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## Melatoninergic Ligands : Design, Synthesis and Pharmacological Evaluation of Novel Series of Naphthofuranic Derivatives

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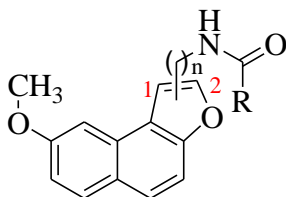
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sur-Seine, France.

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## Graphical abstract



### Naphthofuranic compounds

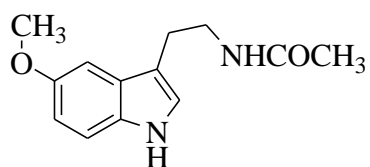
$n = 1, 2$ ;  $X = O, S$

$R = CH_3, c\text{-}C_3H_5, i\text{-}C_3H_7, CH_2F, NHR^1$  ( $R^1 = H, CH_3, C_2H_5\dots$ )

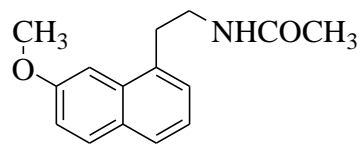
### Abstract

Following our research for new melatonergic ligands, herein we report the design, synthesis and biological evaluation of new series of naphthofuranic derivatives as  $MT_1$  and  $MT_2$  ligands. Binding affinity results of the prepared compounds revealed good binding affinities at both  $MT_1$  and  $MT_2$  receptors. Particularly, compound **6a** behaves as  $MT_1$  partial agonist and  $MT_2$  full agonist and exhibits an excellent binding affinity at  $MT_2$  ( $K_i = 0.09$  nM). Moreover, lateral chain displacement from position 1 to 2 of the furane core has no effect on the binding affinity at  $MT_1$  and  $MT_2$ , while elongation of this side chain, lead to decreased melatonergic binding affinities.

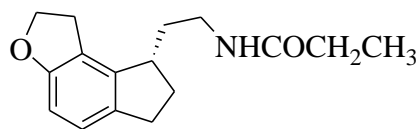
**Key Words:** Melatonin, Naphthofuranic derivatives,  $MT_1$ ,  $MT_2$ , Partial agonist



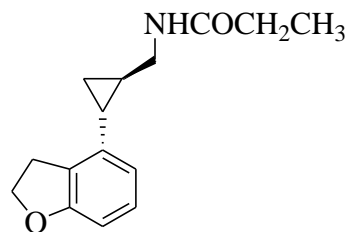
**Melatonin**



**Agomelatine**



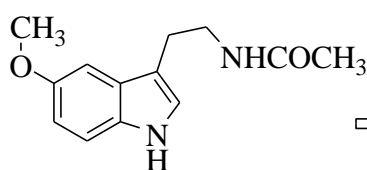
**Ramelteon**



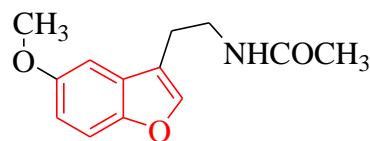
**Tasimelteon**

**Figure 1.** Melatonin and some of its analogues.

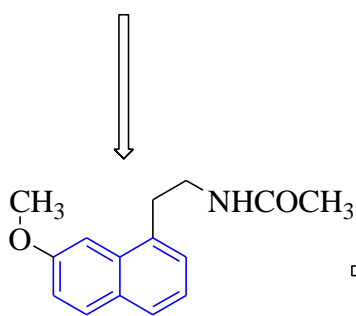
**Chart 1**



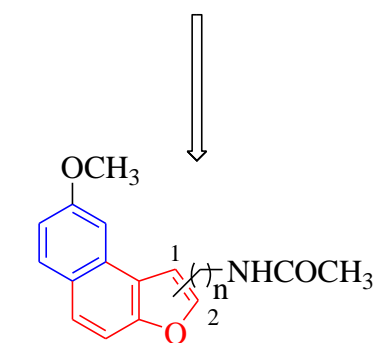
**Melatonin**



**S21767**



**Agomelatine**



**Naphthofuranic compounds**

## 1. Introduction

The neurohormone melatonin (Figure 1) was discovered and characterized as *N*-acetyl-5-methoxytryptamine more than fifty years ago by Aaron Lerner.<sup>1</sup> Its biosynthetic route was then established starting from the *L*-tryptophan acid following a circadian rhythm with high plasmatic concentration at night and low circulating levels during the day time. Melatonin is mainly secreted in humans by the pineal gland located in the hypothalamic suprachiasmatic nucleus (SCN),<sup>2</sup> but it is also synthesized by other regions of the CNS and by other tissues and cells such as the retina,<sup>3</sup> skin, bone marrow, lymphocytes,<sup>4</sup> and gastrointestinal tract.<sup>5</sup> Melatonin seems to play a major role in various physiological processes including, modulation of hormones secretion,<sup>6</sup> regulation of sleep-wake cycle<sup>7</sup> and cardiovascular functions,<sup>8</sup> pain perception,<sup>9</sup> immune system and core body temperature control.<sup>10</sup> Furthermore, melatonin was shown to be involved in several pathological processes such as sleep disturbances and insomnia,<sup>11</sup> cancer and inflammation,<sup>12</sup> neurodegenerative diseases,<sup>13</sup> diabetes,<sup>14</sup> depression and anxiety.<sup>15</sup> The endogenous role and mechanism of action of melatonin have not yet been fully elucidated.

The therapeutic potential of melatonin and most of its physiological effects are mainly mediated *via* activation of two of its receptors belonging to the superfamily of G-protein-coupled receptors (GPCRs) and named MT<sub>1</sub> and MT<sub>2</sub>. Both MT<sub>1</sub> and MT<sub>2</sub> have been cloned revealing that these two receptors are coupled to G<sub>ai</sub> proteins (Morgan et al., 1994).<sup>16</sup> These receptors exhibit a sub-nanomolar binding affinity for melatonin.<sup>17</sup> A third binding site of melatonin named MT<sub>3</sub> was characterized as the human quinone reductase 2 and displays a low binding affinity for melatonin.<sup>18</sup> Later it was shown that these three melatonergic binding sites were localized in different compartments of the human body.<sup>19</sup> In the central nervous system in SCN, cortex, *pars tuberalis*,<sup>20</sup> and peripherally in kidney, adipocytes, retina, blood vessels, gut, testes, bone marrow cells and human lymphocytes among others.<sup>21</sup>

In order to provide a better understanding of the melatonergic system and its physiological roles, during the last decades, much of the research was focused on the discovery of new analogs of melatonin. Moreover, the characterization of melatonin receptor-mediated functions requires potent and selective ligands for MT<sub>1</sub> and MT<sub>2</sub>. Nowadays, a lot of work has been done in this area,<sup>22</sup> however despite the large number of high affinity non-selective ligands, pronounced receptor subtype selective ligands especially for MT<sub>1</sub> remain a real challenge. Accordingly, after more than fifty years of research only few melatonergic compounds are marketed or under clinical trials. Hence, circadin, ramelteon, agomelatine and tasimelteon constitute the only melatonin analogs that are commercialized up to now (Figure 1).<sup>23</sup> For decades, our lab was involved in a large research program that consists in the pharmacomodulation of melatonin by replacing the indole scaffold by different bioisostere nucleus such as benzofurane, benzothiophene, isoquinoline, phthalazine, and naphthalene among others. In parallel, other modulations targeting different positions of the aromatic nucleus, the lateral acetamide chain and the methoxy group were performed.<sup>24,25</sup>

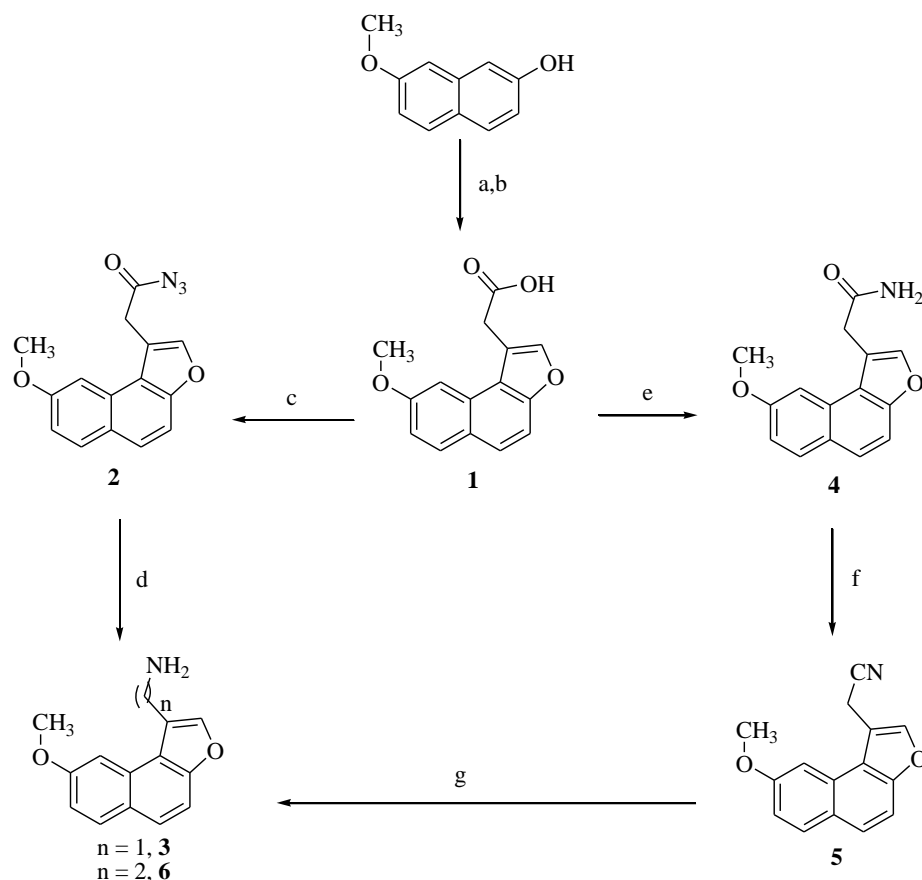
The substitution of the indole of melatonin with naphthalene and benzofurane nucleus was of major interest. First, agomelatine, the naphthalenic analogue of melatonin has showed a very good binding affinity at both MT<sub>1</sub> and MT<sub>2</sub>. Furthermore, this ligand is the only of the bioisosteric analogues of melatonin owning a serotonergic 5-HT<sub>2C</sub> binding affinity. Secondly, the benzofuran analogue of melatonin, the *N*-acetyl-2-(5-methoxybenzo[*b*]furan-3-yl) ethylamine (Chart 1), is a good ligand for the melatonin receptors and is metabolically more stable than melatonin.<sup>26</sup> In order to study the effect of the fusion of these two scaffolds on both the melatonergic affinity and activity, herein, we report the synthesis and pharmacological evaluation of a new series of naphthofuranic derivatives as new MT<sub>1</sub>/MT<sub>2</sub> ligands. The new obtained naphthofuranic derivatives were then submitted to a series of

additional modulations applied to the lateral chain and the acetamide function (Chart 1). We report hereafter the synthetic strategies and biological results of the prepared compounds.

## 2. Results and discussion

**2.1. Chemistry.** The synthetic strategy applied in order to prepare key amines **3** and **6** is shown in scheme 1 starting from 7-methoxy-beta-naphthol. Hence, two main routes were performed starting from the acid **1** issued from the condensation of 7-methoxynaphthol with ethyl 4-chloro-3-oxobutanoate in sulfuric acid according to the Pechmann procedure<sup>27</sup> followed by the transformation of the resulted chromenone into naphthofuranic derivative **1** by treatment with sodium hydroxide.<sup>28a</sup> Route 1 consists of azide **2** preparation from **1** by treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDCI) and sodium azide. Submission of the azide **2** to a Curtius rearrangement<sup>28b</sup> led to the corresponding isocyanate; this non isolated intermediate was subsequently hydrolysed under acid conditions to provide the desired amine **3**. In route 2, the acid **1** was first converted into amide **4** *via* treatment with oxalyl chloride and aqueous ammonia. Dehydration of **4** through treatment by trifluoroacetic anhydride (TFAA) led to nitrile **5**. Chemical reduction of compound **5** using lithium aluminum hydride and aluminum chloride yielded amine **6** in good yield.

**Scheme 1.** Synthesis of amines **3** and **6**.



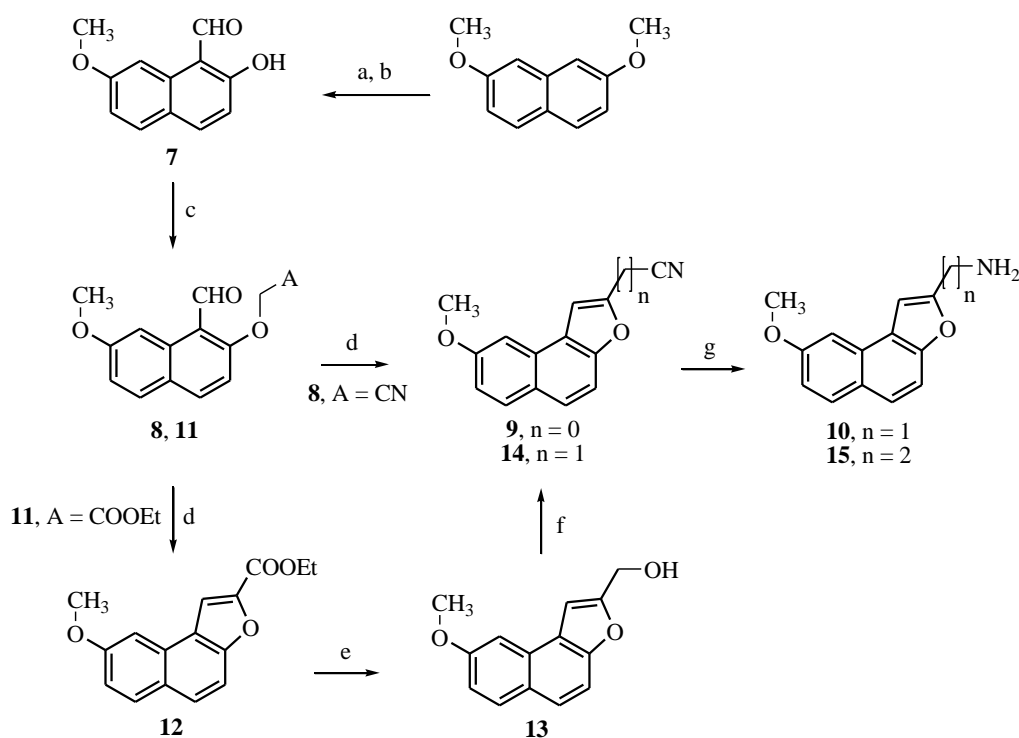
*Reagents:* a) Ethyl 4-chloro-3-oxobutanoate, H<sub>2</sub>SO<sub>4</sub>, rt, 57% ; b) 6M NaOH, 80 °C, 61% ; c) EDCI, NaN<sub>3</sub>, DCM/DMF, rt, 52% ; d) 12M HCl, toluene, reflux, 80% ; e) (i) (COCl)<sub>2</sub>, DCM/DMF, 0 °C to rt, (ii) NH<sub>4</sub>OH, Et<sub>2</sub>O, 0 °C, 78% ; f) TFAA, NEt<sub>3</sub>, THF, 0 °C to rt, 92% ; g) LiAlH<sub>4</sub>, AlCl<sub>3</sub>, Et<sub>2</sub>O/DCM, 0 °C to rt, 74%.

In Scheme 2 is outlined the synthetic sequence used for the preparation of amines **10** and **15**. First, formylation of 2,7-dimethoxynaphthalene according to Vilsmeier-Haack conditions furnished aldehyde **7**.<sup>29</sup> Selective demethylation *via* aluminum chloride treatment of the 2-methoxy resulted into naphthol **7**. *O*-alkylation of this later compound with the appropriate alkyl bromide in the presence of potassium carbonate led to derivatives **8** and **11**. Submission of **8** and **11** to a base-catalysed cyclisation reaction led to naphthofuranic derivatives **9** and **12**. Reduction of the ester **12** by treatment with lithium aluminum hydride provided the alcohol



**13.** Activation of compound **13** with thionyl chloride in the presence of pyridine followed by nucleophilic substitution with sodium cyanide gave the nitrile **14** in a good yield. Finally, chemical reduction of nitriles **9** and **14**, by a mixture of lithium aluminum hydride and aluminum chloride gave the desired amines **10** and **15**.

**Scheme 2.** Synthesis of compounds **10** and **15**.

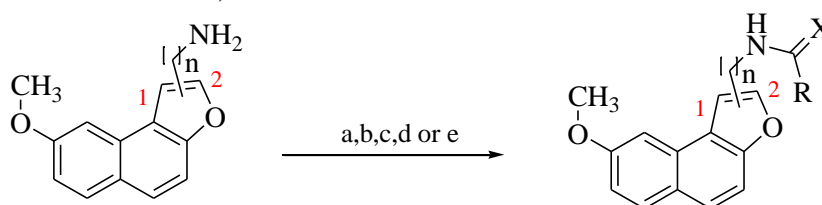


*Reagents:* a) *N*-methylformanilide, POCl<sub>3</sub>, DCM, reflux, 90% ; b) AlCl<sub>3</sub>, DCM, rt, 86% ; c) K<sub>2</sub>CO<sub>3</sub>, BrCH<sub>2</sub>A, acetone, rt, 100% for **8** and 97% for **11** ; d) K<sub>2</sub>CO<sub>3</sub>, DMF, 60 °C, 75% for **9** and 70% for **12** ; e) LiAlH<sub>4</sub>, THF, rt, 79% ; f) (i) SOCl<sub>2</sub>, pyridine, DCM, rt ; (ii) NaCN, TBABr, DCM/H<sub>2</sub>O, rt, 42% ; g) LiAlH<sub>4</sub>, AlCl<sub>3</sub>, Et<sub>2</sub>O/DCM, 0 °C to rt, 90% for **10** and 72% for **15**.

Synthesis of the desired final amides, ureas, and thioureas **3a-d**, **6a-c**, **10a-i** and **15a-c** was carried out as illustrated in Scheme 3. Hence, *N*-acylated compounds **3a-c**, **6a-b**, **10a-b** and **15a-b** were obtained from the corresponding amines by reaction with the appropriate acid chlorides according to a variant of the Schotten-Baumann procedure.<sup>30</sup> Fluorinated derivatives

**3d** and **10c** were prepared by reaction of the corresponding free amine with ethyl fluoroacetate in 2,2,2-trifluoroethanol. Urea **10d** and thiourea **10g** were obtained from **10** by treatment with potassium cyanate and potassium thiocyanate respectively. Otherwise, the reaction of **10** with *N*-methylphenylcarbamate, synthesized as previously described in the literature,<sup>31</sup> furnished methyl urea **10e**. Finally, alkylureas (**6c**, **10f**, **15c**) and alkythioureas (**10h**, **10i**) were prepared from the corresponding amines by treatment with alkyl isocyanate or alkylisothiocyanate in dry dichloromethane.

**Scheme 3.** Synthesis of amides, ureas and thioureas.



Compound	Isomer	n	X	R	Conditions	Yield (%)
<b>3a</b>	1	1	O	CH <sub>3</sub>	a	68
<b>3b</b>	1	1	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	a	53
<b>3c</b>	1	1	O	<i>i</i> -C <sub>3</sub> H <sub>7</sub>	a	55
<b>3d</b>	1	1	O	CH <sub>2</sub> F	b	40
<b>6a</b>	1	2	O	CH <sub>3</sub>	a	72
<b>6b</b>	1	2	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	a	83
<b>6c</b>	1	2	O	NHC <sub>2</sub> H <sub>5</sub>	e	84
<b>10a</b>	2	1	O	CH <sub>3</sub>	a	79
<b>10b</b>	2	1	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	a	80
<b>10c</b>	2	1	O	CH <sub>2</sub> F	b	45
<b>10d, 10g</b>	2	1	O, S	H	c	62
<b>10e</b>	2	1	O	NHCH <sub>3</sub>	d	40
<b>10f</b>	2	1	O	NHC <sub>2</sub> H <sub>5</sub>	e	65
<b>10h</b>	2	1	S	NHCH <sub>3</sub>	e	63
<b>10i</b>	2	1	S	NHC <sub>2</sub> H <sub>5</sub>	e	72
<b>15a</b>	2	2	O	CH <sub>3</sub>	a	74
<b>15b</b>	2	2	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	a	53
<b>15c</b>	2	2	O	NHC <sub>2</sub> H <sub>5</sub>	e	54

*Reagents:* a) RCOCl, K<sub>2</sub>CO<sub>3</sub>, EtOAc/H<sub>2</sub>O, 0 °C to rt ; b) FCH<sub>2</sub>COOEt, CF<sub>3</sub>CH<sub>2</sub>OH, reflux ; c) KNCO, H<sub>2</sub>O/HCl, rt for **10d** and KNCS, dioxane/THF, 60 °C for **10g** ; d) *N*-methylphenylcarbamate, DMSO, 60 °C ; e) R<sub>1</sub>NCX, DCM, rt.

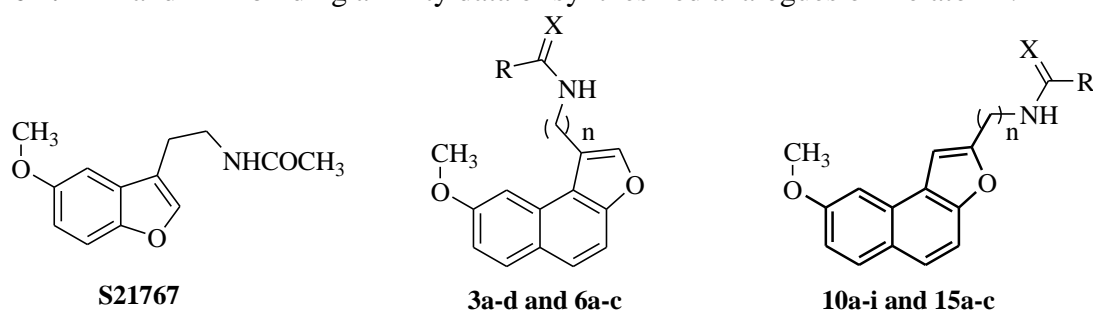
### 3. Pharmacology

In this paper, we describe the design and synthesis of a new series of naphthofuranic derivatives as melatonin MT<sub>1</sub> and MT<sub>2</sub> ligands. The new synthesized derivatives are composed of two position isomers, differently substituted at position 1 or 2 of the naphthofuranic nucleus. To determine their binding affinities and functional activities, the synthesized compounds were assayed at human MT<sub>1</sub> and MT<sub>2</sub> melatonin receptors stably transfected in Chinese Hamster Ovarian (CHO) cells, using 2-[<sup>125</sup>I]iodomelatonin as radioligand.<sup>32</sup> In tables 1-2, are shown the chemical structures and obtained biological results.

**3.1. Results and Discussion.** The binding affinity results of the prepared acetamides **3a** and **6a** revealed that this type of structure owns a good melatonergic binding affinity at both MT<sub>1</sub> and MT<sub>2</sub> receptors, and a very good affinity at MT<sub>2</sub> with regard to both melatonin and agomelatine (Table 1). Particularly, compound **6a** exhibits an excellent binding affinity and a selectivity of 31 fold to MT<sub>2</sub> receptor subtype than to MT<sub>1</sub>. Modulation of the acetamide group of both **3a** and **6a** has led to the synthesis of cyclopropanamides (**3b**, **6b**), isopropylamide (**3c**), and fluoroacetamide (**3d**) derivatives with lower binding affinity at both MT<sub>1</sub> and MT<sub>2</sub>. Similarly, substitution of the acetamide by an ethylurea (**6c**) produced the decrease of the melatonergic binding affinity. Furthermore and in order to fine tune the effect of the lateral chain position on the melatonergic binding affinity, compounds **10a** and **15a**, isomers of **3a** and **6a**, were synthesized and biologically evaluated. From the obtained results (Table 1), we can conclude that the lateral chain displacement from position 1 to 2 in this naphthofuranic series has no effect on the binding affinity at MT<sub>1</sub> and MT<sub>2</sub>. Interestingly, replacement of the acetamide group by bulky amides (**10b**, **15b**) and fluoroacetamide (**10c**) in

this series (isomers 2) has no significant effect on binding affinity in comparison with isomers 1. However, its replacement by ureas and thioureas lead to the decrease of the binding affinity especially at MT<sub>1</sub> producing the appearance of a weak MT<sub>2</sub> selectivity (Table 1). Finally, as an observed general rule herein, the elongation of the side chain, lead to decreased melatoninergetic binding affinities, despite its position in position 1 or 2 of the naphthofuranic ring. Additional docking investigations are necessary to study the conformation of these two isomers in the pocket of the binding site.

**Table 1.** MT<sub>1</sub> and MT<sub>2</sub> binding affinity data of synthesized analogues of melatonin.



Compound		n	X	R	<b>K<sub>i</sub> (nM) [ I95] (n)</b>	
					<i>h</i> -MT <sub>1</sub>	<i>h</i> -MT <sub>2</sub>
Amides	<b>Melatonin</b>	-	-	-	0.23 [ 0.21; 0.26 ](136)	0.52 [0.4; 0.58 ] (136)
	<b>Agomelatine</b>	-	-	-	0.12 [ 0.12; 0.12 ](2)	0.21 [0.08; 0.57 ] (2)
	<b>S21767</b>	-	-	-	0.15 [0.15] (1)	0.34 [0.34] (1)
	<b>3a</b>	1	O	CH <sub>3</sub>	4.6 [2.2; 9.7] (2)	0.7 [0.5; 1.1] (2)
	<b>6a</b>	2	O	CH <sub>3</sub>	2.8 [2.1; 3.8] (2)	0.09 [0.04; 0.2] (2)
	<b>10a</b>	1	O	CH <sub>3</sub>	2.1 [1.9; 2.3] (2)	0.4 [0.4; 0.4] (2)
	<b>15a</b>	2	O	CH <sub>3</sub>	10 [9; 10] (2)	1.7 [1.0; 2.6] (2)
	<b>3b</b>	1	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	20 [16; 24] (2)	15 [14; 17] (2)
	<b>6b</b>	2	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	23.6 [107; 522] (2)	13.3 [7.5; 23.2] (2)
	<b>10b</b>	1	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	1.9 [1.7; 2.3] (2)	0.6 [0.5; 0.7] (2)
	<b>15b</b>	2	O	<i>c</i> -C <sub>3</sub> H <sub>5</sub>	73 [67; 79] (2)	11 [11; 11] (2)
	<b>3c</b>	1	O	<i>i</i> -C <sub>3</sub> H <sub>7</sub>	158 [116; 217] (2)	24 [11; 57] (2)
	<b>3d</b>	1	O	CH <sub>2</sub> F	9.9 [6.1; 16] (3)	2.1 [0.9; 5.2] (2)
	<b>10c</b>	1	O	CH <sub>2</sub> F	5.7 [5.6; 5.7] (2)	0.7 [0.5; 1.0] (2)
Ureas	<b>10d</b>	1	O	NH <sub>2</sub>	nd	nd
	<b>10e</b>	1	O	NHCH <sub>3</sub>	nd	nd
	<b>6c</b>	2	O	NHC <sub>2</sub> H <sub>5</sub>	157 [99; 249] (2)	226 [104; 493] (3)
	<b>10f</b>	1	O	NHC <sub>2</sub> H <sub>5</sub>	17 [16; 18 ] (2)	4.2 [4.2; 4.2] (2)
	<b>15c</b>	2	O	NHC <sub>2</sub> H <sub>5</sub>	210 [191; 230] (2)	46 [39; 54] (2)
Thio ureas	<b>10g</b>	1	S	NH <sub>2</sub>	nd	nd
	<b>10h</b>	1	S	NHCH <sub>3</sub>	43 [30; 48] (2)	0.8 [0.5; 1.1] (2)
	<b>10i</b>	1	S	NHC <sub>2</sub> H <sub>5</sub>	140 [120; 155] (2)	7 [4; 11] (2)

K<sub>i</sub> (nM) values are geometric mean values (with 95% confidence limits shown in brackets) of at least (n) separate experiments performed in duplicate.

nd: not determined.

The intrinsic activity evaluation results<sup>7</sup> are shown in Table 2 and reveal that most of the synthesized derivatives exhibit a partial agonist activity. Besides, acetamide **3a** acts a partial agonist at both MT<sub>1</sub> and MT<sub>2</sub> with EC<sub>50</sub> of 4.8 and 1.4 nM respectively. More interestingly, the acetamide **6a** behaves as MT<sub>1</sub> partial agonist and MT<sub>2</sub> full agonist with EC<sub>50</sub> of 4.6 and 0.7 nM respectively, hence representing one of the most interesting compounds of this series. Finally, with regard to the agomelatine profile, the prepared compounds were tested for their binding affinities at serotonin 5-HT<sub>2C</sub> receptor subtype and revealed a binding affinity at the micromolar range for most of the compounds (data not shown).

**Table 2.** Intrinsic activity of synthesized compounds

Compound	<i>h</i> -MT <sub>1</sub>		<i>h</i> -MT <sub>2</sub>	
	EC <sub>50</sub> (nM) [I95] ( <i>n</i> )	E <sub>max</sub> (%) ± ESM ( <i>n</i> )	EC <sub>50</sub> (nM) [I95] ( <i>n</i> )	E <sub>max</sub> (%) ± ESM ( <i>n</i> )
<b>Agomelatine</b>	1.4 [0.7; 2.5] (4)	99 ± 6 (4)	0.18 [0.08; 0.39] (3)	91 ± 7 (3)
<b>3a</b>	4.8 [2.5; 9.1] (2)	77 ± 2 (2)	1.4 [0.83; 2.3] (3)	67 ± 3 (3)
<b>6a</b>	4.6 [2; 11] (3)	39 ± 4 (3)	0.7 [0.3; 1.8] (2)	101 ± 1 (2)
<b>10a</b>	7.7 [4.8; 12] (2)	28 ± 7 (2)	0.98 [0.43; 2.2] (2)	27 ± 4 (2)
<b>15a</b>	>10000 (2)	nd (2)	>10000 (2)	nd (2)
<b>3b</b>	1.0 [0.8; 1.3] (2)	57 ± 8 (2)	8.8 [7.2; 1.1] (3)	62 ± 4 (3)
<b>6b</b>	5.2 [3.8; 7.2] (3)	56 ± 14 (3)	3.2 [2.8; 3.6] (2)	68 ± 7 (2)
<b>10b</b>	1.0 [0.8; 1.3] (2)	10 ± 2 (2)	0.98 [0.80; 1.2] (2)	20 ± 1 (2)
<b>15b</b>	>10000 (2)	nd (2)	5.8 [2.7; 13] (2)	9 ± 3 (2)
<b>3c</b>	8.1 [8.1; 8.2] (2)	57 ± 8 (2)	8.8 [7.2; 11] (3)	62 ± 4 (3)
<b>3d</b>	29 [20; 41] (2)	85 ± 0 (2)	3.4 [3.1; 3.7] (3)	68 ± 4 (3)
<b>10c</b>	9.5 [4.3; 21] (2)	28 ± 3 (2)	1.1 [1.1; 1.2] (2)	31 ± 3 (2)
<b>6c</b>	75 [32; 177] (3)	77 ± 10 (3)	6.2 [3.6; 11] (2)	93 ± 1 (2)
<b>10f</b>	> 10000 (3)	nd (3)	10 [8; 13] (3)	19 ± 2 (3)

EC<sub>50</sub> values are geometric mean values (with 95% confidence limits in parentheses)

E<sub>max</sub> values are arithmetic mean ± S.E.M.

nd: not determined.

## 4. Conclusions

In conclusion we report herein the synthesis and binding affinity results of new series of naphthofuran derivatives as melatonin ligands. Besides acetamides showed good binding affinities at both MT<sub>1</sub> and MT<sub>2</sub> receptor subtypes in comparison with the other amides and ureas. Most of the synthesized compounds displayed a weak MT<sub>2</sub> selectivity except **6b** and **6c**. Furthermore, no noticeable difference in terms of binding affinities was observed between naphthofuran isomers 1 and 2.

## 5. Experimental section

**5.1. Chemistry.** Melting points were determined on a Buchi SMP-20 capillary apparatus and are uncorrected. FT-IR spectra were recorded on a Thermo Nicolet Avatar 320 FT-IR spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on an AMX 300 Bruker or DPX 300 Advance spectrometer. Chemical shifts are reported in ppm (parts per million) relative to that of (CH<sub>3</sub>)<sub>4</sub>Si and *J* values are reported in hertz (Hz). Reactions and resulted products were monitored via TLC on Merck precoated silica gel 60 F-254. LC-MS spectra were performed on a LC-Surveyor MSQ spectrometer and were recorded in the APCI positive mode (Toho Bioscience column, TSK gel Super ODS, 4.6 mm DI x 5.0 cm) eluted in a gradient of H<sub>2</sub>O/CH<sub>3</sub>CN.

**2-(8-Methoxynaphto[2,1-*b*]furan-1-yl)acetyl azide (2).** To a solution of compound (**1**) (1.05 g, 4 mmol) in dichloromethane (DCM) (20 mL) and some drops of DMF were added sodium azide (0.22 g, 4.4 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (0.88 g, 4.4 mmol). The mixture was stirred for 3 h at room temperature and washed with a saturated aqueous solution of NaHCO<sub>3</sub> and water. The organic layer was dried over MgSO<sub>4</sub>, filtered

and evaporated under *vacuo*. The resulting solid was recrystallized from acetonitrile to give **2** (52%) as a yellow solid; mp 114-116 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)

δ : 7.87 (d, 1H, 9.0 Hz), 7.72 (s, 1H), 7.68 (d, 1H, 8.7 Hz), 7.58 (d, 1H, 2.4 Hz), 7.51 (d, 1H, 8.7 Hz), 7.16 (dd, 1H, 9.0 Hz and 2.4 Hz), 4.07 (s, 2H), 4.00 (s, 3H) ; IR (ν, cm<sup>-1</sup>, KBr) : 2131 (N<sub>3</sub>), 1703 (CO) ; LC-MS : *m/z* = 282 (MH<sup>+</sup>).

**(8-Methoxynaphtho[2,1-*b*]furan-1-yl)methylamine hydrochloride (3)**. A solution of **2** (1.44 g, 5 mmol) in toluene (25 mL) was refluxed for 1h. Then, 12M solution of HCl (10 mL) was added and the mixture was refluxed for an additional 30 min. The solvent was evaporated and the resulting solid was recrystallized from acetonitrile to afford **3** (80%) as a white solid; mp 246-248 °C ; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 8.73 (br s, 3H), 8.13 (s, 1H), 8.00 (d, 1H, 8.7 Hz), 7.82 (d, 1H, 9.0 Hz), 7.64 (d, 1H, 8.7 Hz), 7.46 (d, 1H, 2.4 Hz), 7.22 (dd, 1H, 9.0 Hz and 2.4 Hz), 4.63 (m, 2H), 3.99 (s, 3H) ; IR (ν, cm<sup>-1</sup>, KBr) : 3427-2605 (NH<sub>3</sub><sup>+</sup>Cl<sup>-</sup>) ; LC-MS : *m/z* = 228 (MH<sup>+</sup>).

**2-(8-Methoxynaphtho[2,1-*b*]furan-1-yl)acetamide (4)**. To a solution of **1** (1.05 g, 4 mmol) in DCM (40 mL) and some drops of DMF at 0 °C was added oxalyl chloride (0.62 mL, 6 mmol). The mixture was stirred for 2 h at room temperature and concentrated *in vacuo*. Et<sub>2</sub>O (10 mL) was added and the mixture was cooled at 0 °C to add an aqueous solution of ammonia 30% (10 mL). The mixture was extracted with EtOAc, washed with water, dried over MgSO<sub>4</sub> and evaporated *in vacuo*. The resulting solid was recrystallized from acetonitrile to give **4** (78%) as a brown solid ; mp 208-210 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.86 (d, 1H, 9.0 Hz), 7.72-7.69 (m, 2H), 7.56 (d, 1H, 2.4 Hz), 7.52 (d, 1H, 8.7 Hz), 7.16 (dd, 1H, 9.0 Hz et 2.4 Hz), 5.57 (br s, 2H), 4.00 (s, 2H), 3.97 (s, 3H) ; IR (ν, cm<sup>-1</sup>, KBr) : 3386-3177 (NH<sub>2</sub>), 1654 (CO) ; LC-MS : *m/z* = 256 (MH<sup>+</sup>).

**2-(8-Methoxynaphtho[2,1-*b*]furan-1-yl)acetonitrile (5)**. To a solution of **4** (1.54 g, 6 mmol) in THF (60 mL) were added carefully at 0 °C triethylamine (1.69 mL, 12 mmol) and



trifluoroacetic anhydride (0.97 mL, 7.2 mmol). The mixture was stirred at 0 °C for 1h and at room temperature for an additional hour, evaporated *in vacuo* and poured into water. The mixture was acidified with a 3M solution of HCl, and extracted with EtOAc. The organic layer was washed with an aqueous solution of K<sub>2</sub>CO<sub>3</sub> and water, dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The resulting solid was recrystallized from toluene to afford **5** (92%) as a yellow solid ; mp 163-164 °C ; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 8.09 (s, 1H), 8.00 (d, 1H, 9.0 Hz), 7.83 (d, 1H, 8.7 Hz), 7.61-7.66 (m, 2H), 7.22 (dd, 1H, 9.0 Hz and 2.4 Hz), 4.57 (s, 2H), 3.99 (3H, s) ; IR (ν, cm<sup>-1</sup>, KBr) : 2237 (CN) ; LC-MS : *m/z* = 238 (MH<sup>+</sup>).

**2-(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethylamine (6)**. To a suspension of LiAlH<sub>4</sub> (0.44 g, 12 mmol) in Et<sub>2</sub>O (20 mL) at 0 °C was added a solution of AlCl<sub>3</sub> (1.59 g, 12 mmol) in Et<sub>2</sub>O (20 mL). After 5 minutes of stirring, **5** (1.43 g, 6 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added dropwise at 0 °C. Then, the resulting mixture was stirred at room temperature during 1 h. An aqueous solution of sodium hydroxide 10% was added carefully and the mineral solid was filtered and washed with Et<sub>2</sub>O. The filtrate was washed with water and dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to afford **6** (74%) as a yellow solid ; mp 88-90 °C ; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 7.95 (d, 1H, 8.7 Hz), 7.84 (s, 1H), 7.73 (d, 1H, 9.0 Hz), 7.62 (d, 1H, 2.4 Hz), 7.56 (d, 1H, 9.0 Hz), 7.16 (dd, 1H, 8.7 Hz and 2.4 Hz), 5.75 (br s, 2H), 3.93 (s, 3H), 3.09 (m, 2H), 2.99 (m, 2H); IR (ν, cm<sup>-1</sup>, KBr) : 2941-2835 (NH<sub>2</sub>) ; LC-MS : *m/z* = 242 (MH<sup>+</sup>).

**(1-Formyl-7-methoxynaphthalen-2-yloxy)acetonitrile (8)**. To a solution of 2-hydroxy-7-methoxy-naphthalene-1-carbaldehyde (**7**) (10.11 g, 50 mmol) in acetone (230 mL) were added K<sub>2</sub>CO<sub>3</sub> (13.82 g, 100 mmol) and bromoacetonitrile (4.53 mL, 65 mmol). The mixture was stirred at reflux for 5 h. After filtration and evaporation of the filtrate, the residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and water. The organic layer was washed with water, dried over

MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to afford **8** (100%) as a white solid ; mp 136-138 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 10.84 (s, 1H), 8.76 (d, 1H, 2.4 Hz), 8.01 (d, 1H, 9.0 Hz), 7.68 (d, 1H, 9.0 Hz), 7.13 (dd, 1H, 9.0 Hz and 2.4 Hz), 7.09 (d, 1H, 9.0 Hz), 4.99 (s, 2H), 3.97 (s, 3H) ; IR (ν, cm<sup>-1</sup>, KBr) : 2133 (CN), 1670 (CO) ; LC-MS : *m/z* = 242 (MH<sup>+</sup>).

**8-Methoxynaphtho[2,1-*b*]furan-2-carbonitrile (9)**. To a solution of **8** (12.06 g, 50 mmol) in dimethylformamide (150 mL) was added potassium carbonate (27.64 g, 200 mmol). The mixture was stirred for 16 h at 60 °C and poured into cold water. The resulting solid was dried and recrystallized from toluene to afford **9** (75%) as a white solid ; mp 151-153 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.89 (s, 1H), 7.87 (d, 1H, 9.0 Hz), 7.85 (d, 1H, 9.0 Hz), 7.51 (d, 1H, 9.0 Hz), 7.42 (d, 1H, 2.4 Hz), 7.24 (dd, 1H, 9.0 Hz et 2.4 Hz), 4.01 (s, 3H) ; IR (ν, cm<sup>-1</sup>, KBr) : 2219 (CN) ; LC-MS : *m/z* = 224 (MH<sup>+</sup>).

**(8-Methoxynaphtho[2,1-*b*]furan-2-yl)methylamine (10)**. To a suspension of LiAlH<sub>4</sub> (1.14 g, 30 mmol) in Et<sub>2</sub>O (15 mL) at 0 °C was added a solution of AlCl<sub>3</sub> (4 g, 30 mmol) in Et<sub>2</sub>O (15 mL). After 5 min of stirring, a solution of **9** (3.35 g, 15 mmol) in DCM (10 mL) was added dropwise at 0 °C. The resulting mixture was stirred at room temperature during 1 h. An aqueous solution of sodium hydroxide 10% was added carefully and the mineral solid was filtered and washed with Et<sub>2</sub>O. The filtrate was washed with water and dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to afford **10** (90%) as a white solid ; mp 201-203 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.82 (d, 1H, 9.0 Hz), 7.61 (d, 1H, 9.0 Hz), 7.47 (1H, d, 9.0 Hz), 7.37 (d, 1H, 2.7 Hz), 7.13 (dd, 1H, 9.0 Hz and 2.7 Hz), 6.98 (s, 1H), 4.06 (s, 2H), 3.97 (s, 3H), 1.68 (br s, 2H); IR (ν, cm<sup>-1</sup>, KBr): 2941-2835 (NH<sub>2</sub>); LC-MS : *m/z* = 228 (MH<sup>+</sup>).

**Ethyl 2-(1-formyl-7-methoxynaphthalen-2-yloxy)acetate (11)**. A mixture of **7** (10.11 g, 50 mmol), potassium carbonate (13.82 g, 100 mmol) and ethyl bromoacetate (7.21 mL, 65 mmol) in acetone (230 mL) was refluxed for 5 h. Then, the mixture was filtered. After

concentration of the filtrate, the residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and water. The organic layer was dried over MgSO<sub>4</sub>, filtered, concentrated under reduced pressure and recrystallized from methanol to give **11** (97%) as a white solid ; mp 115-117 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 11.00 (s, 1H), 8.86 (d, 1H, 2.4 Hz), 7.97 (d, 1H, 9.0 Hz), 7.68 (d, 1H, 9.0 Hz), 7.10 (dd, 1H, 2.4 Hz and 9.0 Hz), 6.97 (d, 1H, 9.0 Hz), 4.87 (s, 2H), 4.30 (q, 2H, 7.2 Hz), 3.99 (s, 3H), 1.31 (t, 3H, 7.2 Hz) ; IR (ν, cm<sup>-1</sup>, KBr) : 1773 (COOEt), 1658 (CHO) ; LC-MS : *m/z* = 289 (MH<sup>+</sup>).

**Ethyl 8-methoxynaphtho[2,1-*b*]furan-2-carboxylate (12).** To a solution of **11** (14.41 g, 50 mmol) in dimethylformamide (150 mL) was added potassium carbonate (27.64 g, 200 mmol). This mixture was stirred for 16 h at 60 °C, then poured into cold water and the resulting solid was filtered, dried and recrystallized from acetonitrile to give **12** (70%) as a white solid ; mp 67-69 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 8.00 (s, 1H), 7.87 (d, 1H, 9.0 Hz), 7.80 (d, 1H, 9.0 Hz), 7.57 (d, 1H, 9.0 Hz), 7.48 (d, 1H, 2.4 Hz), 7.19 (dd, 1H, 9.0 Hz and 2.4 Hz), 4.49 (q, 2H, 7.2 Hz), 4.00 (s, 3H), 1.48 (t, 3H, 7.2 Hz) ; IR (ν, cm<sup>-1</sup>, KBr) : 1735 (CO) ; LC-MS : *m/z* = 271 (MH<sup>+</sup>).

**(8-Methoxynaphtho[2,1-*b*]furan-2-yl)methanol (13).** To a solution of **12** (9.46 g, 35 mmol) in THF (150 mL) was added LiAlH<sub>4</sub> (2.7 g, 70 mmol) at 0 °C. The mixture was stirred at room temperature during 3 h and hydrolyzed by addition of an aqueous solution of sodium hydroxide 10%. The resulting mineral solid was filtered and washed with THF. The filtrate was concentrated under reduce pressure and partitioned between CH<sub>2</sub>Cl<sub>2</sub> and water. The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to afford **13** (79%) as a white solid ; mp 112-114 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.85 (d, 1H, 9.0 Hz), 7.66 (d, 1H, 9.0 Hz), 7.50 (d, 1H, 9.0 Hz), 7.40 (d, 1H, 2.7 Hz), 7.15 (dd, 1H, 9.0 Hz and 2.7 Hz), 7.14 (s, 1H), 4.87 (s, 2H), 3.99 (s, 3H), 1.93 (br s, 1H) ; IR (ν, cm<sup>-1</sup>, KBr) : 3261 (OH) ; LC-MS : *m/z* = 229 (MH<sup>+</sup>).

**(8-Methoxynaphtho[2,1-*b*]furan-2-yl)acetonitrile (14).** To a solution of **13** (4.57 g, 20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) at room temperature were added pyridine (0.49 mL, 6 mmol) and SOCl<sub>2</sub> (1.45 mL, 20 mmol). The mixture was stirred during 5 hours. Water (20 mL) and DCM (20 mL) were added then, addition of NaCN (4.90 g, 100 mmol) followed by TBABr (16.12 g, 50 mmol). After 16 h of stirring at room temperature, the layers were separated and the aqueous solution was extracted twice with DCM. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/DCM 1/1) to afford **14** (42%) as a yellow solid; mp 84-86 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.84 (d, 1H, 9.0 Hz), 7.67 (d, 1H, 9.0 Hz), 7.47 (d, 1H, 9.0 Hz), 7.37 (d, 1H, 2.4 Hz), 7.20 (s, 1H), 7.16 (dd, 1H, 9.0 Hz and 2.4 Hz), 4.01 (s, 2H), 3.99 (s, 3H) ; IR (ν, cm<sup>-1</sup>, KBr) : 2237 (CN) ; LC-MS : *m/z* = 238 (MH<sup>+</sup>).

**2-(8-Methoxynaphtho[2,1-*b*]furan-2-yl)ethylamine (15).** To a suspension of LiAlH<sub>4</sub> (0.31 g, 8 mmol) in Et<sub>2</sub>O (20 mL) at 0 °C was added a solution of AlCl<sub>3</sub> (1.06 g, 8 mmol) in ether (20 mL). After 5 minutes of stirring, **14** (0.79 g, 4 mmol) in DCM (10 mL) was added dropwise at 0 °C. The resulting mixture was stirred at room temperature during 1 h. An aqueous solution of sodium hydroxide 10% was added carefully and the formed mineral was filtered and washed with ether. The filtrate was washed with water and dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to afford **15** (72%) as a yellow solid ; mp 64-66 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.82 (d, 1H, 9.0 Hz), 7.60 (d, 1H, 9.0 Hz), 7.46 (d, 1H, 9.0 Hz), 7.37 (d, 1H, 2.4 Hz), 7.12 (dd, 1H, 9.0 Hz and 2.4 Hz), 6.93 (s, 1H), 3.98 (s, 3H), 3.16 (mt, 2H), 3.02 (mt, 2H), 1.69 (br s, 2H) ; IR (ν, cm<sup>-1</sup>, KBr) : 3358 (NH<sub>2</sub>); LC-MS : *m/z* = 242 (MH<sup>+</sup>).

**General procedure for synthesis of amides 3a-c, 6a-b, 10a-b and 15a-b.** To a solution of corresponding amine **3**, **6**, **10** or **15** (1 mmol) in EtOAc (30 mL) and water (10 mL) were

added  $K_2CO_3$  (3 mmol) and the corresponding acid chloride (1.5 mmol) at 0 °C. The mixture was stirred at room temperature during 2 h and the layers were separated. The organic one was washed with a 1M solution of HCl and water, dried over  $MgSO_4$ , filtered and concentrated under reduced pressure.

***N*-((8-Methoxynaphtho[2,1-*b*]furan-1-yl)methyl)acetamide (3a).** Recrystallized from acetonitrile (68%) as a white solid ; mp 198-200 °C ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  : 7.86 (d, 1H, 8.7 Hz), 7.71-7.69 (m, 2H), 7.51 (d, 1H, 9.0 Hz), 7.48 (d, 1H, 2.4 Hz), 7.16 (dd, 1H, 9.0 Hz and 2.4 Hz), 5.67 (br s, 1H), 4.86 (d, 2H, 4.8 Hz), 3.95 (s, 3H), 2.04 (s, 3H) ;  $^{13}C$  NMR (300 MHz,  $DMSO-d_6$ )  $\delta$  : 169.88, 158.96, 154.11, 143.79, 130.93, 129.55, 126.21, 125.70, 120.40, 119.38, 116.84, 110.52, 103.32, 55.80, 34.51, 23.03 ; IR ( $\nu$ ,  $cm^{-1}$ , KBr) : 3287 (NH), 1644 (CO) ; LC-MS :  $m/z$  = 270 ( $MH^+$ ).

***N*-((8-Methoxynaphtho[2,1-*b*]furan-1-yl)methyl)cyclopropanecarboxamide (3b).** Recrystallized from acetonitrile (53%) as a white solid ; mp 250-252 °C ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  : 7.87 (d, 1H, 8.7 Hz), 7.72 (s, 1H), 7.71 (d, 1H, 8.7 Hz), 7.53 (d, 1H, 9.0 Hz), 7.51 (d, 1H, 2.4 Hz), 7.17 (dd, 1H, 9.0 Hz and 2.4 Hz), 5.84 (br s, 1H), 4.89 (d, 2H, 4.8 Hz), 3.95 (s, 3H), 1.36 (mt, 1H), 1.06 (mt, 2H), 0.79 (mt, 2H) ;  $^{13}C$  NMR (300 MHz,  $DMSO-d_6$ )  $\delta$  : 173.33, 158.59, 154.18, 143.97, 130.87, 129.49, 126.13, 125.80, 120.47, 119.22, 116.91, 110.60, 103.49, 55.69, 34.63, 13.96, 6.78 (2C) ; IR ( $\nu$ ,  $cm^{-1}$ , KBr) : 3282 (NH), 1632 (CO) ; LC-MS :  $m/z$  = 296 ( $MH^+$ ).

***N*-((8-Methoxynaphtho[2,1-*b*]furan-1-yl)methyl)isobutyramide (3c).** Recrystallized from acetonitrile (55%) as a white solid; mp 208-209 °C ;  $^1H$  NMR (300 MHz,  $DMSO-d_6$ )  $\delta$  : 8.20 (br s, 1H), 7.99 (s, 1H), 7.94 (d, 1H, 9.0 Hz), 7.77 (d, 1H, 9.0 Hz), 7.60 (d, 1H, 9.0 Hz), 7.44 (d, 1H, 2.4 Hz), 7.15 (dd, 1H, 9.0 Hz and 2.4 Hz), 4.70 (d, 2H, 4.8 Hz), 3.89 (s, 3H), 2.43 (mt, 1H) ; 1.01 (mt, 6H) ;  $^{13}C$  NMR (300 MHz,  $DMSO-d_6$ )  $\delta$  : 176.67, 158.57, 154.06, 143.65, 130.82, 129.58, 126.09, 125.67, 119.51, 116.71, 110.50, 103.52, 103.34, 55.74, 34.32, 34.22,

20.05 (2C); IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3296 (NH), 1636 (CO); LC-MS:  $m/z = 298$  ( $\text{MH}^+$ ).

***N*-(2-(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethyl)acetamide (6a).** Recrystallized from toluene (72%) as a white solid; mp 132-133 °C ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 8.11 (br s, 1H), 7.96 (d, 1H, 9.0 Hz), 7.88 (s, 1H), 7.74 (d, 1H, 9.0 Hz), 7.65 (d, 1H, 2.4 Hz), 7.57 (d, 1H, 9.0 Hz), 7.17 (dd, 1H, 9.0 Hz and 2.4 Hz), 3.96 (3H, s), 3.46 (mt, 2H), 3.16 (mt, 2H), 1.82 (s, 3H) ;  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  : 169.85, 158.50, 153.80, 142.30, 131.03, 129.80, 125.86, 125.63, 120.61, 119.69, 116.22, 110.58, 103.17, 55.79, 39.28, 25.85, 23.07 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3296 (NH), 1628 (CO) ; LC-MS :  $m/z = 284$  ( $\text{MH}^+$ ).

***N*-(2-(8-Methoxynaphtho[2,1-*b*]furan-1-yl)ethyl)cyclopropanecarboxamide (6b).** Recrystallized from toluene (83%) as a white solid ; mp 160-161 °C ;  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  : 8.33 (br s, 1H), 7.95 (d, 1H, 9.0 Hz), 7.88 (s, 1H), 7.75 (d, 1H, 9.0 Hz), 7.64 (d, 1H, 2.4 Hz), 7.57 (d, 1H, 9.0 Hz), 7.17 (dd, 1H, 9.0 Hz and 2.4 Hz), 3.95 (s, 3H), 3.50 (mt, 2H), 3.18 (mt, 2H), 1.54 (mt, 1H), 0.58-0.73 (m, 4H) ;  $^{13}\text{C}$  NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  : 173.18, 158.55, 153.86, 142.39, 131.10, 129.84, 125.96, 125.77, 120.63, 119.82, 116.29, 110.61, 103.20, 55.80, 39.28, 26.00, 14.18, 6.68 (2C) ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3288 (NH), 1634 (CO) ; LC-MS :  $m/z = 310$  ( $\text{MH}^+$ ).

***N*-((8-Methoxynaphtho[2,1-*b*]furan-2-yl)methyl)acetamide (10a).** Recrystallized from acetonitrile (79%) as a white solid ; mp 197-199 °C ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  : 7.83 (d, 1H, 9.0 Hz), 7.64 (d, 1H, 9.0 Hz), 7.46 (d, 1H, 9.0 Hz), 7.37 (d, 1H, 2.4 Hz), 7.14 (dd, 1H, 9.0 Hz and 2.4 Hz), 7.09 (s, 1H), 5.95 (br s, 1H), 4.68 (d, 2H, 5.7 Hz), 3.98 (s, 3H), 2.08 (s, 3H) ;  $^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  : 170.1, 158.3, 152.9, 152.8, 130.3, 128.9, 125.3, 125.0, 122.7, 116.5, 109.6, 103.4, 102.6, 55.4, 37.2, 23.2 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3281 (NH), 1625 (CO) ; LC-MS :  $m/z = 270$  ( $\text{MH}^+$ ).

***N*-((8-Methoxynaphtho[2,1-*b*]furan-2-yl)methyl)cyclopropanecarboxamide (10b).**

Recrystallized from acetonitrile (80%) as a white solid ; mp 190-192 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.83 (d, 1H, 9.0 Hz), 7.64 (d, 1H, 9.0 Hz), 7.47 (d, 1H, 9.0 Hz), 7.38 (d, 1H, 2.4 Hz), 7.14 (dd, 1H, 9.0 Hz and 2.4 Hz), 7.09 (s, 1H), 6.10 (br s, 1H), 4.70 (d, 2H, 5.7 Hz), 3.98 (s, 3H), 1.41 (mt, 1H), 1.05 (mt, 2H), 0.76 (mt, 2H) ; <sup>13</sup>C NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 173.5, 158.9, 155.4, 152.8, 131.0, 129.4, 125.7, 125.4, 123.6, 117.3, 110.4, 104.0, 103.9, 56.3, 37.1, 14.5, 7.4 (2C) ; IR (ν, cm<sup>-1</sup>, KBr) : 3264 (NH), 1633 (CO) ; LC-MS : *m/z* = 296 (MH<sup>+</sup>).

***N*-(2-(8-Methoxynaphtho[2,1-*b*]furan-2-yl)ethyl)acetamide (15a).** Recrystallized from toluene (74%) as a white solid ; mp 64-66 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.84 (d, 1H, 9.0 Hz), 7.63 (d, 1H, 9.0 Hz), 7.47 (d, 1H, 9.0 Hz), 7.37 (d, 1H, 2.4 Hz), 7.14 (dd, 1H, 9.0 Hz and 2.4 Hz), 6.93 (s, 1H), 5.71 (br s, 1H), 3.99 (s, 3H), 3.71 (q, 2H, 6.3 Hz), 3.10 (t, 2H, 6.3 Hz), 1.99 (s, 3H) ; <sup>13</sup>C NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 170.2, 158.7, 156.4, 152.7, 131.0, 129.3, 125.7, 124.8, 123.88, 117.1, 110.4, 103.9, 103.4, 56.2, 38.1, 29.3, 23.5 ; IR (ν, cm<sup>-1</sup>, KBr) : 3280 (NH), 1534 (CO) ; LC-MS : *m/z* = 284 (MH<sup>+</sup>).

***N*-(2-(8-Methoxynaphtho[2,1-*b*]furan-2-yl)ethyl)cyclopropanecarboxamide (15b).**

Recrystallized from toluene (53%) as a white solid ; mp 160-162 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.84 (d, 1H, 9.0 Hz), 7.63 (d, 1H, 9.0 Hz), 7.48 (d, 1H, 9.0 Hz), 7.37 (d, 1H, 2.4 Hz), 7.14 (dd, 1H, 9.0 Hz and 2.4 Hz), 6.94 (s, 1H), 5.86 (br s, 1H), 3.99 (s, 3H), 3.74 (q, 2H, 6.3 Hz), 3.10 (t, 2H, 6.3 Hz), 1.31 (mt, 1H), 0.99 (mt, 2H), 0.73 (mt, 2H) ; <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>) δ : 173.7, 158.2, 155.1, 152.7, 130.3, 128.6, 125.3, 124.3, 123.0, 116.4, 109.7, 102.6, 102.5, 55.4, 38.0, 29.0, 14.8, 7.2 ; IR (ν, cm<sup>-1</sup>, KBr) : 3286 (NH), 1637 (CO) ; LC-MS : *m/z* = 310 (MH<sup>+</sup>).

**General procedure for synthesis of fluoroacetamide 3d and 10c.** To a solution of corresponding free amine **3** or **10** (8 mmol) in trifluoroethanol (30 mL) was added ethyl

fluoroacetate (32 mmol). The mixture was refluxed during 12 h then, hydrolyzed and extracted with Et<sub>2</sub>O. The organic layer was washed with a 1M solution of HCl and water, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure.

**2-Fluoro-N-((8-methoxynaphtho[2,1-*b*]furan-1-yl)methyl)acetamide (3d).** Recrystallized from cyclohexane (40%) as a white solid ; mp 152-153 °C ; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 8.70 (br s, 1H), 8.00 (s, 1H), 7.95 (d, 1H, 9.0 Hz), 7.77 (d, 1H, 9.0 Hz), 7.60 (d, 1H, 9.0 Hz), 7.48 (d, 1H, 2.6 Hz), 7.12 (dd, 1H, 9.0 Hz and 2.6 Hz), 4.90 (d, 2H, 37.8 Hz), 4.80 (mt, 2H), 3.89 (s, 3H) ; IR (ν, cm<sup>-1</sup>, KBr) : 3439 (NH), 1657 (CO) ; LC-MS : *m/z* = 288 (MH<sup>+</sup>).

**2-Fluoro-N-((8-methoxynaphtho[2,1-*b*]furan-2-yl)methyl)acetamide (10c).** Recrystallized from acetonitrile (45%) as a white solid ; mp 204-206 °C ; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ : 7.84 (d, 1H, 9.0 Hz), 7.66 (d, 1H, 9.0 Hz), 7.48 (d, 1H, 9.0 Hz), 7.38 (d, 1H, 2.7 Hz), 7.14 (dd, 1H, 9.0 Hz et 2.7 Hz), 7.13 (s, 1H), 6.81 (br s, 1H), 4.89 (d, 2H, 47.1 Hz), 4.77 (d, 2H, 5.7 Hz), 3.98 (s, 3H) ; <sup>13</sup>C NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 168.3 (d, J<sub>C-F</sub> 73.8 Hz), 158.9, 154.8, 152.8, 131.0, 129.4, 125.7, 125.4, 123.5, 117.3, 110.4, 104.1, 104.0, 80.9 (d, J<sub>C-F</sub> 714.0 Hz), 56.3, 36.6 ; IR (ν, cm<sup>-1</sup>, KBr) : 3310 (NH), 1651 (CO) ; LC-MS : *m/z* = 288 (MH<sup>+</sup>).

**N<sup>1</sup>-((8-Methoxynaphtho[2,1-*b*]furan-2-yl)methyl)urea (10d).** To a solution of **10** (0.45 g, 2 mmol) in water (10 mL) and 1M solution of HCl (1 mL) was added potassium cyanate (0.2 g, 2.4 mmol). The mixture was stirred at room temperature during 2 h then, hydrolyzed and extracted with EtOAc. The organic layer was washed with water, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The resulting solid was treated with diethyl ether to afford **10d** (62%) as a white solid ; mp 227-229 °C ; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ : 7.89 (d, 1H, 9.0 Hz), 7.68 (d, 1H, 9.0 Hz), 7.63 (d, 1H, 2.4 Hz), 7.54 (d, 1H, 9.0 Hz), 7.30 (s, 1H), 7.12 (dd, 1H, 9.0 Hz and 2.4 Hz), 6.74 (br s, 1H), 5.73 (br s, 2H), 4.42 (d, 2H, 5.7 Hz), 3.93



(s, 3H) ;  $^{13}\text{C}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  : 159.0, 158.4, 156.5, 152.3, 130.6, 129.0, 125.3, 124.8, 123.2, 116.8, 110.0, 103.5, 102.8, 55.9, 37.4 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3419 ( $\text{NH}_2$ ), 3341 (NH), 1627 (CO) ; LC-MS :  $m/z$  = 271 ( $\text{MH}^+$ ).

***N*<sup>1</sup>-((8-Methoxynaphtho[2,1-*b*]furan-2-yl)methyl)-*N*<sup>3</sup>-methylurea (10e).** To a solution of **10** (0.45 g, 2 mmol) in DMSO (10 mL) were added *N*-methylphenylcarbamate (0.45 g, 3 mmol) and triethylamine (0.42 mL, 3 mmol). The mixture was stirred at 60 °C during 3 h then, hydrolyzed and extracted with DCM. The organic layer was washed with a 1M solution of HCl and water, dried over  $\text{MgSO}_4$ , filtered and concentrated under reduced pressure. The resulting solid was recrystallized from acetonitrile to afford **10e** (40%) as a white solid ; mp 201-203 °C ;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  : 7.90 (d, 1H, 9.0 Hz), 7.68 (d, 1H, 9.0 Hz), 7.65 (d, 1H, 2.1 Hz), 7.55 (d, 1H, 9.0 Hz), 7.29 (s, 1H), 7.12 (dd, 1H, 9.0 Hz and 2.1 Hz) ; 6.57 (br s, 1H, 5.4 Hz), 5.92 (br q, 1H, 4.6 Hz), 4.44 (d, 2H, 5.4 Hz), 3.93 (s, 3H), 2.60 (d, 3H) ;  $^{13}\text{C}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  : 158.9, 158.4, 156.6, 152.3, 130.6, 129.0, 125.3, 124.8, 123.2, 116.8, 110.0, 103.5, 102.8, 55.9, 37.5, 26.9 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3273 (NH), 1625 (CO) ; LC-MS :  $m/z$  = 285 ( $\text{MH}^+$ ).

**General procedure for synthesis of alkyl urea 6c, 10f, 15c and thiourea 10h and 10i.** To a solution of corresponding amine **6**, **10** or **15** (1 mmol) in DCM (10 mL) were added triethylamine (2 mmol) and the corresponding alkyl isocyanate or isothiocyanate (1.2 mmol) at 0 °C. The mixture was stirred at room temperature during 2 h and hydrolyzed.  $\text{CH}_2\text{Cl}_2$  was added and the organic layer was washed with a 1M solution of HCl and water, dried over  $\text{MgSO}_4$ , filtered and concentrated under reduced pressure.

***N*<sup>1</sup>-Ethyl-*N*<sup>3</sup>-(2-(8-methoxynaphtho[2,1-*b*]furan-1-yl)ethyl)urea (6c).** Recrystallized from toluene (84%) as a white solid ; mp 214-216 °C ;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  : 7.96 (d, 1H, 9.0 Hz), 7.88 (s, 1H), 7.75 (d, 1H, 9.0 Hz), 7.68 (d, 1H, 2.4 Hz), 7.58 (d, 1H, 9.0 Hz), 7.17 (dd, 1H, 9.0 Hz and 2.4 Hz), 6.05 (br s, 1H), 5.87 (br s, 1H), 3.98 (s, 3H), 3.43 (mt, 2H),

3.16 (mt, 2H), 3.02 (mt, 2H) ; 0.98 (mt, 3H) ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3332 (NH), 1689 (CO) ; LC-MS :  $m/z = 313$  ( $\text{MH}^+$ ).

***N*<sup>1</sup>-Ethyl-*N*<sup>3</sup>-((8-methoxynaphtho[2,1-*b*]furan-2-yl)methyl)urea (10f).** Recrystallized from toluene (65%) as a white solid ; mp 185-186 °C ; <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  : 7.82 (1H, d, 9.0 Hz), 7.63 (d, 1H, 9.0 Hz), 7.45 (d, 1H, 8.7 Hz), 7.37 (d, 1H, 2.4 Hz), 7.13 (dd, 1H, 8.7 Hz and 2.4 Hz), 7.08 (s, 1H), 4.62 (s, 2H), 3.97 (s, 3H), 3.27 (q, 2H, 7.2 Hz), 1.68 (brs, 2H), 1.16 (t, 3H, 7.2 Hz) ; <sup>13</sup>C NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  : 158.8, 158.6, 156.9, 152.7, 131.0, 129.4, 125.7, 125.2, 123.6, 117.2, 110.4, 104.0, 103.2, 56.3, 37.8, 35.1, 16.5 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr): 3351 (NH), 3288 (NH), 1631 (CO); LC-MS:  $m/z = 299$  ( $\text{MH}^+$ ).

***N*<sup>1</sup>-Ethyl-*N*<sup>3</sup>-(2-(8-methoxynaphtho[2,1-*b*]furan-2-yl)ethyl)urea (15c).** Recrystallized from toluene (54%) as a white solid ; mp 184-186 °C ; <sup>1</sup>H NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  : 7.90 (d, 1H, 9.0 Hz), 7.66 (d, 1H, 9.0 Hz), 7.61 (d, 1H, 2.4 Hz), 7.54 (d, 1H, 9.0 Hz), 7.27 (s, 1H), 7.10 (dd, 1H, 9.0 Hz and 2.4 Hz), 5.97 (br s, 1H), 5.87 (br s, 1H), 3.93 (s, 3H), 3.42 (q, 2H, 6.6 Hz), 3.02-2.98 (m, 4H), 0.97 (t, 3H, 7.2 Hz) ; <sup>13</sup>C NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  : 158.4, 158.3, 156.4, 152.3, 130.6, 128.8, 125.3, 124.3, 123.5, 116.7, 110.0, 103.5, 102.9, 55.8, 38.4, 34.5, 29.9, 16.1 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3317 (NH), 1625 (CO) ; LC-MS :  $m/z = 313$  ( $\text{MH}^+$ ).

***N*<sup>1</sup>-((8-Methoxynaphtho[2,1-*b*]furan-2-yl)methyl)-*N*<sup>3</sup>-methylthiourea (10h).** Recrystallized from toluene (63%) as a white solid ; mp 208-210 °C ; <sup>1</sup>H NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  : 8.04 (br s, 1H), 7.90 (d, 1H, 8.7 Hz), 7.70 (d, 1H, 8.7 Hz), 7.65-7.64 (m, 2H), 7.56 (d, 1H, 9.0 Hz), 7.34 (s, 1H), 7.12 (dd, 1H, 9.0 Hz and 2.7 Hz), 4.89 (d, 2H, 5.1 Hz), 3.93 (s, 3H), 2.87 (s, 3H) ; <sup>13</sup>C NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  : 184.2, 158.4, 154.9, 152.4, 130.6, 129.0, 125.3, 124.9, 123.1, 116.9, 110.0, 103.5 (2C), 55.9, 41.7, 31.03 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3222 (NH), 1227 (CS) ; LC-MS :  $m/z = 301$  ( $\text{MH}^+$ ).

***N*<sup>1</sup>-Ethyl-*N*<sup>3</sup>-((8-methoxynaphtho[2,1-*b*]furan-2-yl)methyl)thiourea (10i).** Recrystallized from toluene (72%) as a white solid ; mp 185-186 °C ; <sup>1</sup>H NMR (300 MHz,  $\text{DMSO-}d_6$ )  $\delta$  :

8.02 (br s, 1H), 7.90 (d, 1H, 9.0 Hz), 7.71-7.68 (m, 2H), 7.65 (d, 1H, 2.7 Hz), 7.55 (d, 1H, 9.0 Hz), 7.35 (s, 1H), 7.12 (dd, 1H, 9.0 Hz and 2.7 Hz), 4.90 (d, 2H, 5.1 Hz), 3.93 (s, 3H), 3.03 (q, 2H, 7.5 Hz), 1.09 (t, 3H, 7.5 Hz) ;  $^{13}\text{C}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  : 181.3, 158.4, 154.9, 152.4, 130.6, 129.0, 125.3, 124.9, 123.1, 116.9, 110.0, 103.5 (2C), 55.9, 45.9, 41.2, 14.9 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3231 (NH), 1229 (CS) ; LC-MS :  $m/z$  = 315 ( $\text{MH}^+$ ).

**$N^1$ -((8-Methoxynaphtho[2,1-*b*]furan-2-yl)methyl)thiourea (10g).** To a solution of **10** (0.45 g, 2 mmol) in dioxane (5 mL) and THF (5 mL) was added potassium thiocyanate (0.23 g, 2.4 mmol). The mixture was stirred at 60 °C during 3 h then hydrolyzed and extracted with EtOAc. The organic layer was washed with a 1M solution of HCl and water, dried over  $\text{MgSO}_4$ , filtered and concentrated under reduced pressure. The resulting solid was treated with diethyl ether to afford **10g** (48%) as a white solid ; mp 233-235 °C ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  : 7.92-7.89 (m, 2H), 7.69-7.61 (m, 2H), 7.54 (d, 1H, 8.7 Hz), 7.42 (br s, 2H), 7.32 (s, 1H), 7.11 (dd, 1H, 9.0 Hz and 2.7 Hz), 4.37 (d, 2H, 4.1 Hz), 3.93 (s, 3H) ;  $^{13}\text{C}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  : 184.0, 158.3, 155.9, 152.3, 130.6, 129.0, 125.3, 124.5, 123.3, 116.7, 110.0, 103.5, 101.9, 55.8, 38.5 ; IR ( $\nu$ ,  $\text{cm}^{-1}$ , KBr) : 3284 (NH), 1226 (CS) ; LC-MS :  $m/z$  = 287 ( $\text{MH}^+$ ).

## 5.2. Pharmacological methods

5.2.1. **Reagents and Chemicals.** 2- $^{125}\text{I}$ -Iodomelatonin (2200 Ci/mmol) was purchased from NEN (Boston, MA). Other drugs and chemicals were purchased from Sigma-Aldrich (Saint Quentin, France).

5.2.2. **Cell Culture.** HEK (provided by A.D. Strosberg, Paris, France) and CHO cell lines stably expressing the human melatonin  $\text{MT}_1$  or  $\text{MT}_2$  receptors were grown in DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/mL penicillin and 100  $\mu\text{g}/\text{ml}$  streptomycin. Grown at confluence at 37 °C (95%  $\text{O}_2$ /5%  $\text{CO}_2$ ), they were harvested

in PBS containing EDTA 2 mM and centrifuged at 1000 x 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 (95 000g, 30 min, 4 °C) and the resulting pellet suspended in 75 mM TRIS (pH 7.5), 12.5 mM MgCl<sub>2</sub> and 2 mM EDTA. Aliquots of membrane preparations were stored at -80 °C until use.

**5.2.3. Binding Assays.** 2-[<sup>125</sup>I]iodomelatonin binding assay conditions were essentially as previously described.<sup>30</sup> Briefly, binding was initiated by addition of membrane preparations from stable transfected HEK or CHO cells diluted in binding buffer (50 mM Tris-HCl buffer, pH 7.4 containing 5 mM MgCl<sub>2</sub>) to 2-[<sup>125</sup>I]-iodomelatonin (25 or 200 pM for MT<sub>1</sub> and MT<sub>2</sub> receptors, respectively, expressed in HEK cells or 20 pM for MT<sub>1</sub> and MT<sub>2</sub> receptors expressed in CHO cells) 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 analysed using the program PRISM (Graph Pad Software Inc., San Diego, CA) to yield IC<sub>50</sub> (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<sub>D</sub>, the dissociation constant of the radioligand characterising the membrane preparation.<sup>33</sup>

[<sup>35</sup>S] GTPγS binding assay was performed according to published methodology.<sup>30</sup> Briefly, membranes from transfected CHO cells expressing MT<sub>1</sub> or MT<sub>2</sub> receptor subtype and compounds were diluted in binding buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 3 μM GDP, 3 mM MgCl<sub>2</sub>, and 20 μg/mL saponin). Incubation was started by the addition of 0.2 nM [<sup>35</sup>S]GTPγS to membranes (20 μg/ml) and drugs, and further followed for 1 h at room temperature. For experiments with antagonists, membranes were pre-incubated with both the

melatonin (3 nM) and the antagonist for 30 min prior the addition of [<sup>35</sup>S]GTPγS. Non specific 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 [<sup>35</sup>S]GTPγS binding (expressed in dpm) were for CHO-MT<sub>1</sub> or MT<sub>2</sub> 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 non specific 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<sub>50</sub> (Effective concentration 50 %) and E<sub>max</sub> (maximal effect) for agonists. Antagonist potencies are expressed as  $K_B = IC_{50} / 1 + ([Ago]/EC_{50} ago)$ , where IC<sub>50</sub> is the inhibitory concentration of antagonist that gives 50% inhibition of [<sup>35</sup>S] GTPγS binding in the presence of a fixed concentration of melatonin ([Ago]) and EC<sub>50</sub> ago is the EC<sub>50</sub> of the molecule when tested alone. I<sub>max</sub> (maximal inhibitory effect) was expressed as a percentage of that observed with melatonin at 3 nM for MT<sub>2</sub> receptor.

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