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Original article

New ligands at the melatonin binding site MT_3

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Abstract

The third melatonin binding site, MT3 is a non-classical one since it is not a seven transmembrane domains receptor, but an enzyme, quinone reductase 2. A major concern for the study of the physiological role of this site is the lack of specific ligands, permitting to more accurately dissect the pathways linked to the activation of MT3. Indeed, in the course of finding new ligands, we identified a new series of compounds with affinity to the binding site in the nM range, particularly 2,3-dimethoxy 7-hydroxy 10-methyl 5H 10H indeno(1,2-b)indol-10-one (DMHMIO), with a Ki of 190 pM. Based on slightly different and novel synthons compared to most of the compounds used in melatonin pharmacology studies, these compounds offer new perspective for the description of the melatonin pathways, so much more by not having any affinity towards the MT1 and MT2 'classical' melatonin receptors.

Keywords: Melatonin; MT3 binding site; Ligands; Binding; Quinone reductase 2

1. Introduction

Melatonin is an indole-derived neurohormone of long standing interest which is produced in the pineal gland and is derived from serotonin [1]. Because of its nocturnal synthesis, melatonin is suspected to relay the circadian rhythm and the information on the photoperiod to the peripheral organs for daily and seasonal physiological regulations [2,3]. Several cellular targets of melatonin have been detected since 1986, after the synthesis of 2-iodomelatonin [4], a very potent melatonin agonist that was rapidly used as 2-[^{125}I]-iodomelatonin. These melatonin binding sites MT_1 [5], MT_2 [6] and a Mel1c (only present in *X. laevis* [7]) have been cloned. They are G-protein coupled seven-transmembrane domain receptors and are characterized by subnanomolar affinities for melatonin and 2-iodomelatonin. In addition, there is evidence for a nanomolar melatonin binding site in Hamster brain [8,9] and kidney [9,10], called MT_3. Its specific ligands over MT_1 and MT_2 known to date include mainly 5-methoxycarbonylamino-*N*-acetyltryptamine (MCA-NAT) [11] and prasozin [9,11]. Besides its original pharmacology, MT_3 is characterized by fast kinetics of ligand association/dissociation [8,10,11], raising difficulties for affinity measurements. We recently stressed the need for more specific chemical tools in order to better understand the characteristics and the role of this binding site [3]. We reported several times on the search of new ligands to MT_3 [12,13]. These compounds were essentially bicyclic chemicals that loosely resembled the indolic melatonin.

In the present work, we report a series of new chemical entities as potent ligands of MT_3. They are indeno- and isoindolo-indol-10-one derivatives. These tetracyclic compounds are quite far from the previous reference compounds at this binding site and present affinities for MT_3 in the low nanomo-

Abbreviations: DMF, dimethylformamide; DMSO, dimethylsulfoxide.

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lar range. These tools are now ready for pharmacological and physiopathological studies on classical melatonin models.

2. Chemistry

Indeno (1,2-b) indol-10-one and isoindolo (2,1-a) indol-10-one have been already described in the literature [14]. Their syntheses start from 3-(o-iodo benzoyl) indole and 1-(o-iodo benzoyl) indole, respectively. It is difficult to prepare substituted iodobenzoyl derivatives as some migration of the benzoyl moiety leads to non-selective cyclization, making this method unusable in the present case.

Compounds **1** and **2** have been prepared by mean of a shared intermediate, following the scheme described in Fig. 1. Phtalides **3** are brominated in carbon tetrachloride or dichloromethane using *N*-bromosuccinimide following a very classical process [15]. Crude bromophtalides **4** are then substituted by triphenylphosphine to give Wittig reagents **5**. Condensation with a suitable 5-substituted 2-nitro benzaldehyde **6** gives the benzylidene phtalides **7** as a mixture of Z and E isomers. Compounds **7** can be either catalytically reduced to amines **8**, or rearranged to indan-1,3-diones **11** by action of a strong base as similarly previously described by Eskola and Hyrkko [16] and by Estevez et al. [17]. Aminobenzylidene lactones **8** are rearranged to 2-carboxyphenyl indoles **9** under the action of a strong base and then cyclized to give isoindolo indoles **10** by elimination of water and deprotected to compounds **1** by hydrogenolysis when necessary. Indan-1,3-diones **11** are reduced and spontaneously cyclized to give indeno indoles **12** in a one step procedure. Compound **12** was finally deprotected to compound **2** by hydrogenolysis. One easily understands that compound **11** lead to a single derivative if substitution of **11** is symmetrical and can lead to a mixture of compound **12** if the substitution is not symmetrical, due to a possible attack of the amine (resulting from nitro reduction) on both carbonyl functions, necessitating a chromatographic separation. In some case, substitution can induce the preferential

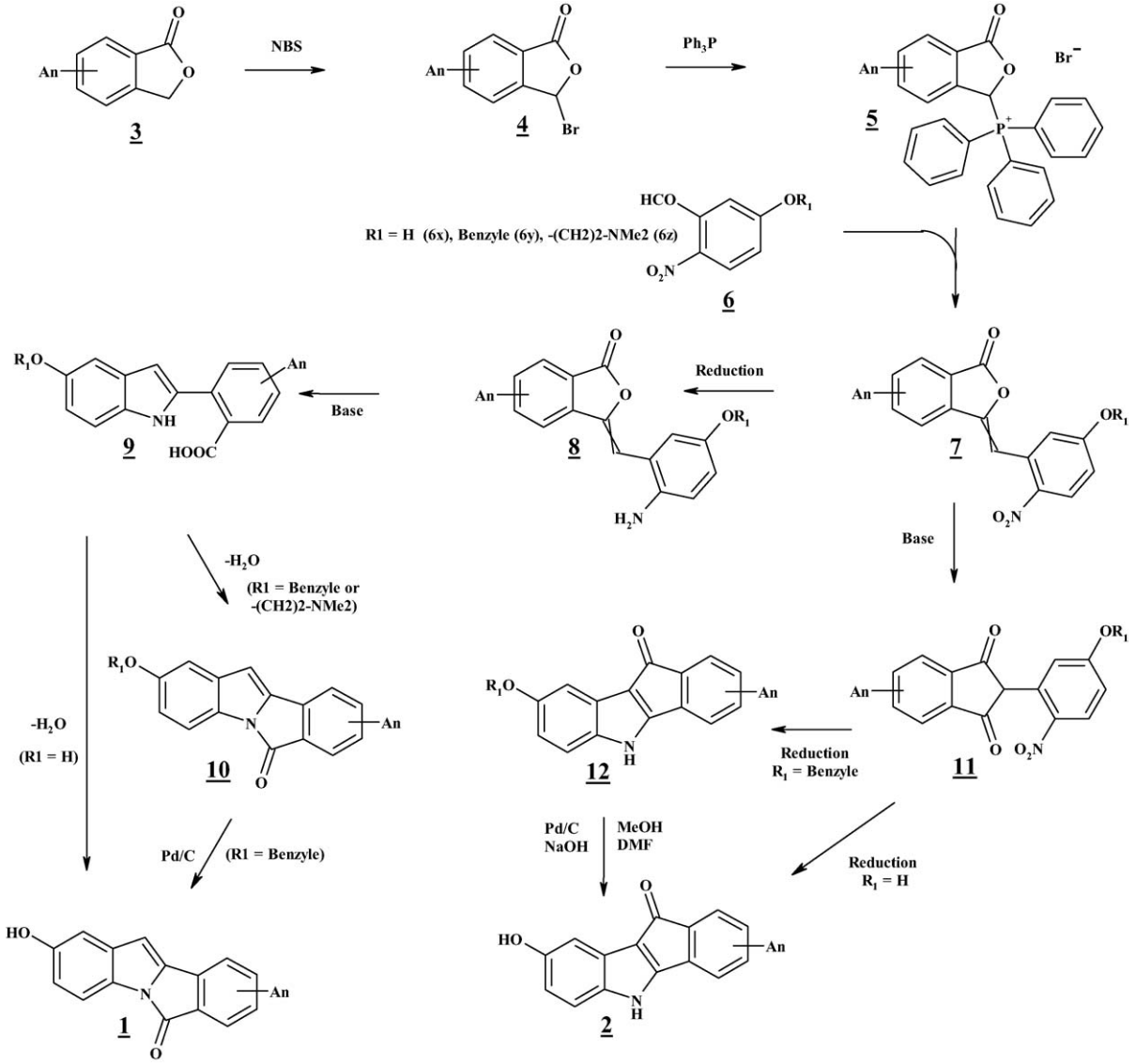


Fig. 1. General synthetic pathway for the obtention of compounds **1** and **2**.

formation of one isomer. One could have tried to transform in a one step procedure, phtalides **3** to 2-phenyl indane-1,3-diones **11** by reaction with the appropriate benzaldehyde under the action of MeONa, as described by Shapiro et al. [19]. We needed to isolate the nitrobenzylidenes **7** as key intermediates to produce either isoindolo-indoles **1** or indeno-indoles **2**. In these conditions of Shapiro et al. [19], intermediates **7**, probably formed during the reaction, cannot be isolated and therefore spontaneously evolved to compound **11**.

Methoxy-substituted compound **1** or **2** can be demethylated to give the polyphenolic derivatives **14** and **15**, respectively. Selective monodemethylation of polymethoxylated derivatives can be achieved with a low to moderate yield if the methoxy substituent is ortho or para to a carbonyl group (ketone or lactame) as already described in [20]. Using an excess of demethylating reagent allows complete demethylation, but the yields remain low because of the instability of the compounds in strong acid reaction mixtures. Most of the time, the reaction leads to a mixture of methoxy and hydroxy derivatives. When possible, these products have been separated by chromatography for characterization in order to illustrate more precisely their structure/activity relationships. In some case (compound **15**), using $BeCl_2$ as described by Sharghi and Tamaddon [21] gives a selective reaction with an acceptable yield. Indeno-indoles 10-ones **2** are selectively substituted on the indolic nitrogen to give compound **16**, or selectively iodinated with a very low yield, after *N*-methylation, to give **18** in an attempt to prepare a radioiodinated ligand for MT_3 receptors, by using the so-called Kirsch and Sluzarek reagent as described by Boussard et al. [22]. We also have access to reduced indeno-indoles **17** directly from the *O*-benzylated derivatives **12**. This is achieved by a simple modification of the debenzylation solvent (acetic acid instead of NaOH, in DMF/methanol mixture). Some of these transformations are very efficient, some others give very poor yield, but the products have been isolated and characterized to be tested in the pharmacological assay. All these transformations are listed in Fig. 2. A particular case (formation of compound **14d** from a different intermediate) is mentioned below in Fig. 3. Treatment with methanol of the crude Wittig reaction mixture resulting from **5g** and **6x**, leads to partial methanolyis of the lactone as indicated in Fig. 3. The isolations of compounds **7gx** (expected) and **13** were achieved with low yield, for characterization. Further reduction of **13** with Raney nickel followed by base treatment led directly to the expected di-hydroxy isoindoloindole **14d** described with others polyphenolic compounds in Table 10.

3. Biology

The activity of the compounds was determined by binding assays on MT_3-rich membrane from hamster brain, using iodomelatonin as the radioligand, as classical for the study of this

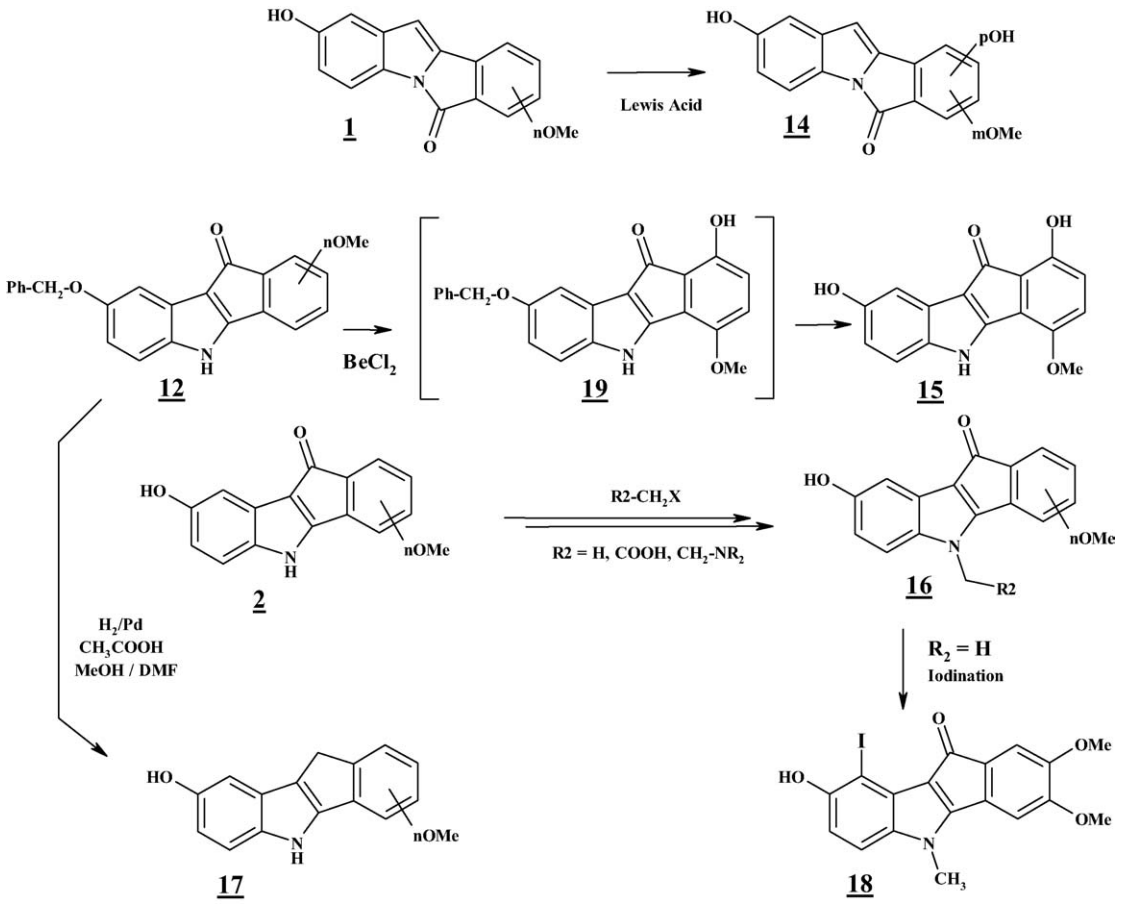


Fig. 2. Schematic pathways for the transformations of compounds **1** and **2**.

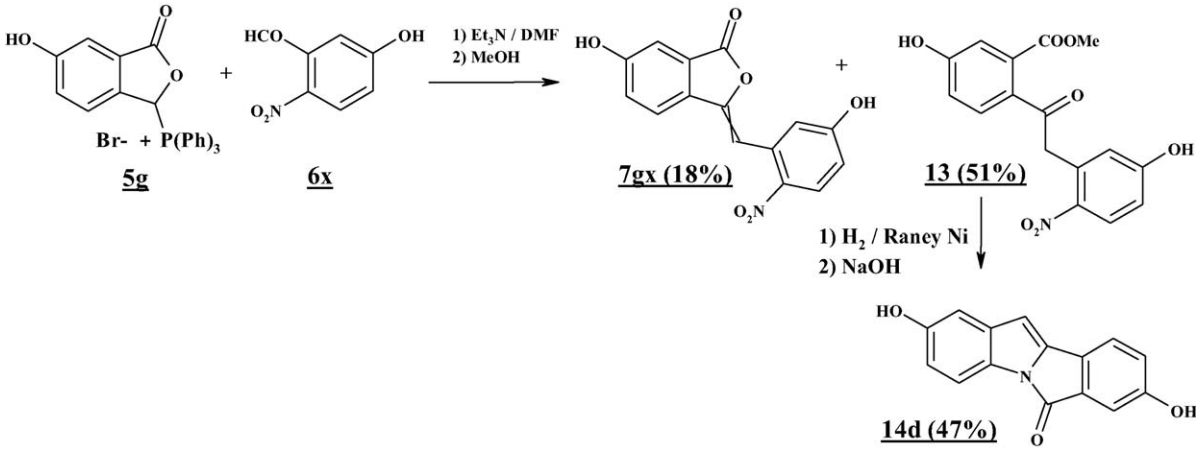


Fig. 3. Particular case: preparation of compound 3,8-dihydroxy-isoindolo(1,2-a)indol-10-one (**14d**).

binding site [9]. We demonstrated [13], after Molinari et al. [11], that the experimental binding data obtained with iodo-melatonin are completely correlated with those obtained with iodo-MCA-NAT. All these results presented here were confirmed with [^{125}I]MCA-NAT [11,13] (data not shown). The compounds have no affinity towards the melatonin receptors. MT_3 is not a membrane-bound receptor, the binding test cannot be complemented with a functional one. Indeed, despite suggestion that MT_3 might be linked to phosphoinositide production in RMPI cell lines [23], in other cell lines where the binding site can be measured, no IP production could be correlate to MT_3 specific ligands. A more integrated functional assay has been described for a MT_3 ligand in vivo, as it reduces the intraocular pressure [24,25]. We were not able to test our compounds in similar assays.

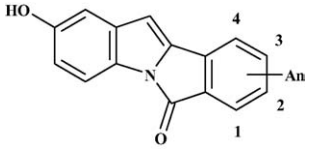
4. Results and discussion

A survey of the literature rapidly leads to an apparent paucity of tools to study the MT_3 melatonin binding site (see Boutin et al. [3] for review). Indeed, apart from the initially described, poorly active and non-specific prasozin [9,10] or attempts to synthesize loose derivatives of the melatonin core [11,12], the compounds able to interact with MT_3 are rather scarce. We recently published [13] a series of compounds that helped to understand the complex relationship between MT_3, the melatonin binding site and QR2, the enzyme that is actually this binding site [26]. Exploring further the SAR of molecules binding the MT_3 site is crucial for its understanding since it will provide new potent molecules that are specific towards the MT_3 binding site (i.e. with no affinity for MT_1 and MT_2). To keep the system as simple as possible, we started by trying to find compounds of new structures with affinity for MT_3. Such compounds were found in a classical screening program. A posteriori, a loose structural similarity can be depicted between these compounds and melatonin, as they might be thought of as two fused indole moieties. Furthermore, it is easy to dock some of these compounds inside the catalytic site of QR2 (A. Gohier, G. Ferry, J.A. Boutin, in preparation), since QR2 has been crystallized [27], and that co-crystallizations were also reported with resveratrol [28] and CB 1954 [29].

Overall variations around compound **2** gave more potent compounds than variations around compound **1**. Some of these compounds have remarkable affinities towards MT_3, far better than melatonin itself or MCANAT. For example, the compounds **1ax, 16aj** or **16cj** have subnanomolar affinities for MT_3 (with Ki of 430, 190 and 790 pM, respectively, see Tables 1B,2B and 3B). The minute modifications of the core, tetracyclic structures, led to some interesting observations concerning the SAR of this series of compounds.

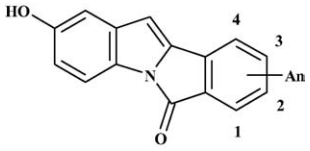
In brief, methoxylation of position 2 in compound **1** led to inactive compounds unless, methoxylation occurred also at position 3 (Table 1A). Furthermore, the presence of hydroxyl, as opposed to methoxyl moieties at most of the positions of this aromatic ring, led to less potent ligands (Table 1B). Since the presence oh two hydroxy onto the molecules might suggest a possibility of a quinone-like structure, we determined if the compounds were able to act as substrates of the QR2 enzyme, as we previously described for other quinones [30]. Having rules out this possibility, we turned to the moderate hydrophobic nature of methoxy residues, as compared to hydroxy residues. We concluded that the presence at some position (particularly, the position 2) of slightly more hydrophobic residue (such as methoxy) were not favorable for the fixation of the

Table 1A
Structure–activity relationship for compound **1** derivatives: influence of methoxy substitution and of demethylation



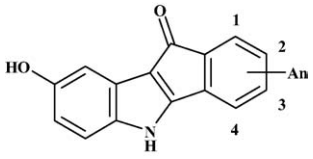
	An	Ki (nM)
1ax	2,3-diOMe	0.43
1bx	2,4-diOMe	> 100
1cx	1,4-diOMe	2.8
1dx	2-Ome	> 100
1fx	4-Ome	32

Table 1B
Structure–activity relationship for compound **1** derivatives: influence of methoxy substitution and of demethylation



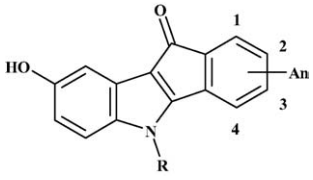
	An	Ki (nM)		An	Ki (nM)		An	Ki (nM)
1ax	2,3-diOMe	0.43	*14k*	3-OH 2-OMe	15			
1cx	1,4-diOMe	2.8	*14j*	1-OH 4-OMe	6.8	*14i*	1,4-diOH	36
1dx	2-OMe	> 100	*14g*	2-OH	1.9			
			14k	1-OH	> 100			

Table 2A
Structure–activity relationship for compound **2**: influence of demethylation and of *N*-methylation



	An	Ki (nM)		An	Ki (nM)
2c	1,4-diOMe	3	*15*	1-OH 4-OMe	2

Table 2B
Structure–activity relationship for compound **2**: influence of demethylation and of *N*-methylation



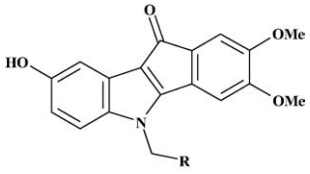
An		R	Ki (nM)		R	Ki (nM)
2,3-diOMe	*2a*	H	1.3	*16aj*	Me	0.19
1,4-diOMe	*2c*	H	3	*16cj*	Me	0.79

de-methylated compound—compare the IC50s of compound **1dx**, > 100 nM, and its de-methylated counterpart, the compound **14g**, 1.9 nM (see Table 1B). Nevertheless, the hydroxy residue at position 1, was deleterious to the fixation of the compound (compound **14k**, IC50 > 100 nM, see Table 2A). We intend to better understand these complicated situations by using either docking techniques on the available crystals, or to co-crystallisation approaches.

For compound **2**, the most striking variation was on the nitrogen, the methylation of which permitted to obtain **16aj**, the most active compound identified so far (Table 2B). Indeed, any other variations on that nitrogen diminished the potency of the new derivatives (see Table 3A). On the same core molecule, attempts were made to reduce the carbonyl. The replacement of this carbonyl by methylene considerably limited the potency of the molecule (Table 2B).

Furthermore, in a failing attempt to obtain an iodinated MT_3 radioligand, we tried to synthesize a derivative with an iodine

Table 3A
Structure–activity relationship for compound **2**: influence of nitrogen substituent nature, of ketone reduction and of iodination



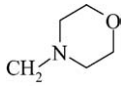
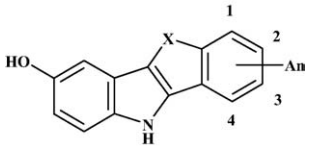
	R	Ki (nM)
16aj	H	0.19
16ak	CH_2-NMe_2	32
16al		26
16an	CH_2-COOH	100

Table 3B
Structure–activity relationship for compound **2**: influence of nitrogen substituent nature, of ketone reduction and of iodination



An		X	Ki (nM)		X	Ki (nM)
2,4-diOMe	*2b*	CO	0.58	*17b*	CH_2	45
1,4-diOMe	*2c*	CO	3	*17c*	CH_2	38

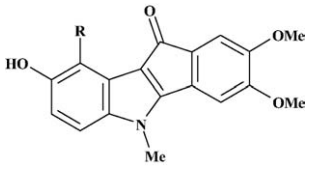
atom (i.e. susceptible to be radiolabeled) but this transformation led to an almost inactive compound (Table 3C).

Finally, as expected from our knowledge of MT and MT2 molecular pharmacology, none of these compounds showed significant affinity towards these receptors (see Table 4).

5. Conclusion

The present report describes structures and synthesis details on new ligands of the MT_3 melatonin binding site. Provided more data will be gathered on their broad specificity (i.e. outside the melatoninergic pharmacology), these substances will permit to launch a series of studies of the physio-pathological

Table 3C
Structure–activity relationship for compound **2**: influence of nitrogen substituent nature, of ketone reduction and of iodination



	R	Ki (nM)		R	Ki (nM)
16aj	H	0.19	*18*	I	> 100

Table 4
Lack of affinity of MT_3 compounds for the MT_1 and MT_2 melatonin receptors

Compound	MT1 IC50 (μM)	MT2 IC50 (μM)
1fx	> 10	> 10
14k	> 10	> 10
1dx	> 10	> 10
14g	> 10	> 10
1bx	> 10	> 10
16cj	> 10	> 10
16aj	> 10	> 10
16al	> 10	0.56
16ak	> 10	> 10
16an	> 10	> 10

Compounds, dissolved in DMSO were tested at full range of concentrations from 0.1 nM up to 10 μM against [^{125}I] 2-idomelatonin binding, as described previously in [19].

role of *MT_3*, as for instance on intraocular pressure or the numerous properties of *MT_3*/QR2 [31].

6. Experimental protocols

6.1. General

All compounds were submitted to standard analytical methods and gave satisfactory results. ^{1}H NMR spectra were performed on an AC200 (if not specified otherwise) spectrometer (Brucker, Wissembourg, France). The following abbreviations are used in description: s: singlet, d: doublet, t: triplet, dd: double doublet, sl: singlet large, m: multiplet, ex: signal exchangeable with D_2O. δ are expressed in ppm, using TMS as a reference. Infrared spectra were performed on an IF25 of IF28 spectrometer (Brucker), using samples dispersed in Nujol. Frequencies are expressed in cm^{-1}. Melting points (instant) were determined on a Leica-Reichert Kofler apparatus (Leica-Reichert, Rueil-Malmaison, France). Preparative chromatography was performed using 230–400 mesh Merck silica gel 60 (Merck-Darmstadt, Germany). Kugelrohr apparatus was a GKR-51 (Büchi, Switzerland).

6.2. Typical procedures for the preparation of 2,3-dimethoxy 7-hydroxy [10H] isoindolo (1,2-a) indol-10-one (**1a**) and 2,3-dimethoxy 8-hydroxy 5H indeno (1,2-b) indol-10-one (**2a**)

For each step, a table (see Tables 5–16) gives the results for the different substituted derivatives obtained.

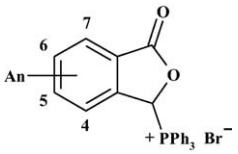
6.2.1. Procedure for preparation of 2,3-dimethoxy 7-hydroxy [10H] isoindolo (1,2-a) indol-10-one (**1a**)

*6.2.1.1. Triphenyl (5,6-dimethoxy phtalid-3-yl) phosphonium bromide **5a***. An amount of 62 g of 5,6-dimethoxy phtalide **3a** was dissolved in 800 ml of carbon tetrachloride. The mixture was refluxed by heating with an halogen lamp (500 W). Sixty-eight grams (1.2 equiv.) of *N*-bromosuccinimide were then added by portions. After this addition was completed, the reflux was maintained for 2 additional hours. The mixture was then cooled down to 10 °C. The solid precipitate of succinimide was collected by filtration. The solvent of the filtrate was then distilled under reduced pressure. Eight hundred milliliters of anhydrous toluene were added to the residue and this mixture, filtered again to eliminate remaining succinimide. The crude dimethoxy bromophtalide **4a** could be isolated after the distillation of toluene, as an oily brown residue. 83.7 g (1 eq) of triphenylphosphine was added to the toluene solution of **4a** and this mixture, heated to reflux for 16 h. After cooling, the crystalline precipitate of Wittig reagent **5a** was collected by filtration, washed three times with 100 ml of anhydrous toluene and then with boiling isopropanol. Finally it was washed with cold diethyl ether and dried under vacuum. One hundred twenty-eight grams (75%) of triphenyl (5,6-dimethoxy phtalid-3-yl) phosphonium bromide **5a** were obtained in this way. Table 5 summarizes the analytical data concerning all the compounds prepared in this way. Wittig reagent **5a** must react with the suitable nitrobenzaldehyde obtained by substitution of the phenol [18].

*6.2.1.2. 5-Benzyloxy 2-nitro benzaldehyde (**6y**)*. A solution of 20 g of 5-hydroxy 2-nitrobenzaldehyde **6x** in 60 ml of DMF was added in 5 min at room temperature to a suspension of 33 g (2 equiv.) of potassium carbonate in 20 ml of DMF and 160 ml of diisopropyl ether. This mixture was then refluxed for 2 hours, and kept at room temperature. 13.8 ml of benzyl chloride was added and the reaction mixture was refluxed for 15 hours. After cooling, potassium salts were filtered out and washed with DMF. Filtrate was kept to dryness. The residue was triturated with diethyl ether and filtrated. The benzyloxy derivative crystallized slowly from filtrate and was collected by filtration. 24.5 g (79%) of 5-benzyloxy 2-nitro benzaldehyde **6y** were obtained in this way. 5-(2-Dimethylaminoethoxy) 2-nitro benzaldehyde (**6z**) was prepared following the same procedure, replacing benzyl chloride by 2-chloroethyl dimethyl amine, and diethyl ether by methylene chloride. The pure compound was obtained by washing the methylene chloride solution with water, drying it with magnesium sulfate and eliminating the solvent under reduced pressure. Table 6 summarizes the analytical data concerning all the compounds prepared in the same way.

*6.2.1.3. 5,6-Dimethoxy 3-(2-nitro 5-hydroxy benzylidene) phtalide (mixture of E/Z isomers) (**7ax**)*. One hundred grams of Wittig reagent **5a** was added by small portions to a stirred solution of 31.2 g (1 equiv.) of 5-hydroxy 2-nitro benzaldehyde

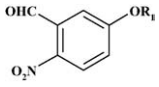
Table 5
Analytical data



	A	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
5a	5,6-Dimethoxy	~260	75	1780	$CDCl_3$ + DMSO: 9.3(s 1H), 7.8(m 15H), 6.5(s 1H), 7.2(s 1H), 3.9(s 3H), 3.6(s 3H)
5b	4,6-Dimethoxy	> 260	82	1772	DMSO: 8.5(s 1H), 7.9(m 15H), 6.9(dd 2H), 3.8(s 3H), 3.3(s 3H)
5c[a]	4,7-Dimethoxy	> 260	78	1780	DMSO: 8.5(s 1H), 7.7(m 15H), 7.4(d 1H), 7.2(d 1H), 3.8(s 3H), 3.35(s 3H)
5d	6-Methoxy	> 260	56	1785	DMSO: 8.5(s 1H), 7.9(m 15H), 7.4(m 2H), 6.8(d 1H), 3.8(s 3H)
5e	7-Methoxy	> 260	36	1788	$CDCl_3$ + DMSO: 9.75(s 1H), 7.85–7.65(m 15H), 7.55(t 1H), 7.0(d 1H), 6.65(dd 1H), 3.9(s 3H)
5f	4-Methoxy	247	38	1777	$CDCl_3$: 9.75(s 1H), 8.1–7(m 18H), 3.4(s 3H)
5g[b]	6-Hydroxy	> 260	55	1770	DMSO: 8.5(s 1H), 8.1–7.7(m 15H), 7.2(dd 1H), 7.1(d 1H), 6.7(d 1H)

[a] Bromination of phtalide **4c** has been realized in methylene chloride.
[b] Compound **5g** has been isolated from 6-acetoxy phtalide, the hydroxy group is liberated from ester function during treatment with isopropanol.

Table 6
Analytical data



	R1	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
6x	H	Commercially available			
6y	Benzyl	73	79	1705	DMSO: 10.5(s 1H), 8.2(d 1H), 7.4(m 6H), 7.2(dd 1H), 5.2(s 2H)
6z	$-(CH2)_2-N(Me)_2$	Oil	50	2824, 2774, 1700, 1517, 1331 (film)	$CDCl_3$: 10.45(s 1H), 8.15(d 1H), 7.35(d 1H), 7.18(dd 1H), 4.2(t 2H), 2.8(t 2H), 2.35(s 6H)

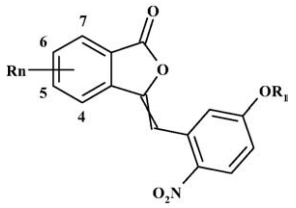
6x in 1000 ml of DMF and 26 ml (1 equiv.) of triethylamine at room temperature. The reaction mixture was heated to 90 °C for 90 min, and then cooled to room temperature and kept to dryness under reduced pressure. The residue was triturated with diethyl ether. The solid was filtrated, treated with methanol under reflux, cooled to room temperature and filtered again to give 44.8 g (70%) of compound **7ax** as a mixture (75:25) of E and Z isomers of 5,6-dimethoxy 3-(2-nitro 5-hydroxy benzylidene) phtalide. Table 7 summarizes the analytical data concerning all the compounds prepared in the same way.

*6.2.1.4. 5,6-Dimethoxy 3-(5-hydroxy 2-amino benzylidene) phtalide (**8ax**)*. Fifteen grams of compound **7ax** were dissolved in 460 ml of DMF and reduced in a Parr apparatus with 36 ml of Raney nickel suspension under mild hydrogen pressure (3–5 atm) until the reduction was complete. The mixture was then filtered under nitrogen atmosphere. The nickel residue was rinsed with DMF, and the filtrate kept to dryness, washed with diethyl ether and dried under vacuum. 12.7 g (93%) of almost pure 5,6-dimethoxy 3-(5-hydroxy 2-amino benzylidene) phtalide **8ax** were obtained. Table 8 summarizes the analytical data concerning all the compounds prepared in the same way.

*6.2.1.5. 5-Hydroxy 2-(2-carboxy 4,5-dimethoxy phenyl) indole (**9ax**)*. An amount of 12.7 g of compound **8ax** was added to 95 ml of ethanol. 81 ml (2 eq) of 1 N NaOH solution were added, the reaction mixture turned to dark red color. It was refluxed for 45 min. After cooling on an ice bath, 100 ml of 1 N HCl solution were added while stirring. pH decreased to ~1. The mixture was kept at room temperature for 1 hour, the precipitate was collected by filtration, washed with diethyl ether and dried. A 7 g fraction of compound **9ax** was obtained. The aqueous phase of the filtrate was concentrated to 1/3. A dark gummy mass formed and was dissolved in a neutral aqueous methanol mixture. This solution was extracted with diethyl ether. The last acidic aqueous phase was also extracted with diethyl ether. All the ethereal phases were added, treated with active charcoal, and concentrated to obtain a dark gray residue. This residue was washed with cold diethyl ether and dried. An additional 2.6 g of compound was obtained. 9.6 g (76%) of 5-hydroxy 2-(2-carboxy 4,5-dimethoxy phenyl) indole **9ax** were obtained in this way. Table 9 summarizes the analytical data concerning all the compounds obtained in the same way.

*6.2.1.6. 2,3-Dimethoxy 7-hydroxy 10H isoindolo (1,2-a) indol-10-one (**1ax**)*. Method 1: *Cyclo-dehydration using catalytic APTS*. Two grams of compound **9ax** and a catalytic amount of *p*-toluene sulfonic acid were added to 80 ml of toluene. The mixture was refluxed with stirring for 12 hours in a reactor

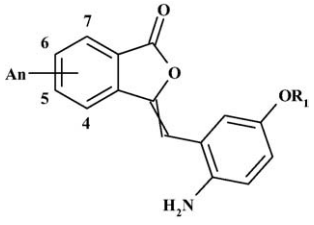
Table 7
Analytical data



	R1	R	M.p.[a] (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
7ax	H	5,6-Dimethoxy	253	70	3248, 1760, 1660	DMSO: 11.2(m(ex) 1H), 8.2(d 1H), 7.4(s 1H), 7.15(d 1H), 7.1(s 1H), 7.0(dd 1H), 6.7(s 1H), 3.9(s 3H), 3.4(s 3H)
7ay	Benzyl	5,6-Dimethoxy	190	75	1761, 1660, 1570, 1358	$CDCl_3$: 8.3(d 1H), 7.3(m 7H), 7.2(dd 1H), 7.1(s 1H), 6.4(s 1H), 5.1 (s 2H), 4.0(s 3H), 3.6(s 3H)
7by	Benzyl	4,6-Dimethoxy	192	69	1774, 1518, 1507, 1334	$CDCl_3$: 8.05(d 1H), 7.75(s 1H), 7.55–7.3(m 6H), 7.0(S 1H), 6.95 (dd 1H), 6.75(s 1H), 5.2(s 2H), 4.0(s 3H), 3.9(s 3H)
7cx	H	4,7-Dimethoxy	> 260	85	3357, 1746, 1589, 1333	DMF (300 MHz): 11.4(sl(ex) 1H), 8.15(d 1H), 7.65(d 1H), 7.6(d 1H), 7.5(s 1H), 7.35(d 1H), 7.0(dd 1H), 4.15(s 3H), 4.0(s 3H)
7cy	Benzyl	4,7-Dimethoxy	168	77	1781, 1636	$CDCl_3$: 8.05(d 1H), 7.75(d 1H), 7.6(s 1H), 7.55–7.3(m 5H), 7.15 (d 1H), 6.95(m 2H), 5.75(s 2H), 4(2s 6H)
7dx	H	6-Methoxy	225	51	3323, 1740, 1514, 1328	DMSO: 12.1–10.5(sl(ex) 1H), 8.25(d 1H), 7.45(d 1H), 7.3(dd 1H), 7.1(d 1H), 7.05(m 3H), 3.9(s 3H)
7ez	$(CH_2)_2NMe_2$	7-Methoxy	134	73	1779, 1503	$CDCl_3$: 8.25(d 1H), 7.4(t 1H), 7.1(m 3H), 6.9(d 1H), 6.6(d 1H), 4.15(t 2H), 3.95(s 3H), 2.75(t 2H), 2.35(s 6H)
7fx	H	4-Methoxy	> 260	57	3251, 1740, 1660	DMSO: 11(m(ex) 1H), 8.1(d 1H), 7.7(m 1H), 7.6(m 2H), 7.5(d 1H), 7.4(s 1H), 6.9(dd 1H), 4.1(s 3H)
7gx[b]	H	6-Hydroxy	-	6	-	DMSO: 8.25(dd 1H), 7.2–6.95 (m 6H)

[a] Melting points are given only as an indication, as the compounds **7** are most of the time obtained as mixtures of E and Z isomers.
[b] **7gx** has been isolated with a very low yield (see Scheme 3), and is crystallized with 30% DMF.

Table 8
Analytical data

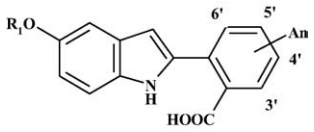


	R1	A	M.p.[a] (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
8ax	H	5,6-Dimethoxy	231	93	1741	DMSO: 8.65(s(ex) 1H), 7.35(2s 1H), 7.15(s 1H), 6.8(2s 1H), 6.7–6.5(m 3H), 5.05(s(ex) 1H), 4.05(s(ex) 1H), 4.0(s 3H), 3.7(s 3H)
8by	Benzyl	4,6-Dimethoxy	141	84	1752	$CDCl_3$: 7.6–7.25(m 6H), 6.95(d 1H), 6.85–6.6(m 4H), 5.05(s 2H), 4(s 3H), 3.9(s 3H), 3.6(sl(ex) 2H)
8cx	H	4,7-Dimethoxy	> 260	80	1723, 1619	DMSO: 8.6(s(ex) 1H), 7.4(d 1H), 7.3(d 1H), 7.1(d 1H), 6.9(s 1H), 6.6(d 1H), 6.5(dd 1H), 4.7(m(ex) 2H), 3.9(2s 6H)
8dx	H	6-Methoxy	203	94	1777	DMSO: 8.6(s(ex) 1H), 7.5(d 1H), 7.4(d 1H), 7.35(dd 1H), 6.65(m 4H), 4.6(s(ex) 2H), 3.9(s 3H)
8ez	$(CH_2)_2NMe_2$	7-Methoxy	148	75	1773, 1638	$CDCl_3$: 7.4(t 1H), 7–6.7(m 5H), 6.6(sl 1H), 4(s 3H), 4(t 2H), 3.5(m (ex) 2H), 2.7(t 2H), 2.3(s 6H)
8fx	H	4-Methoxy	234	91	1756, 1666	DMSO: 8.6(m(ex) 1H), 7.5(m 3H), 7.3(d 1H), 6.9(s 1H), 6.6(m 2H), 4.7(m(ex) 2H), 4.1(s 3H)

[a] Melting points are given only as an indication, as the compounds **7** are most of the time obtained as mixtures of E and Z isomers.

equipped with a Dean-Stark apparatus. The reaction mixture was then cooled to room temperature and filtered. The precipitate was washed two times with 5 ml of cold toluene, three times with 5 ml of cold methanol and then with 5 ml of diethyl ether. This material was then dissolved in the minimum of THF, and this solution filtered through a Millipore membrane. The THF solution was kept to dryness under reduced pressure, the residue refluxed for a few minutes with 10 ml of methanol, the mixture cooled on an ice bath, filtered again and the residue finally washed with diethyl ether and dried under vacuum.

Table 9
Analytical data



	R1	A	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
9ax	H	4′,5′-Dimethoxy	160	76	1666, 1641	DMSO (300 MHz): 12.6(sl(ex) 1H), 10.9(m(ex) 1H), 8.6(m(ex) 1H), 7.3(s 1H), 7.2(d 1H), 7.1(s 1H), 6.8(sl 1H), 6.6(d 1H), 6.3(sl 1H), 3.8(2s 6H)
9by	Benzyl	4′,6′-Dimethoxy	210	98	1688	DMSO: 12.6(s(ex) 1H), 10.9(m(ex) 1H), 7.4(m 5H), 7.3(d 1H), 7.1 (d 1H), 6.8(m 3H), 6.2(sl 1H), 5.1(s 2H), 3.9(2s 6H)
9cx	H	3′,6′-Dimethoxy	140	91	1700	DMSO (300 MHz): 12.8(m(ex) 1H), 10.7(sl(ex) 1H), 8.6(s(ex) 1H), 7.2(d 1H), 7.1(2d 2H), 6.8(d 1H), 6.6(dd 1H), 6.3(d 1H), 3.7 (2s 6H)
9dx	H	4′-Methoxy	210	91	1711	DMSO: 11(m(ex) 2H), 8.65(sl(ex) 1H), 7.6(d 1H), 7.1(d 1H), 6.8(d 1H), 6.6(dd 1H), 6.3(s 1H), 3.8(s 3H)
9ez	$(CH_2)_2NMe_2$	3′-Methoxy	> 260	81	1580	DMSO[a]: 15–14(m(ex) 2H), 7.5(t 1H), 7.3(m 2H), 7.1(m 2H), 6.8 (dd 1H), 6.6(sl 1H), 4.3(m 2H), 3.9(s 3H), 3.5(m 2H), 2.9(s 6H)
9fx	H	6′-Methoxy	234	75	1722, 1627	DMSO (300 MHz): 12.6(m(ex) 1H), 10.7(sl(ex) 1H), (8.6(s(ex) 1H), 7.4(t 1H), 7.2(m 3H), 6.8(d 1H), 6.6(dd 1H), 6.1(sl 1H), 3.8(s 3H)

[a] With addition of 2 drops of CF_3COOD.

1.2 g (64%) of 2,3-dimethoxy 7-hydroxy 10H isoindolo (1,2-a) indol-10-one **1ax** was obtained this way. In one case, purification has been completed by chromatography on silica, developed with dichloromethane.

Method 2: *Cyclo-dehydration using dicyclohexyl-carbo-diimide*. Eight grams of compound **9ax** were dissolved in 175 ml of DMF and placed in a reactor. 6.37 g of dicyclohexyl-carbodiimide were added under stirring and the mixture, refluxed for 90 min. The mixture, cooled at room temperature, was filtered. The filtrate was kept to dryness under reduced pressure and the residue triturated, first with diethyl ether, then with dichloromethane. The solid obtained was added to the first precipitate and this mixture was purified by distillation under vacuum in a Kugelrohr apparatus to separate it from dicyclohexyl-urea. The compound obtained was finally crystallized with isopropanol and washed with petroleum ether to give 5.46 g (73%) of 2,3-dimethoxy 7-hydroxy 10H isoindolo (1,2-a) indol-10-one **1ax**. In some cases (compounds **10by** and **10ez**), the distillation has been replaced by chromatography on silica, developed with a mixture of dichloromethane and methanol (95:5). Table 10 summarizes the analytical data concerning all the compounds obtained in the same way.

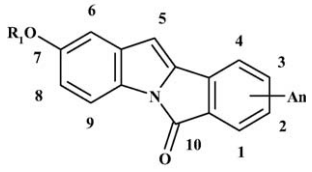
*6.2.1.7. 2,4-Dimethoxy 7-hydroxy 10H isoindolo (2,1-a) indol-10-one (**1bx**)*. 4.1 g of compound **10by** (see Table 10) were dissolved in a mixture of 170 ml of DMF and 55 ml of methanol, and the solution was placed in a Parr apparatus. 410 mg of palladium catalyst (10% on C) were introduced under nitrogen flow and the mixture was hydrogenated under a 4–6 atm pressure at 50 °C until the theoretical absorption was complete. After cooling, the catalyst was eliminated by filtration and the filtrate was dried under reduced pressure. The residue was triturated with an excess of diethyl ether for 24 hours and filtered again. This new residue was then triturated with 25 ml of a 1:1 mixture of methanol and dichloromethane for 10 hours and filtered. This final residue was dried under vacuum. 2.75 g (88%) of 2,4-dimethoxy 7-hydroxy 10H isoindolo (2,1-a) indol-10-one **1bx** were obtained. Table 10 summarizes the analytical data concerning the compounds obtained in the same way.

*6.2.1.8. 1,7-Dihydroxy 4-methoxy isoindolo (1,2-a) indol-10-one (**14c**) and 1,4,7-Trihydroxy isoindolo (1,2-a) indol-10-one (**14c′**)*. Demethylation of methoxy derivatives can be achieved using different demethylating agents but the reaction remains incomplete, or produces a large proportion of degradation compounds if an excess of reagent is used. The following examples are detailed for illustrating one process.

0.44g of 1,4-dimethoxy derivative **1cx** was added to a mixture of 20 ml of acetic acid and 0.65 ml of 48% aqueous solution of bromhydric acid. The mixture was refluxed for 16 h, and then dried under reduced pressure. The residue was triturated with a large excess of diethylether and filtrated. The precipitate was purified by chromatography on silica, eluating with a mixture of dichloromethane and methanol (98:2 to 95:5). Forty milligrams of 1,4,7-trihydroxy isoindolo (1,2-a) indol-10-one **14c′** were isolated. The filtrate was chromatographied separately and developed with the same solvent to finally produce 40 mg of 1,7-dihydroxy 4-methoxy isoindolo (1,2-a) indol-10-one **14c** and 60 mg of remaining starting material **1cx**. Table 11 summarizes the analytical data concerning the compounds obtained in the same way.

*6.2.1.9. 6-Hydroxy 3-(2-nitro 5-hydroxy benzylidene) phtalide (**7gx**) and 5-Hydroxy 2-(2-nitro 5-hydroxy phenylacetyl) benzoic acid methyl ester (**13**)*. Five grams of phosphonium bro-

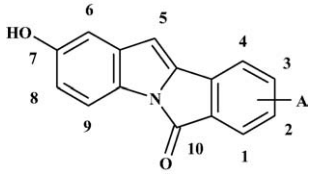
Table 10
Analytical data



	R1	A	M.p. (°C)	Yield (%, method)	IR (Nujol suspension)	NMR (δ ppm)
1ax	H	2,3-Dimethoxy	> 250	64 (1) 73 (2)	1726	DMSO: 9.3(m(ex) 1H), 7.5(d 1H), 7.4(s 1H), 7.3(s 1H), 6.9(sl 1H), 6.7(d 1H), 6.6(s 1H), 4(2s 6H)
10by	Benzyl	2,4-Dimethoxy	194	74 (1)	1720	DMSO: 7.7(d 1H), 7.4(m 5H), 6.9(m 3H), 6.6(d 1H), 6.4(s 1H), 5.1(s 2H), 3.9(2s 6H)
1bx	H	2,4-Dimethoxy	> 250	88[a]	1694	DMSO: 9.3(s(ex) 1H), 7.5(d 1H), 6.9(m 3H), 6.7(dd 1H), 6.4(s 1H), 3.9(2s 6H)
1cx	H	1,4-Dimethoxy	> 250	84 (2)	1716, 1733	DMSO (300 MHz): 9.3(s(ex) 1H), 7.5(d 1H), 7.3(d 1H), 7.1(d 1H), 6.9(d 1H), 6.7(dd 1H), 6.6(s 1H), 3.9(2s 6H)
1dx	H	2-Methoxy	217	57 (2)	1696	DMSO (300 MHz): 9.35(s(ex) 1H), 7.65(d 1H), 7.5(d 1H), 7.25(d 1H), 7.2(dd 1H), 6.9(d 1H), 6.25(dd 1H), 6.2(s 1H), 3.85(s 3H)
10ez	$(CH_2)_2NMe_2$	1-Methoxy	134	70 (2)	1719	DMSO (300 MHz): 7.8(d 1H), 7.4(m 1H), 7.1(d 1H), 7(d 1H), 6.9 (dd 1H), 6.8(d 1H), 6.5(s 1H), 4.1(t 2H), 4(s 3H), 2.7(t 2H), 2.3(s 6H)
1fx	H	4-Methoxy	~250	72 (2)	1697, 1718	DMSO: 9.3(m(ex) 1H), 7.6(d 1H), 7.4(d 1H), 7.3(m 2H), 6.9(d 1H), 6.7(dd 1H), 6.6(s 1H), 4(s 3H)

[a] Yield of debenzylation reaction (from **10by**).

Table 11
Analytical data



	A	M.p. (°C)	Yield (%, reagent)	IR (Nujol suspension)	NMR (δ ppm)
14a	3-Hydroxy 2-methoxy	> 260	6 ($AlCl_3$/toluene)	1693	DMSO (400 MHz): 10.25(s(ex) 1H), 9.3(s(ex) 1H), 7.45(d 1H), 7.22(s 1H), 7.08(s 1H), 6.83(d 1H) 6.7(m 1H), 6.63(s 1H), 3.75(s 1H)
14c	1-Hydroxy 4-methoxy	220	10 (HBr 48% aq CH_3COOH)	1695	DMSO (300 MHz): 10.1(s(ex) 1H), 9.3(s(ex) 1H), 7.5(d 1H), 7.2(d 1H), 6.9(d 1H), 6.8(d 1H), 6.7(m 1H), 6.5(s 1H), 3.9(s 1H)
14c′	1,4-Dihydroxy	> 260	10 (HBr 48% aq CH_3COOH)	1666	DMSO: 9.3(sl(ex) 1H), 8.5(sl(ex) 1H), 7.45(d 1H), 6.85(m 2H), 6.7(dd 1H) 6.45(d 1H), 6.45(s 1H)
14d	2-Hydroxy	> 260	47[a]	1692	DMSO: 10.2(m(ex) 1H), 9.3(m(ex) 1H), 7.5(2d 2H), 7(d + dd 2H), 6.9 (d 1H), 6.7(dd 1H)
14e	1-Hydroxy	250	34[b] (BBr_3 CH_2Cl_2)	1703, 1689	DMSO (300 MHz): 10.5(sl(ex) 1H), 9.2(sl(ex) 1H), 7.5(d 1H), 7.3(t 1H), 7.1(d 1H), 6.8(sl 1H), 6.8(d 1H), 6.7(m 2H)

[a] Prepared from compound **13** as described above.
[b] Prepared from **10ez**, aminoalkyl chain is eliminated during demethylation process.

mide **5g** were placed in a reactor with 57 ml of DMF and 1.4 ml of triethylamine. 1.7 g of 2-nitro 5-hydroxy benzaldehyde **6x** were added by small fractions in 5 min under stirring. The brown reaction mixture obtained was warmed to 90 °C for 40 min, then cooled to room temperature and dried under reduced pressure. The residue was triturated with an excess of diethyl ether. Triethyl ammonium bromine was eliminated by filtration. The ethereal filtrate was dried and the oily residue purified by silica chromatography developed with a mixture of dichloromethane and diethyl ether (95:5 to 90:10) to give 0.57 g (18%) of 6-hydroxy 3-(2-nitro 5-hydroxy benzylidene) phtalide **7gx** (described in Table 7) and 1.68 g (51%) of 5-hydroxy 2-(2-nitro 5-hydroxy phenylacetyl) benzoic acid methyl ester **13** (IR (Nujol suspension): 1690, 1666), NMR (DMSO): 10.9(m(ex) 2H), 8.1(d 1H), 7.9(d 1H), 7(dd 1H), 6.9(m 3H), 4.7(s 2H), 3.7(s 3H).

*6.2.1.10. 3,8-Dihydroxy isoindolo (1,2-a) indol-10-one (**14d**).* 5.9 g of compound **13** were reduced at room temperature with 14.8 ml of Raney nickel suspension in 300 ml of DMF under a 6 atm hydrogen pressure until the absorption was complete. The catalyst was then separated by filtration. The DMF filtrate

was kept to dryness and this residue triturated in an excess of dichloromethane. The concentration of the dichloromethane solution under reduced pressure to about 100 ml, liberated a first precipitate of 2.5 g of the intermediary amine. This filtrate and the precedent residue were added and treated by chromatography on silica, developed with a mixture of dichloromethane and methanol (98:2 to 95:5). A second sample of 1.6 g of amine was isolated from the first eluted fractions. 4.1 g of the crude intermediate amine obtained were dissolved in a mixture of 10 ml of methanol and 20 ml of 1 N NaOH solution. The mixture was stirred at room temperature for 3 hours and then concentrated to about 15 ml at room temperature under reduced pressure. The precipitate obtained was collected, refluxed a few minutes in 10 ml of methanol, cooled and filtered again. This last precipitate was then dissolved in a mixture of acetone and DMF, the solution obtained was filtrated on a Millipore filter. The filtrate was dried, and the residue triturated with diethyl ether and finally dried under vacuum. 2.1 g (47%) of 3,8-dihydroxy isoindolo (1,2-a) indol-10-one **14d** were obtained. Table 11 summarizes the analytical data obtained on all the polyphenolic isoindolo (1,2-a) indol-10-one compounds.

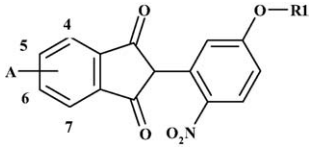
*6.2.2. Procedure for preparation of 2,3-dimethoxy 8-hydroxy 5H indeno (1,2-b) indol-10-one (**2a**)*

*6.2.2.1. 5,6-Dimethoxy 2-(2-nitro 5-hydroxy phenyl) indan-1,3-dione (**11a**)*. Twenty-six grams of compound **7y** were added to a mixture of 60 ml of 4 N NaOH solution and 92 ml of methanol. The dark red mixture was warmed to 40 °C under vigorous stirring for 1 h, cooled down to 0 °C on an ice bath and acidified with 75 ml of a 4 N HCl solution. An abundant white precipitate formed. A vigorous stirring was maintained at room temperature for 12 h. The precipitate was collected by filtration, washed with cold water and dried under vacuum. The purification by silica chromatography, developed with a mixture of dichloromethane and methanol (99:1) gives 19.5 g (75%) of 5,6-dimethoxy 2-(2-nitro 5-benzyloxy phenyl) indan-1,3-dione **11ay**. The same reaction can be performed starting with the deprotected benzylidene phtalide **7x** at room temperature for 16 h. In that case, the crude precipitate obtained after acidification was washed first with water and then with petroleum ether to give an almost pure compound 5,6-dimethoxy 2-(2-nitro 5-hydroxy phenyl) indan-1,3-dione (**11ax**). Table 12 summarizes data concerning all the compounds prepared in the same way.

*6.2.2.2. 2,3-Dimethoxy 8-benzyloxy 5H indeno (1,2-b) indole 10-one (**12a**)*. 19.4 g of compound **11ay** were dissolved in 250 ml of DMF and reduced in a Parr apparatus with 12 ml of Raney nickel suspension under mild hydrogen pressure (3–5 atm) until the reduction was complete. This mixture was then filtered and the nickel residue was washed with DMF. The filtrate was dried under reduced pressure. The residue was then washed with 50 ml of ethanol, 100 ml of diethyl ether and dried under vacuum. 12.6 g (73%) of 2,3-dimethoxy 8-benzyloxy-5H-indeno (1,2-b) indole 10-one **12a** were obtained. Table 13 summarizes the analytical data concerning all the compounds prepared in the same way.

*6.2.2.3. 2,3-Dimethoxy 8-hydroxy 5H indeno (1,2-b) indol-10-one (**2a**)*. 11.3 g of compound **12a** were introduced in a reactor containing 340 ml of methanol, 150 ml of DMF and 59 ml of 1 N NaOH solution. After flushing it out with nitrogen, 3 g of Pd (10% on C) were added and the mixture, hydrogenated under low pressure (<80 mbar) until deprotection was complete. The catalyst was separated by filtration. Sixty milliliters of a 1 N HCl solution were added while stirring on an ice bath. After drying under reduced pressure, the residue was treated with an excess of isopropanol. The sodium chloride was eliminated by filtration, and isopropanol was distilled under reduced pressure. The final residue was washed with a few ml of iced water. 8.3 g (95%) of 2,3-dimethoxy 8-hydroxy 5H indeno (1,2-b) indol-10-one **2a** were obtained as a dark red powder. The same compound could be obtained (80%) by reduction with Raney nickel and spontaneous cyclization of compound **11ax**, using conditions described above for **11ay**. Table 14 summarizes the

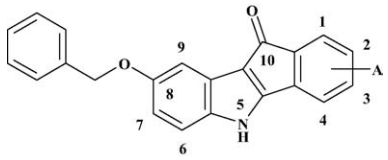
Table 12
Analytical data



	R1	A	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
11ax[a]	H	5,6-Dimethoxy	230	66	1741, 1622, 1586, 1704, 1507, 1339, 1223	DMSO (300 MHz): 11.2(sl(ex) 1H), 8.1(d 1H), 7.45(s 2H), 6.98(dd 1H), 6.95(d 1H), 5.29(s(ex) 1H), 4(s 6H)
11ay	Benzyl	5,6-Dimethoxy	171	75	1741, 1698, 1501, 1342	$CDCl_3$: 8.25(d 1H), 7.4(m+2s 7H), 7.05(dd 1H), 6.95(d 1H), 5.15(s 2H), 4.65(s 1H), 4.05(s 6H)
11by	Benzyl	4,6-Dimethoxy	153	95	1748, 1710	$CDCl_3$: 8.2(d 1H), 7.4(m 5H), 7.1–6.8(m 4H), 5.2(s 2H), 4.6(m 1H), 4(2s 6H)
11cy	Benzyl	4,7-Dimethoxy	213	66	1742, 1710	$CDCl_3$ (300 MHz): 8.3(d 1H), 7.4(m 5H), 7.3(s 2H), 7.1(dd 1H), 7 (d 1H), 5.2(s 2H), 4.6(m 1H), 4(s 6H)

[a] Prepared from **7x**.

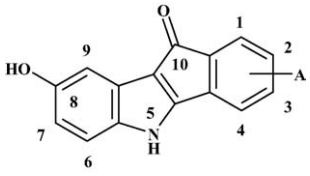
Table 13
Analytical data



	A	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
12a	2,3-Dimethoxy	235	73	1669	DMSO: 12.2(m(ex 1H), 7.4(m 6H), 7(m 2H), 6.7(dd 1H), 5.1(s 2H), 3.9 (s 6H)
12b[a]	2,4-Dimethoxy	-	(10[a])	1658	$CDCl_3$ (300 MHz): 8.5(m(ex) 1H), 7.5(d 2H), 7.4(m 3H), 7.3(d 1H), 7.2 (d 1H), 6.8(dd 1H), 6.8(d 1H), 6.3(d 1H), 5.1(s 2H), 3.9(2s 6H)
12b′[a]	1,3-Dimethoxy	-	(33[a])	1662	DMSO (300 MHz): 12.2(s(ex) 1H), 7.4(m 6H), 7.1(d 1H), 6.8(dd 1H), 6.55(d 1H), 6.49(d 1H), 5.1(s 2H), 3.9(2s 6H)
12c	1,4-Dimethoxy	> 260	63	1683	DMSO (300 MHz): 11.95(m(ex) 1H), 7.5–7.3(m 6H), 7.15(d 2H), 7.05 (s 1H), 6.95(d 1H), 6.8(dd 1H), 5.15(s 2H), 3.9(s 3H), 3.8(s 3H)

[a] As explained, when substitution of the indene part is not symmetrical (**11b**), reaction leads to a mixture, compounds have been incompletely separated (~85% purity) with low yields by chromatography, but signals has been identified in NMR. In the case of **12b**, purification has been completed after debenzylation at the next step.

Table 14
Analytical data



	A	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
2a	2,3-Dimethoxy	> 260	95 (80[a])	1669	DMSO: 12.1(m(ex) 1H), 9(m(ex) 1H), 7.2(d 1H), 7(2s 2H), 6.8(s 1H), 6.6(dd 1H), 3.9(2s 6H)
2b	2,4-Dimethoxy	> 260	26[b]	1668	DMSO: 11.9(sl(ex) 1H), 9.05(m(ex) 1H), 7.15(d 1H), 6.8(d 1H), 6.6(s 2H), 6.55 (dd 1H), 3.9(s 3H), 3.85(s 3H)
2b′	1,3-Dimethoxy	> 260	26[c]	1654	DMSO: 12.1(sl(ex) 1H), 9.05(m(ex) 1H), 7.2(d 1H), 6.87(d 1H), 6.6(dd 1H), 6.55 (d 1H), 6.38(d 1H), 3.85(2s 6H)
2c	1,4-Dimethoxy	> 260	90	1688, 1671	DMSO (300 MHz): 11.8(s(ex) 1H), 9.1(sl(ex) 1H), 7.2(d 1H), 6.95(d 1H), 6.85(s 1H), 6.55(d 1H), 3.9(s 3H), 3.8(s 3H)

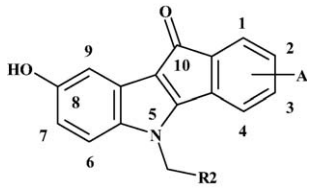
[a] By reduction and cyclization of the non-benzylated phenyl indane dione **11ax**.
[b] By chromatographic separation from the remaining isomer **2b′** and crystallization in isopropanol.
[c] By chromatographic separation from the remaining isomer **2b** and crystallization in isopropanol.

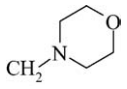
analytical data concerning all the compounds prepared in the same way. Indeno indoles **2** were selectively substituted on the indolic nitrogen by using potassium carbonate in acetone or DMF.

*6.2.2.4. 2,3-Dimethoxy 8-hydroxy 5-carboxymethyl indeno (1,2-b) indol-10-one (**16an**)*. Two grams of compound **2a** were dissolved in 15 ml of DMF and 5 ml of acetone. 0.94 g (1 equiv.) of potassium carbonate were added. The mixture was stirred at 47 °C for 12 h. 1.13 g of methyl bromacetate in 5 ml of acetone were added and the temperature maintained for 4 h. After cooling at room temperature, the reaction medium was filtered, the precipitate washed with a large amount of diethylether. The solvent phase was washed with water, dried with magnesium sulfate and kept to dryness. The residue was washed first with isopropanol and then with a minimum amount of diethyl ether. 1.95 g (75%) of 2,3-dimethoxy 8-hydroxy 5-methoxycarbonylmethyl indeno (1,2-b) indol-10-one **16am** were obtained. The free carboxylic acid was obtained by treating the ester **16am** with 1 equiv. of a 1 N NaOH solution in the same volume of ethanol at room temperature for 30 min, and then by acidifying it with 1.05 equiv. of a 1 N HCl solution. The precipitate obtained was filtrated, washed with iced water and finally with petroleum ether. Seventy percent of 2,3-dimethoxy 8-hydroxy 5-carboxymethyl indeno (1,2-b) indol-10-one **16an** are obtained (described in Table 15 as its sodium salt). The same general procedure could be used with *N*-(2-chloro ethyl) morpholine hydrochloride or *N*-(2-chloro ethyl) dimethylamine hydrochloride (using 2 equiv. of potassium carbonate). So this is for methylation using a slight excess of methyl iodide. Table 15 summarizes the analytical data concerning all the N-substituted derivatives **16**.

The 1,4-dimethoxy derivative has been selectively demethylated on position -1, starting from the *O*-benzyl derivative **12c**, the demethylated product has been identified but not purified and immediately submitted to classical debenzylation.

Table 15
Analytical data



	A	R2	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
16aj	2,3-Dimethoxy	H	> 260	49	1659	DMSO (300 MHz): 9.2(s(ex) 1H), 7.28(d 1H), 7.15(s 1H), 6.98(s 1H), 6.82(d 1H), 6.6(dd 1H), 3.98(s 3H), 3.91(s 3H), 3.8(s 3H)
16ak	2,3-Dimethoxy	CH_2–NMe_2	216	40	1667	DMSO (300 MHz): 9.23(m(ex) 1H), 7.32(d 1H), 7.07(s 1H), 7.0(s 1H), 6.82(d 1H), 6.58(dd 1H), 4.45(t 2H), 3.9(s 3H), 3.82(s 3H), 2.65(t 2H), 2.22(s 6H)
16al	2,3-Dimethoxy		228–232	40	1666	DMSO: 9.3(s(ex) 1H), 7.35(d 1H), 7.1(s 1H), 7.0(s 1H), 6.8(d 1H), 6.6(dd 1H), 4.45 (t 2H), 3.85(s 3H), 3.8(s 3H), 3.45(m 4H), 2.7(t 2H), 2.4(m 4H)
16am	2,3-Dimethoxy	COOMe	251	75	1735, 1693	DMSO (300 MHz): 9.25(s(ex) 1H), 7.25(d 1H), 7.02(s 2H), 6.85(d 1H), 6.6(dd 1H), 5.4(s 2H), 3.9–3.8(3s 9H)
16an	2,3-Dimethoxy	COONa	> 260	52 (2 steps)	1681, 1625	DMSO: 9.25(s(ex) 1H), 7.12(d 1H), 6.95(s 1H), 6.92(s 1H), 6.80(d 1H), 6.52(dd 1H), 4.53(s 2H), 3.8(2s 6H)
16cj	1,4-Dimethoxy	H	> 260	56	1666	DMSO (300 MHz): 9.2(m(ex) 1H), 7.3(d 1H), 7.15(d 1H), 7.0(d 1H), 6.9(s 1H), 6.65(dd 1H), 4.05(s 3H), 3.9(s 3H), 3.8(s 3H)
16b'k	1,3-Dimethoxy	CH_2–NMe_2	264	41	1676	DMSO (500 MHz): 9.1(m(ex) 1H), 7.35(d 1H), 6.85(s 1H), 6.65(s + dd 2H), 6.4(s 1H), 4.4(t 2H), 3.85(2s 6H), 2.65(t 2H), 2.2(s 6H)

*6.2.2.5. 1-Hydroxy 4-methoxy 8-benzyloxy 5H indeno (1,2-b) indol-10-one (**19**)*. 1.5 g of compound **12c** was refluxed for 4 days with 0.9 g of beryllium chloride in 150 ml of anhydrous toluene. 0.9 g were then added and the reflux maintained for 3 more days. The mixture was cooled at room temperature and filtered. 1-Hydroxy 4-methoxy 8-benzyloxy 5H indeno (1,2-b) indol-10-one **19** has not been purified but only characterized by NMR (400 MHz) $CDCl_3$: 8.7(s(ex) 1H), 8.2(s(ex) 1H), 7.4(m 5H), 7.3(d 1H), 7.2(d 1H), 6.85(dd 1H), 6.8(d 1H), 6.72(d 1H), 5.1(s 2H), 3.9(s 3H).

*6.2.2.6. 1,8-Dihydroxy 4-methoxy 5H indeno (1,2-b) indol-10-one (**15**)*. Compound **19** obtained above was debenzylated under conditions described for compound **2a**. 1.26 g (88%) of 1,8-Dihydroxy 4-methoxy 5H indeno (1,2-b) indol-10-one **15** were isolated. M.P.: >>260 °C, IR: 1616, 1653, NMR (300 MHz) DMSO: 11.9(m(ex) 1H), 8.8(m(ex) 2H), 7.2(d 1H), 7(d 1H), 6.85(d 1H), 6.7(d 1H), 6.55(dd 1H), 3.9(s 3H).

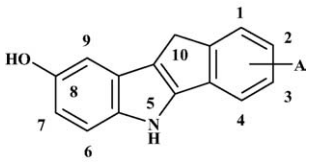
*6.2.2.7. 2,3-Dimethoxy 8-hydroxy 9-iodo 5-methyl indeno (1,2-b) indol-10-one (**18**)*. Five hundred milligrams of compound **16aj** were dissolved at 40 °C in a mixture of *n*-heptane (10 ml), anhydrous DMSO (50 ml) acetic acid (15 ml). To this red solution were added simultaneously: 210 mg of potassium iodide, 150 mg potassium iodate and 500 mg of iodine. After 6 h at the same temperature, the reaction mixture was cooled to –5 °C, and 1.5 ml of a 50% solution of sodium bisulfite, added. After warming up the mixture to room temperature, the volatile solvents were eliminated under reduced pressure. This mixture was injected in a preparative HPLC developed with a mixture of DMSO/H_2O (65:35 to 73:27) on a LICHROPREP RP-18 column. The fractions containing the compound were collected and concentrated under a 0.02 mm vacuum. The residue was triturated with water to eliminate some remaining DMSO and finally with diethyl ether. One hundred and seventy milligrams (25%) of 2,3-dimethoxy 8-hydroxy 9-iodo 5-methyl indeno (1,2-b) indol-10-one **18** were obtained. IR (Nujol): 1661, NMR (300 MHz) DMSO: 9.9(s(ex) 1H), 7.35(d 1H), 7.2(s 1H), 7(s 1H), 6.7(d 1H), 3.9(2s 6H), 3.8(s 3H).

*6.2.2.8. 1,4-Dimethoxy 8-hydroxy 10H indeno (1,2-b) indole (**17c**)*. Six grams of compound **12c** were dissolved in a mixture of 200 ml of DMF, 40 ml of methanol and 180 ml of acetic acid. 0.6 g of Pd (10% on C) were added under a nitrogen flow. The mixture was hydrogenated under low pressure (< 80 mbar) at room temperature until the absorption was complete. This mixture was then filtered. After keeping filtrate to dryness under reduced pressure, the residue was triturated with 100 ml of diethyl ether and filtrated. The precipitate was triturated with a large excess of isopropanol and filtrated. Isopropanol filtrate was kept to dryness and the residue, purified by silica chromatography, developed with a mixture of dichloromethane and methanol (99:1). Some impurities were eliminated by this purification process and 2.7 g (59%) of 1,4-dimethoxy 8-hydroxy 10H indeno (1,2-b) indole **17c** were obtained this way. Table 16 summarizes the data of the compounds obtained by the same way.

6.3. Membranes

As a first step towards describing a precise and complete pharmacology of the best compounds synthesized during the

Table 16
Analytical data



	A	M.p. (°C)	Yield (%)	IR (Nujol suspension)	NMR (δ ppm)
17c	1,4-Dimethoxy	193	59	Not characteristic	DMSO: 10.9(sl(ex) 1H), 8.65(m(ex) 1H), 7.25(d 1H), 6.9(d 1H), 6.8(s 1H), 6.75(d 1H), 6.55(d 1H), 3.9(s 3H), 3.8(s 3H), 3.5(s 2H)
17b	2,4-Dimethoxy	197–198	56	Not characteristic	DMSO (300 MHz): 10.8(sl(ex) 1H), 8.6(m(ex) 1H), 7.2(d 1H), 6.8(2d 2H), 6.6(d 1H), 6.5 (dd 1H), 3.9(2s 6H), 3.6(s 2H)

present work, we chose to characterize their capacity to interfere with the *MT3* binding of iodo-melatonin. In brief, male Syrian hamster brains were obtained from Charles River Breeding Laboratories. Organs were carefully dissected, intensively washed in ice-cold PBS and snap-frozen in liquid nitrogen. They were maintained at –80 °C until further use. Membranes were prepared by the following procedure, all steps being performed at 4 °C. The tissues were thawed, chopped using a surgical blade and resuspended in 5 volumes of 50 mM Tris–HCl (pH 7.5) containing 250 mM sucrose, 1 mM $CaCl_2$ and protease inhibitors as a cocktail commercialized by Boehringer Mannheim (one tablet of Complete™ in 50 ml). The nuclei and unbroken material were pelleted twice by 10 min centrifugation at 280 × *g*. The supernatants were pooled, fivefold diluted with 20 mM Tris–HCl (pH 7.5) containing 1 mM $CaCl_2$ and protease inhibitors and were subjected to a final, 60-min, centrifugation at 400,000 × *g*. The membrane pellets were resuspended with a Dounce as a 2–4 mg ml^{-1} protein suspension, as measured by the method of Lowry et al. [32] adapted for membrane proteins (DC Protein assay, BioRad) using BSA as a standard. Membrane preparations were flash-frozen in liquid nitrogen and were stored at –280 °C until use. Membranes from HEK293 cells expressing MT_1 or MT_2 and membranes from Syrian hamster kidney or brain are referred to as MT_1, MT_2 and *MT3* membranes, respectively, throughout the text.

6.4. Binding

Binding experiments on MT1 and MT2 membranes were realized as described before [33]. Briefly, samples (10 mg of proteins) were incubated for 2 h at 37 °C with 25 pM (MT1) or 170 pM (MT2) 2-[^{125}I]-melatonin (2200 Ci $mmol^{-1}$, NEN, Boston, MA) in the presence (non-specific binding) or not (total binding) of 10 mM melatonin and with varying concentrations of test drugs. Incubations were carried out in triplicates in 96-well microplates and were terminated by filtration through 96-well format glass-fiber plates (GF/B Unifilter, Packard) using a Filtermate (Packard) apparatus. Membranes were then washed three times with 2 ml of 50 mM Tris–HCl (pH 7.5) buffer before the addition of 30 ml per well of scintillation liquid (Microscint 20, Packard) and counting in a β scintillation counter (TopCount NXT, Packard).

Binding experiments on *MT3* kidney membranes were performed in 20 mM Tris–HCl (pH 7.5) buffer containing 1 mM $CaCl_2$ (binding buffer) in a final volume of 150 ml. Filtrations were performed through 96-well glass-fiber supports (GF/B Unifilter, Packard) presoaked for 2 hours before use in 0.3% (v:v) polyethyleneimine and rinsed extemporaneously three times with 200 ml per well of binding buffer. After sample filtration, the filters were rinsed once with 100 ml per well of binding buffer. The filtration plates were disposed directly onto a Multiscreen filtering apparatus (Millipore) connected to a vacuum pump, allowing rapid filtration after the samples were loaded using a 96-well pipetting device (Transtar, Costar). Radioactivity was measured as described above. Unless otherwise stated, incubations were performed for 30 min at room temperature (20–25 °C) using 2-[^{125}I]-I-MCANAT as the specific radioligand. Results are expressed as the specific binding, i.e. the total binding corrected for the non-specific binding.

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