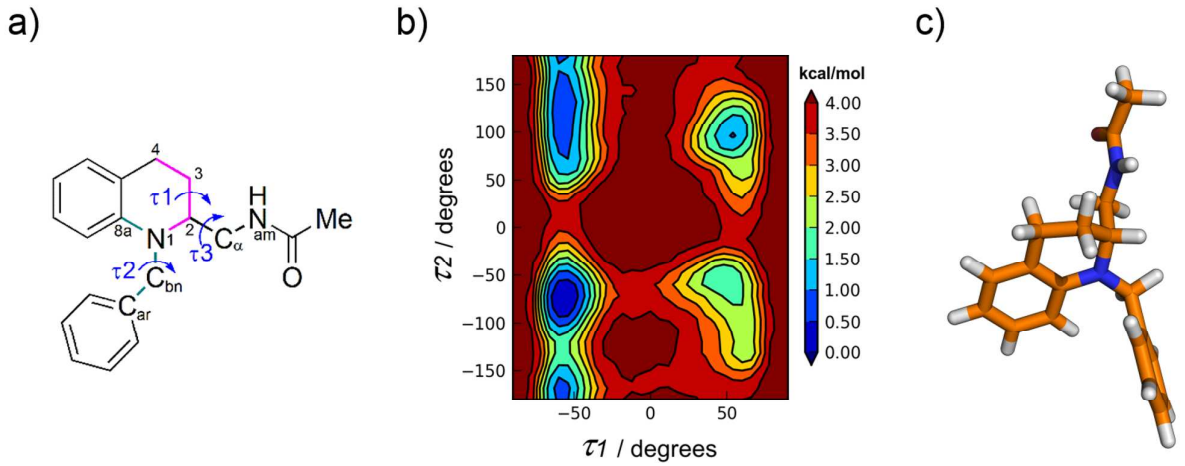


Figure 3. a) Representation of τ1 (N_1-C_2-C_3-C_4, pink bonds), τ2 (C_{8a}-N_1-C_{bn}-C_{ar}, green bonds) and τ3 (N_1-C_2-C_α-N_{am}) dihedral angles. b) Free-energy surface for compound **8e** obtained after 20 ns of

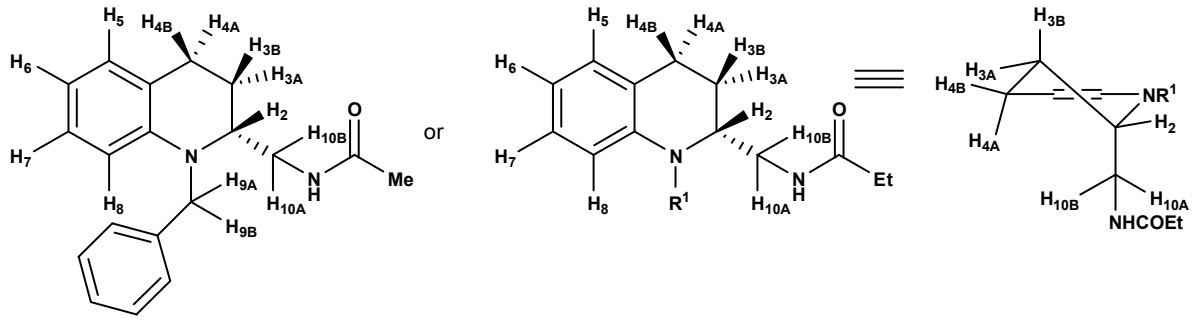
well-tempered metadynamics simulation. c) Representation of one of the conformations of compound **8e** corresponding to the free-energy minimum ($\tau1$ = -57.7°, $\tau2$ = -71.1°).

To evaluate the reliability of the metadynamics simulation, we also performed a well-tempered metadynamics of compound **8e** in methanol and we compared the results with the information obtained from NMR experiments in CD_3OD. The FES obtained after 20 ns of metadynamics simulation in methanol is qualitatively the same as that obtained with water solvation (Supplementary Figure S2). Analysis of 1D and 2D NMR data for compound **8e** in CD_3OD revealed a high prevalence of half-chair conformation for the tetrahydroquinoline ring wherein the C2 amide side chain is axially arranged (Table 2 and Supplementary Figure 3; detailed description of ^1H spectrum is reported in the Supplementary Material). $^1H–^1H$ Vicinal coupling constant analysis showed J values = 3.0 and 4.8 for H_2 with H_{3A} and H_{3B}, respectively, indicative of a gauche arrangement of H_2 with both H_3 protons and consistent with the ring conformation depicted in Figure 3C. Additionally, J values for protons H_3 and H_4 are consistent with an antiperiplanar arrangement of H_{4A} and H_{3B} ($J_{3B,4A}$ = 13.2, Table 2). 2D NOESY data of compound **8e** (Supplementary Figure 4) are also consistent with the prevalence of conformations with axial amide side chain. In fact, strong NOE contacts of the same intensities were recorded for H_{3A} and H_{3B} with both H_2 and H_{4B}: similar distances among these couples of protons are only consistent with equatorial arrangements of H_2 and H_{4B}. NMR data also supported the conformational equilibrium simulated for the benzyl group and the amide side chain. The presence of NOE contacts for the two methylene hydrogens H_9 with protons H_2, H_8 and H_{10A} suggests that the benzyl substituent is rather free to rotate and can assume different conformations. NOE signals observed for H_9 protons with H_{10A}, but not with H_{10B}, as well as strong NOE contacts between H_{4A} and H_{10B},but not with H_{10A}, reveal limited conformational freedom of the amide side chain. This is further supported by the two different values of J recorded for H_2 with H_{10A} and H_{10B}, with H_{10A} spending most of the time in gauche to H_2, closer to the benzyl protons H_9, while H_{10B} was principally anti to H_2, pointing

toward H_{4a}. This preferred arrangement of the side chain, corresponding to $\tau 3 \sim 180°$ was captured by the plain MD simulation (Supplementary Figure 5).

The reliability of the results obtained from MD simulations, tested on these tetraquidroquinolines as well as on tetralin[26] 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, CD_3OD, 25 °C) for Compounds **6d**, **8e** and **8i** (*R* enantiomers depicted).



8e **8i**, **6d**

Protons[a]	**8e** ***J* (Hz)**	**8i** (R^1 = Me) ***J* (Hz)**	**6d** (R^1=H) ***J* (Hz)**
$^3J_{2,3A}$	3.0[b]	3.2	9.2
$^3J_{2,3B}$	4.8[b]	4.4	3.2
$^3J_{3A,4B}$	3.6	3.2	9.2
$^3J_{3B,4B}$	3.6	4.8	5.4
$^3J_{3B,4A}$	13.2	12.8	5.6
$^3J_{3A,4A}$	5.4	5.7	6.0
$^3J_{2,10A}$	4.8[b]	5.0	5.2
$^3J_{2,10B}$	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 H_2 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 MT_1 and MT_2 receptors. The eutomer (-)-**8e** showed about 60-fold higher MT_2 binding affinity than (+)-**8e** and greater potency in activating the MT_2 receptor. As shown in Figure 4, the enantiomer (*R*)-**8e** perfectly fits the bioactive conformation of melatonin, which suggests it should correspond to the eutomer (-)-**8e**.

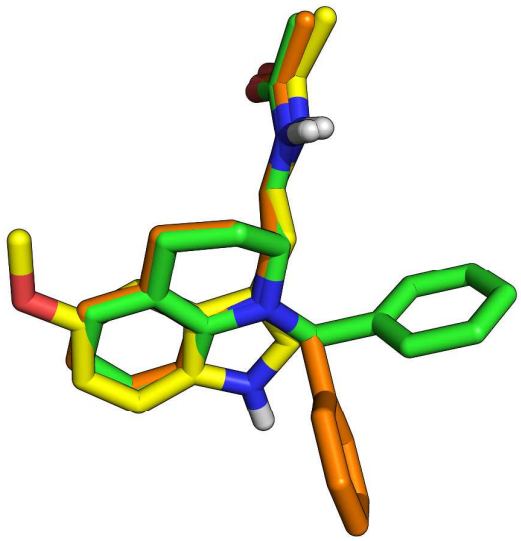


Figure 4. Superposition of melatonin (yellow carbons) and two representative conformations of compound **8e** with the benzyl group in an out-of-plane arrangement (τ2= -71.1°, orange carbons) and in an in-plane arrangement (τ2= -172.7°, green carbons).

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

consequences on the binding profile and SARs. This is supported by 1D and 2D NMR experiments performed on the *N*-methyl (**8i)** and the *N*-unsubstituted (**6d**) derivatives (Table 2 and Supplementary Figures 6-8). Vicinal coupling constants as well as NOE contacts recorded for compound **8i** highlighted the same preferential axial arrangement of the amide side chain seen for the benzyl derivative **8e**. On the other hand, in the case of **6d** experimental *J* values can be interpreted by assuming an equilibrium between two half chair conformations of the tetrahydroquinoline ring, one with equatorial amide side chain and the other with axial amide, inverting rapidly on the NMR time scale. In particular the $J_{2\text{-}3A}$ value ≈ 9 Hz must be considered as the average *J* value of both arrangements. A well-tempered metadynamics simulation performed on compound **6d** provided results consistent with these NMR data and estimated almost equal free-energy content for the axial and the equatorial arrangements of the amide side chain (Supplementary Figure 9). Therefore, while both the *N*-methyl and the *N*-benzyl substituent promote the conformations which fit the pharmacophore model, the lower potency observed for the N-unsubstituted derivative **6d** might be due, at least in part, to the presence of the equatorial species in solution. According to MD simulations and NMR experiments, the benzyl group of compound **8e** can alternatively be arranged perpendicular to the tetrahydroquinoline ring, with an out-of-plane hindrance typical of MT_2-selective antagonists, or coplanar with the tetrahydroquinoline, occupying a region of space where substituents of melatonin receptor agonists are usually present, like the phenyl ring of 2-phenylmelatonin (Figure 4). We speculate that this conformational flexibility could be responsible for the MT_2 selectivity of compound **8e**, and also for the residual intrinsic activity. Indeed, selectivity for the MT_2 receptor should be guaranteed by the bulky benzyl group that could hardly fit the MT_1 binding site, which is supposed to have reduced tolerance toward out-of-plane space occupancy. On the other hand, at the MT_2 receptor compound **8e** could also assume an in-plane conformation of the benzyl group, leading to receptor activation. Of course, this behavior is likely to involve some conformational flexibility of the receptor.

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 MT_1 and MT_2 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 MT_2 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 MT_1 and MT_2 binding affinities. The positive effect of the 5-methoxy group was particularly relevant at the MT_2 receptor, leading to compound **8a** with picomolar binding affinity, more than 10,000-fold MT_2-selectivity and a full agonist behavior at the MT_2 receptor. This compound is the most selective MT_2 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 MT_1 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

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

We also explored different side chains which could represent an alternative to the amide one, focusing on ethylurea and thioethylurea derivatives. The ethylurea side chain, with either a methyl (**6m**) or a hydrogen atom (**6i**) at the tetrahydroquinoline nitrogen, led to significant decreases of binding affinity, as it had been observed for agomelatine[28] and 1,6-dihydro-2*H*-indeno[5,4-*b*]furan derivatives.[29] Insertion of a 5-methoxy group on the tetrahydroquinoline scaffold (**6l**, **6h** and **6n**) did not produce the expected increase of binding affinity. The three thioethylurea derivatives **13**, **6k** and **6j** were even less potent than the corresponding urea derivatives **6m**, **6i** and **6l**, respectively.

Conclusions

Starting from molecular superpositions on previously developed pharmacophore models, the scaffold of *N*-anilinoethylamide melatonin receptor ligands, which had provided potent and MT_2-selective antagonists or partial agonists, was partially constrained to a 2-acylaminomethyl-tetrahydroquinoline nucleus. This nucleus proved to be an excellent new scaffold to develop new melatonergic selective agonists and the benzyl substituent on the tetrahydroquinoline nitrogen favored the proper arrangement of the amide side chain, mimicking the active conformation of melatonin.

Superposition models, supported by extended conformational analysis by MD simulations and NMR experiments, allowed to predict the suitable position for a methoxy group on the tetrahydroquinoline ring, leading to the benzyl derivative **8a** (UCM1014), an extremely potent and selective full agonist at MT_2 receptor.

As a final remark, metadynamics simulations proved to give reliable results on the free-energy profile and conformational preferences of these compounds, at much lower computational cost than classical plain molecular dynamics.

Experimental section

General procedures. Melting points were determined on a Buchi B-540 capillary melting point apparatus and are uncorrected. ^{1}H 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 $(M+1)^+$ 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.

5-Methoxyquinoline-2-carbonitrile (4a). White solid, mp 113-4 °C (EtOAc-petroleum ether); 95% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.05 (s, 3H), 7.00 (dd, 1H, J_1=J_2=4.5), 7.69 (d, 1H, *J*=8.5), 7.74-7.77 (m, 2H), 8.73 (d, 1H, *J*=8.5 Hz). ESI MS (*m/z*): 185 $(M+H)^+$.

6-Methoxyquinoline-2-carbonitrile (4b). White solid, mp 176-7 °C (EtOH-H_2O); 88% yield. Physicochemical data were in agreement to those previously reported.[30]

7-Methoxyquinoline-2-carbonitrile (4c). White solid, mp 187-8 °C (EtOAc-petroleum ether); 74% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.99 (s, 3H), 7.36 (dd, 1H, *J*=2.5 and 9.0 Hz), 7.46 (d, 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 (*m/z*): 185 $(M+H)^+$.

Synthesis of *N*-[(1,2,3,4-tetrahydroquinolin-2-yl)methyl]alkanamides (6a-g). A solution of the suitable quinoline-2-carbonitrile **4a-d** (1 mmol) in THF (5 mL) and 2M NH_3 in EtOH (0.6 mL) was hydrogenated over Raney nickel (4 atm) for 16 h at 60 °C. 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 (Na_2SO_4) and evaporated under reduced pressure to give the corresponding crude oily methanamine **(5a-d)** which was used without any further purification.

Et_3N (0.14 mL) and the suitable anhydride or acyl chloride (1 mmol) were added to a cold solution of the appropriate above crude methanamine **5a-d** 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 $NaHCO_3$ and with brine. After drying over Na_2SO_4, the solvent was removed by distillation *in vacuo* to give a crude product that was purified by flash chromatography.

***N*-[(5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (6a).** Flash chromatography: silica gel, EtOAc as eluent. White solid, mp 108-9 °C (Et_2O-petroleum ether); 54% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.17 (t, 3H, *J*=7.5 Hz), 1.61-1.75 (m, 1H), 1.87-1.96 (m, 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), 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). ^{13}C NMR (50 MHz, $CDCl_3$) 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, 9.9. ESI MS (*m/z*): 249 $(M+H)^+$.

***N*-[(6-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (6b).** Flash chromatography: silica gel, EtOAc as eluent. Oil; 50% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.18 (t, 3H, *J*=7.5 Hz), 1.65-176 (m, 1H), 1.84-1.95 (m, 1H), 2.25 (q, 2H, *J*=7.5 Hz), 2.73-2.86 (m, 2H), 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 (100).

***N*-[(7-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (6c)**. Flash chromatography: silica gel, EtOAc/cyclohexane 9:1 as eluent. Amorphous solid; 47% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.17 (t, 3H, *J*=7.5 Hz), 1.66-1.77 (m, 1H), 1.83-1.95 (m, 1H), 2.26 (q, 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, *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 $(M+H)^+$.

***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]propionamide (6d).** Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent. Beige solid, mp 67-8 °C (Et_2O-petroleum ether); 63% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 1.16 (t, 3H, *J*=7.5 Hz), 1.65 (dddd, 1H, J_1=6.0, J_2≈J_3=9.0 and 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 (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, *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,

J_3=6.5 and J_4=9.0 Hz, H_2), 6.52-6.56 (m, 2H, arom), 6.88-6.90 (m, 2H, arom). ^{13}C NMR (50 MHz, $CDCl_3$) 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 (*m/z*): 219 $(M+H)^+$.

^{1}H NMR spectrum of compound **6d** is depicted in Supplementary Figure S8.

***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]acetamide (6e).** Flash chromatography: silica gel, EtOAc/MeOH 96:4 as eluent. Oil; 73% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.58-1.77 (m, 1H), 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 (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).

***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]cyclopropanecarboxamide (6f).** Flash chromatography: silica gel, EtOAc/cyclohexane 8:2 as eluent. Oil; 73% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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 (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), 6.94-7.02 (m, 2H). ESI MS (*m/z*): 231 $(M+H)^+$.

***N*-[(1,2,3,4-Tetrahydroquinolin-2-yl)methyl]cyclobutanecarboxamide (6g).** Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent. Oil; 34% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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, 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 $(M+H)^+$.

General procedure for the synthesis of (1,2,3,4-tetrahydroquinolin-2-yl)ureido derivatives (6h-k). The suitable ethyl isocyanate (1 mmol) was added to a cold solution of the appropriate above crude (1,2,3,4-tetrahydroquinolin-2-yl)methanamine (**5a** or **5d**) (from 1 mmol) in CH_2Cl_2 (6 mL)

and the resulting mixture was stirred at room temperature for 30 min. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography.

1-Ethyl-3-[(5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]urea (6h). Flash chromatography: silica gel, EtOAc as eluent. White solid, mp 131-2 °C (EtOAc-petroleum ether); 60% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.14 (t, 3H, *J*=7.2 Hz), 1.58-1.74 (m, 1H), 1.85-1.97 (m, 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 s, 1H), 4.63 (br s, 1H), 6.22 (app t, 2H), 6.95 (dd, 1H, J_1=J_2=8.0 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 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*): 263 (M^+), 162 (100).

1-Ethyl-3-[(1,2,3,4-tetrahydroquinolin-2-yl)methyl]urea (6i). Flash chromatography: silica gel, EtOAc as eluent. White solid, mp 86-7 °C (Et_2O); 49% yield. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (t, 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), 3.41-3.48 (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, J_1=J_2=7.0 Hz), 6.95-7.02 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.9, 143.8, 129.2, 126.8, 121.4, 117.6, 114.6, 51.8, 45.6, 35.3, 25.9, 25.4, 15.4. ESI MS (*m/z*): 234 $(M+H)^+$.

1-Ethyl-3-[(5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]thiourea (6j). Flash chromatography: silica gel, cyclohexane-EtOAc 8:2 as eluent. White solid, mp 115-6 °C (EtOAc-petroleum ether); 63% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.22 (t, 3H, *J*=7.2 Hz), 1.63-1.81 (m, 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 (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 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 182.3, 157.8, 144.1, 127.0, 110.1, 108.4, 100.3, 55.3, 50.4, 49.2, 39.1, 24.9, 19.3, 14.1. EI MS (*m/z*): 279 (M^+), 162 (100).

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 (Et_2O-petroleum ether); 65% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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). ^{13}C NMR (50 MHz, $CDCl_3$) 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), $NaHCO_3$ (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 (Na_2SO_4). 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. ^{1}H NMR (200 MHz, $CDCl_3$) 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). ^{13}C NMR (50 MHz, $CDCl_3$) 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. ^{1}H NMR (200 MHz, $CDCl_3$) 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). ^{13}C NMR (50 MHz,

$CDCl_3$) 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), Et_3N (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 (Na_2SO_4). 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. ^{1}H NMR (200 MHz, $CDCl_3$) 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). ^{13}C NMR (50 MHz, $CDCl_3$) 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 NH_3 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 (Na_2SO_4) and evaporated under reduced pressure to give crude (5-methoxyquinolin-2-yl)methanamine which was used without any further purification.

Et_3N (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 $NaHCO_3$ and with brine. After drying over

Na_2SO_4, the solvent was removed by distillation *in vacuo,* and the residue was purified by silica gel flash chromatography (EtOAc as eluent) and crystallization; Beige solid, mp 117-8 °C (CH_2Cl_2-petroleum ether); 48% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26 (t, 3H, *J*=7.5 Hz), 2.40 (q, 2H, *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, *J*=8.5 Hz), 7.61-7.65 (m, 2H), 8.54 (d, 1H, *J*=8.5 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.9, 156.4, 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 $(M+H)^+$.

***N*-[(1-Benzyl-5-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (8a).** This product was obtained starting from **6a** and using the above N^1-benzylation procedure described for compound **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent and crystallization. White solid, mp 154-5 °C (CH_2Cl_2-petroleum ether); 89% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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), 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), 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), 7.23-7.37 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.9, 157.3, 145.2, 139.7, 128.4, 126.8, 126.7, 126.5, 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 $(M+H)^+$.

***N*-(1-Benzyl-6-methoxy-1,2,3,4-tetrahydroquinolin-2-yl-methyl)propionamide (8b).** This product was obtained starting from **6b** and using the above N^1-benzylation procedure described for compound **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 1:1 as eluent and crystallization. White solid, mp 147-8 °C (EtOAc-hexane); 78% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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 (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). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.8, 151.6, 139.8, 139.1, 128.7, 127.1, 127.0, 124.0, 115.5, 114.5, 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).

***N*-[(1-Benzyl-7-Methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (8c).** This product was obtained starting from **6c** and using the above N^1-benzylation procedure described for compound **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent and crystallization. White solid, mp 129-30 °C (EtOAc-petroleum ether); 90% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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 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, 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). ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.0, 159.2, 145.4, 139.1, 129.5, 128.7, 127.0, 126.5, 114.6, 101.2, 99.2, 57.3, 55.4, 55.1, 41.4, 29.5, 23.6, 23.0, 9.7. ESI MS (*m/z*): 339 $(M+H)^+$.

***N*-[(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (8d).** This product was obtained starting from **6d** and using the above N^1-benzylation procedure described for compound **6n**. Flash chromatography: silica gel, EtOAc/cyclohexane 7:3 as eluent and crystallization. White solid, mp 108-9° C (Et_2O-petroleum ether); 92% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05 (t, 3H *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 *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), 7.21-7.38 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.9, 144.6, 139.3, 129.1, 128.7, 127.3, 127.0, 126.6, 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 $(M+H)^+$.

***N*-[(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]acetamide (8e).** This product was obtained starting from **6e** and using the above N^1-benzylation procedure described for compound **6n**. Flash chromatography: silica gel, EtOAc as eluent and crystallization. Greyish solid, mp 120-1 °C (Et_2O-petroleum ether); 59% yield. ^{1}H NMR (600 MHz, CD_3OD) δ 1.84 (tt, 1H $J_1 = J_2 = 4.8$, $J_3 = J_4 = 13.2$, H_{3B}), 1.85 (s, 3H, CH_3), 1.96 (ddt, 1H $J_1 = J_2 = 3.0$, $J_3 = 5.4$, $J_4 = 13.2$ Hz H_{3A}), 2.65 (ddd, 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, $J =$

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, H_2), 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, H_8), 6.46 (td, 1H, $J_1 = J_2$ = 7.2, J_3 = 0.6 Hz, H_6), 6.81 (bt, 1H, $J_1 = J_2$ = 7.8 Hz, H_7), 6.89 (d, 1H, J = 7.2 Hz, H_5), 7.12-7.15 (m, 1H, Ph), 7.18-7.24 (m, 4H, Ph). ^{13}C NMR (50 MHz, $CDCl_3$) 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).

^{1}H NMR and NOESY spectra of compound **8e** are depicted in Supplementary Figures S3 and S4. A description of the ^{1}H 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:** $[\alpha]^{25}$=+74.3 at λ=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:** $[\alpha]^{25}$ -74.8 at λ=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 (CH_2Cl_2-petroleum ether); 58% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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). ^{13}C NMR (50 MHz, $CDCl_3$) 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 $(M+H)^+$.

***N*-[(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]cyclobutanecarboxamide (8g).** This product was obtained starting from **6g** and using the above N^1-benzylation procedure described for compound **6n**. Flash chromatography: silica gel, cyclohexane/EtOAc 7:3 as eluent and crystallization. Amorphous solid; 54% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.69-2.23 (m, 8H), 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 *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). ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.1, 144.6, 139.2, 129.0, 128.7, 127.2, 126.9, 126.5, 122.0, 116.5, 112.7, 57.5, 55.4, 41.5, 39.8, 25.2, 23.9, 23.4, 18.0. ESI MS (*m/z*): 335 $(M+H)^+$.

***N*-[(1-Methyl-5-methoxy-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (8h).** This product was obtained starting from **6a** and using the above N^1-methylation procedure described for compound **6l**. Flash chromatography: silica gel, EtOAc as eluent and crystallization. White solid, mp 139-40 °C (CH_2Cl_2-petroleum ether); 87% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15 (t, 3H *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, 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 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.1, 157.1, 145.8, 127.0, 109.5, 104.7, 98.8, 57.2, 55.4, 40.6, 39.1, 29.7, 22.7, 17.0, 9.8. ESI MS (*m/z*): 263 $(M+H)^+$.

***N*-[(1-Methyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (8i).** This product was obtained starting from **6d** and using the above N^1-methylation procedure described for compound **6l**. Flash chromatography: silica gel, CH_2Cl_2/acetone 9:1 as eluent and crystallization. White solid, mp 63-4 °C (Et_2O-petroleum ether); 95% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 1.15 (t, 3H, *J*=7.5), 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}), 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, 16.5 Hz, H_{4A}), 3.01 (s, 3H, N-CH_3), 3.19 (m, 1H, H_{10B}), 3.36-3.41 (m, 1H, H_{10A}), 3.43 (dddd, 1H,

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, 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, 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 (100).

^{1}H NMR and NOESY spectra of compound **8i** are depicted in Supplementary Figures S6 and S7.

***N*-[(1-Phenethyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]acetamide (8j).** A solution of **6e** (0.204 g, 1 mmol), Et_3N (0.27 mL) and phenylethyl bromide (0.54 mL, 4 mmol) in dry toluene (5 mL) and dry DMF (1 mL) was heated at 90 °C for 18 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 (Na_2SO_4). After removing the solvent by distillation under reduced pressure, the residue was purified by silica gel flash chromatography (EtOAc as eluent). Amorphous solid; 68% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.63-1.85 (m, 2H), 1.96 (s, 3H), 2.63-2.92 (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), 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 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, 23.7, 23.3, 23.0. EI MS (*m/z*): 308 (M^+), 236 (100).

***N*-[(1-Propyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]acetamide (8k).** This product was prepared according the above described procedure for **8j** starting from **6e** and using 1-iodopropane instead of phenylethyl bromide as alkylating reagent. Flash chromatography: silica gel, EtOAc as eluent and crystallization. White solid, mp 106-7 °C (CH_2Cl_2-petroleum ether); 43% yield. ^{1}H NMR (200 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), 2.69-2.79 (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 (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, 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).

***N*-{[1-(2-Methylamino)-2-oxoethyl]-1,2,3,4-tetrahydroquinolin-2-yl]methyl}propionamide (8l).** 2-Chloro-*N*-methylacetamide[32] (0.162 g, 1.5 mmol) was added to a solution of **6d** (0.218 g, 1 mmol) and Et_3N (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 (Na_2SO_4) 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. ^{1}H NMR (200 MHz, $CDCl_3$) 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 (d, 1H *J*=8.0 Hz),), 6.74 (dd, (d, 1H $J_1 \approx J_2$=7.0 Hz), 7.02-7.12 (m, 2H). ESI MS (*m/z*): 290 $(M+H)^+$.

1,2,3,4-Tetrahydroquinoline-2-carbonitrile[22] (9). $NaCNBH_3$ (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 CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4) 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. ^{1}H NMR (200 MHz, $CDCl_3$) 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 trifluoromethansulfonate

(0.125 mL, 0.84 mmol) in CH_3CN (13 mL) and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water and then extracted with EtOAc (3x). The combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give a crude oily residue which was purified by silica gel flash chromatography (cyclohexane/EtOAc 9:1 as eluent). Oil; 55% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.21-2.38 (m, 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 ($J_1 \approx J_2$=7.5 Hz), 6.81-7.39 (m, 7H). ESI MS (*m/z*): 235 $(M+H)^+$.

***N*-[(1-Phenyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]propionamide (11).** A solution of **10** (0.234 g, 1 mmol) in THF (5 mL) and propionic anhydride (1.2 mL, 9 mmol) was hydrogenated (4 atm) over Raney nickel for 5 h at 60 °C. 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 (Na_2SO_4) and evaporated under reduced pressure to give the crude desired product which was purified by crystallization. Beige solid, mp 86-7 °C (Et_2O-petroleum ether); 69% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.14 (t, 3H, *J*=7.5 Hz), 1.93-2.17 (m, 2H), 2.25 (q, 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 (br s, 1H), 6.71-6.83 (m, 2H), 6.93-7.02 (m, 1H), 7.06-7.37 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 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, 23.5, 9.8. EI MS (*m/z*) 294 (M^+), 208 (100).

1-Methyl-1,2,3,4-tetrahydroquinoline-2-carbonitrile[23] (12). Sodium cyanoborohydride (0.08 g, 1.3 mmol) and a 37% aqueous solution of HCHO (0.5 mL) were added to a solution of **9** (0.5 mmol), in MeOH (3 mL) and AcOH (to pH = 5), and the resulting mixture was stirred at room temperature for 16 h. An aqueous solution of 30% NaOH was added, and the aqueous phase was extracted with EtOAc. After drying over Na_2SO_4, the combined organic layers were concentrated by distillation under reduced pressure to give a crude residue which was purified by filtration on

silica gel (cyclohexane/EtOAc 8:2 as eluent). Oil; 58% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.25-2.35 (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, 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).

1-Ethyl-3-[(1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)methyl]thiourea (13). A solution of **12**[23] (1 mmol) in THF (5 mL) and 2M NH_3 in EtOH (0.6 mL) was hydrogenated over Raney nickel (4 atm) for 2 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 (Na_2SO_4) and evaporated under reduced pressure to give the corresponding crude (1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)methanamine which was used without any further purification. Ethyl isothiocyanate (1 mmol) was added to a cold solution of the crude (1-methyl-1,2,3,4-tetrahydroquinolin-2-yl)methanamine in CH_2Cl_2 (6 mL) and the resulting mixture was stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel flash chromatography (cyclohexane/EtOAc 7:3 as eluent) and crystallization. White solid, mp 94-5 °C (Et_2O-petroleum ether); 52% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 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, 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), 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, 116.7, 111.9, 57.5, 46.5, 38.8, 36.3, 24.0, 23.7, 14.0. ESI MS (*m/z*): 264 $(M+H)^+$.

***N*-[2-(1,2,3,4-Tetrahydroquinolin-2-yl)ethyl]propionamide (14)**. A solution of (1,2,3,4-tetrahydroquinolin-2-yl)acetonitrile[24] (1 mmol) in THF (5 mL) and 2M NH_3 in EtOH (0.6 mL) was hydrogenated over Raney nickel (4 atm) for 6 h at 60 °C. 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 (Na_2SO_4) and evaporated under reduced pressure to give the corresponding crude oily amine which was used without any further purification.

Et_3N (0.14 mL) and propionic anhydride (1 mmol) were added to a cold solution of the above crude amine 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 $NaHCO_3$ and with brine. After drying over Na_2SO_4, the solvent was removed by distillation *in vacuo* to give a crude product that was purified by flash chromatography: EtOAc as eluent. Oil; 57% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.20 (t, 3H *J*=7.5 Hz), 1.59-1.78 (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 (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).

***N*-[2-(1-Benzyl-1,2,3,4-tetrahydroquinolin-2-yl)ethyl]propionamide (15).** This product was obtained starting from **14** and using the previous N^1-benzylation procedure described for compound **6n**. The crude product was purified by silica gel flash chromatography: cyclohexane/EtOAc 3:7 as eluent. Beige solid, mp 66-7 °C (Et_2O-petroleum ether); 70% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ 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, 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 (m, 2H), 7.23-7.39 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.8, 144.6, 139.2, 129.2, 128.6, 127.1, 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 (M^+), 91 (100).

Optical resolution of compound (±)-8e

Racemic **(±)-8e** was separated into its constituent enantiomers using a semi-preparative column having triacetylcellulose (TAC) as a chiral stationary phase. A Büchi (Switzerland) borosilicate column (length: 40 cm; internal diameter: 3.0 cm) was filled with a slurry of 100 g of TAC in EtOH/H_2O 7:3 and packed following a previously described procedure.[31] Solvent delivery system: Gilson 307 pump; mobile phase: EtOH/H_2O 7:3; flow rate: 1 mL/min; sample concentration: 1.7% (w/v in EtOH). Ultraviolet detection was achieved at 262 nm using a Waters Lamba-Max Model

480 tunable absorbance detector. Fractions were collected using a Gilson FC 203B fraction collector (12 min/tube). The chromatograms were registered with a Linseis recorder (05.80 L) (range AUFS 2.0 x 20 mV).

The enantiomeric purity of chromatographic fractions was 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. The chromatographic fractions showing a single enantiomer (λ = 262 nm: t_{R1} = 17.09 min; t_{R2}= 19.5 min) were collected, the others recombined and recycled through the TAC column. Repeating the procedure just described several times, it was possible to obtain an enriched fraction (ee >97%) for both enantiomers. The enantiomer enriched fractions were collected, evaporated under reduced pressure, and further purified by flash chromatography (silica gel, EtOAc as eluent) and crystallization (Et_2O-petroleum ether).

Optical rotation analysis was performed using a Perkin-Elmer 241 polarimeter using a Hg lamp (λ=365 nm). α values were determined at 25 °C and are reported in 10^{-1} deg cm^2 g^{-1}; concentration (*c*) is in g per 100 mL. Enantiomeric purity was determined by HPLC on the following apparatus: Shimadzu LC-10AT (liquid chromatograph), Shimadzu SPD-10A (UV detector), Shimadzu C-R6A Chromatopac, using Chiralcel AD-H as column.

Pharmacology

Reagents and Chemicals

2-[^{125}I]-Iodomelatonin (2200 Ci/mmol) was purchased from NEN (Boston, MA). Other drugs and chemicals were purchased from Sigma-Aldrich (Saint Quentin, France).

Cell Culture

CHO cell lines stably expressing the human melatonin MT_1 or MT_2 receptors were grown in DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/mL penicillin and 100 µg/ml streptomycin. Grown at confluence at 37 °C (95% O_2/5% CO_2), they were harvested

in PBS containing EDTA 2 mM and centrifuged at 1000 g for 5 min (4 °C). The resulting pellet was suspended in TRIS 5 mM (pH 7.5), containing EDTA 2 mM and homogenized using a Kinematica polytron. The homogenate was then centrifuged (95000 g, 30 min, 4 °C) and the resulting pellet suspended in 75 mM TRIS (pH 7.5), 12.5 mM $MgCl_2$ and 2 mM EDTA. Aliquots of membrane preparations were stored at -80 °C until use.

Binding Assays

2-[^{125}I]iodomelatonin binding assay conditions were essentially as previously described.[33] Briefly, binding was initiated by addition of membrane preparations from stable transfected CHO cells diluted in binding buffer (50 mM Tris-HCl buffer, pH 7.4 containing 5 mM $MgCl_2$) to 2-[^{125}I]-iodomelatonin (20 pM for MT_1 and MT_2 receptors) and the tested drug. Nonspecific binding was defined in the presence of 1 µM melatonin. After 120 min incubation at 37 °C, reaction was stopped by rapid filtration through GF/B filters presoaked in 0.5% (v/v) polyethylenimine. Filters were washed three times with 1 mL of ice-cold 50 mM Tris-HCl buffer, pH 7.4.

Data from the dose-response curves (7 concentrations in duplicate) were analyzed using the program PRISM (Graph Pad Software Inc., San Diego, CA) to yield IC_{50} (inhibitory concentration 50). Results are expressed as $K_i = IC_{50} / 1 + ([L]/K_D)$, where [L] is the concentration of radioligand used in the assay and K_D, the dissociation constant of the radioligand characterizing the membrane preparation.[34]

Functional Assays:

[^{35}S]GTPγS binding assay was performed according to published methodology.[33] Briefly, membranes from transfected CHO cells expressing MT_1 or MT_2 receptor subtype and compounds were diluted in binding buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 3 µM GDP, 3 mM $MgCl_2$, and 20 µg/mL saponin). Incubation was started by the addition of 0.2 nM [^{35}S]GTPγS to membranes (20 µg/mL) and drugs, and further followed for 1 h at room temperature. Nonspecific

binding was defined using cold GTPγS (10 μM). Reaction was stopped by rapid filtration through GF/B filters followed by three successive washes with ice cold buffer.

Usual levels of [^{35}S]GTPγs binding (expressed in dpm) were for CHO-MT_1 or MT_2 membranes: 2000 for basal activity, 8000 in the presence of melatonin 1 μM, and 180 in the presence of GTPγS 10 μM which defined the nonspecific binding. Data from the dose-response curves (7 concentrations in duplicate) were analyzed by using the program PRISM (Graph Pad Software Inc., San Diego, CA) to yield EC_{50} (Effective concentration 50 %) and Emax (maximal effect) for agonists.

Molecular modeling

Compounds **8e** and **6d** were built in Maestro 9.6[35] and minimized with Macromodel 10.2[36] applying the OPLS2005 force field[37] and the GB/SA continuum solvation model for water[38] to an energy gradient of 0.05 kJ mol^{-1} $Å^{-1}$. The minimized structures were solvated with explicit TIP3P water or methanol molecules with Desmond 3.6,[39] placing simulation box boundaries 10 Å far from ligand atoms on each side. The resulting systems were first equilibrated applying the default relaxation protocol implemented in Desmond 3.6 and then submitted to standard plain molecular dynamics (MD) or well-tempered metadynamics simulations applying the Langevin coupling scheme[40] and setting temperature and pressure at 298 K and 1 atm, respectively. Plain MD simulation was carried out for 1 μs, collecting one snapshot every 10 ps. For metadynamics simulations, dihedral angles τ1 and τ2 (τ1: N1-C2-C3-C4; τ2: C8a-N1-Cbn-Car, see legend of Figure 3) were chosen as the collective variables for compound **8e**, while for compound **6d** only τ1 was considered. The initial height and width of the Gaussian potentials were set to 0.03 kcal mol^{-1} and 2 degrees, respectively, while the time interval between subsequent potentials was set to 0.09 ps.

The convergence of well-tempered metadynamics simulations was assessed by evaluating the time evolution of the relative free-energy levels of the free-energy minima (see Supplementary Figures S9b and S10b). To this aim, well-tempered metadynamics were run for 50 ns, free-energy profiles

were reconstructed after every ns and the simulation was considered to be converged when free-energy differences among global and local minima became approximately constant. For both compounds **8e** and **6d** metadynamics simulations converged approximately after 20 ns. Therefore, only the free-energy profiles obtained at 20 ns for compounds **8e** and **6d** are reported and discussed. The maximum free-energy values were truncated at 4 kcal/mol.

Supporting Information

^{1}H NMR spectra of compounds **8e**, **8i** and **6d**, NOESY spectra of compounds **8e** and **8i,** free-energy surfaces obtained from plain molecular dynamics and metadynamics simulations of compounds **8e** and **6d**, purity of target compounds.

Corresponding author

Phone: +39 0521 905061

Fax: + 39 0521 905006

E-mail: silvia.rivara@unipr.it

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Abbreviations used

DMEM, Dulbecco's modified eagle medium; Emax, maximum activation of receptor; GTPγS, guanosine 5′-O-(3-thiotriphosphate); MD, molecular dynamics; MPLC, medium-pressure liquid chromatography; MT_1, melatonin receptor subtype 1; MT_2, melatonin receptor subtype 2; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; PBS, phosphate-buffered saline; TAC, triacetylcellulose; THF, tetrahydrofuran.

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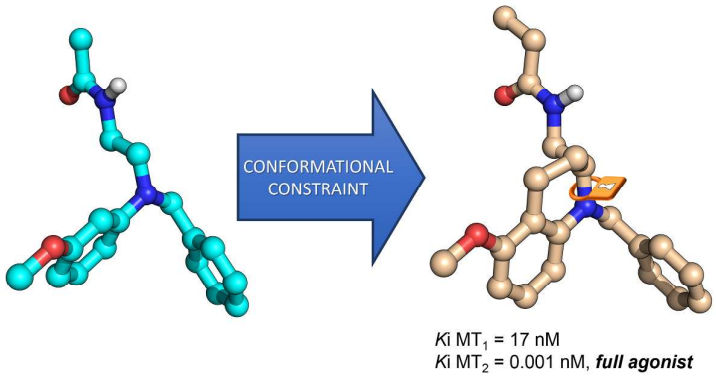
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