1-(2-Alkanamidoethyl)-6-methoxyindole Derivatives: A New Class of Potent Indole Melatonin Analogues

Giorgio Tarzia, Giuseppe Diamantini, Barbara Di Giacomo, and Gilberto Spadoni*

Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Urbino, Piazza Rinascimento 6, I-61029 Urbino, Italy

Daniele Esposti

Istituto di Fisiologia Umana, Università degli Studi di Milano, Via Manzagalli 32, I-20131 Milano, Italy

Romolo Nonno, Valeria Lucini, Marilou Pannacci, Franco Fraschini, and Bojidar Michaylov Stankov

Cattedra di Chemioterapia, Dipartimento di Farmacologia, Università degli Studi di Milano, Via Vanvitelli, 32, I-20129 Milano, Italy

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A new series of indole melatonin analogues, bearing the amido ethyl side chain attached at the N-1 position of the indole nucleus, were synthesized and tested for their affinity for the melatonin receptor isolated from quail optic tecta in a series of *in vitro* ligand-binding experiments using 2-[^{125}I]iodomelatonin as the labeled ligand. The biological activity was evaluated using two models: effects on the forskolin-stimulated cAMP accumulation in explants from quail optic tecta and evaluation of the GTPγS index derived from competition experiments performed in the absence or presence of GTPγS. Compounds **2a** and **2k**−**n**, obtained by shifting the methoxy group and the ethylamido side chain from the C-5 and C-3 positions of melatonin to the C-6 and N-1 positions of the indole nucleus, exhibited an affinity similar to that of melatonin itself, as well as full agonist activity. Optimization of the C-2 substituent by introducing Br, phenyl, or $COOCH_3$ (**2b**−**d**) resulted in a significantly enhanced affinity (in the picomolar range) and improved agonist biological activity. Compounds lacking the methoxy group and bearing an *N*-alicyclic group (**2h**−**j**) behaved as partial agonists or antagonists.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, aMT, **1**), the principal hormone of the pineal gland, has a number of relevant actions.[1] Its activity, mediated through high-affinity G-protein-coupled receptors, is principally related to the regulation of the photoperiodic responses,[2] the entrainment of the mammalian circadian rhythms,[3] the induction of sleep in humans,[4] and retinal physiology.[5] The physiological importance of other claims such as antioxidant[6] and immunomodulatory[7] actions has not yet been substantiated. Despite its great potential, the therapeutic use of aMT is limited due to its very short biological half-life and poor selectivity for its target sites, Mel_{1a}, Mel_{1b}, Mel_{1c}, and Mel_2, although the physiological role of the latter two receptors is still unclear.[8]

We[9] and other authors[10] have shown that the key elements for high binding affinity to the melatonin receptor are the methoxy group and the *N*-alkanamido side chain linked to an appropriate aromatic spacer, as well as their relative spatial positions.

If one considers the NH indole unimportant for the binding to the melatonin receptor (since it can be replaced by other molecular frames[11] without loss of affinity), it can be assumed that the C-3 and N-1 indole positions are equivalent, although *N*-methylmelatonin is 40 times less potent than aMT itself.[12] Thus, as a working hypothesis, we assumed that the above pharmacophoric groups of melatonin can be shifted from C-5 and C-3 to the C-6 and N-1 indole positions without loss of potency.

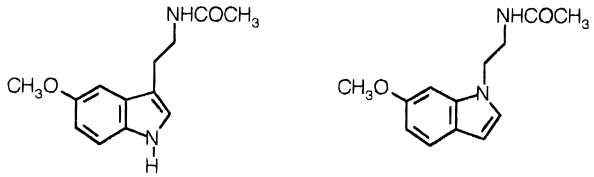

Figure 1.

In order to verify this hypothesis, we studied the synthesis and biological activity of the new indole derivatives **2a**−**n** and **6** containing an alkanamido side chain linked to the N-1 indole position. The rationale of our design is evident upon comparison of the structures of both aMT and the new derivative **2a** (Figure 1).

Furthermore, considering that melatonin is peripherally metabolized to 6-hydroxymelatonin,[13] the compounds of this study might present a distinctly different metabolic behavior and half-life. However, these latter points have not been investigated yet. The minor structural differences of these compounds relative to melatonin could be sufficient to induce selectivity for its target sites.

Chemistry

The novel melatonin analogues **2a**−**n** (Table 1) were synthesized starting from the corresponding indoles **3a**−**h** as reported in Scheme 1. *N*-Cyanomethylation

* Corresponding author: Gilberto Spadoni, Istituto di Chimica Farmaceutica, Università di Urbino, Piazza Rinascimento 6, 61029 Urbino, Italy. (Tel) +39-722-2545. (Fax) +39-722-2737. (E-mail) chimfarm@chim.uniurb.it.

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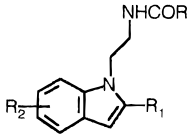
Table 1. Binding Affinity[a] and Biological Activity of Compounds **2a**−**n**, **6**, and **7**

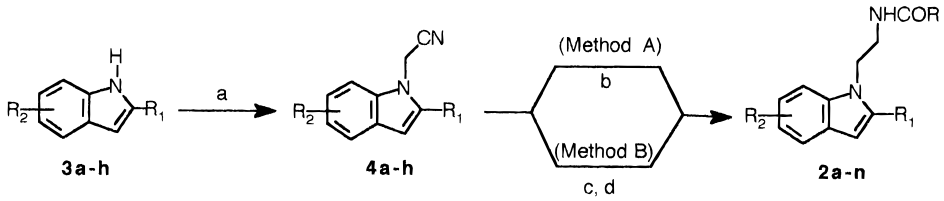


2a-n

compd	R	R_1	R_2	IC_{50}	K_i	relative affinity[b]	GTPγS index[c]	cAMP index[d]	activity[e]
aMT (**1**)				2.2	0.61	1	1	1	A
2a	CH_3	H	6-OCH_3	12	3.4	5.45	0.85	0.85	A
2b	CH_2CH_3	Br	6-OCH_3	0.16	0.044	0.073	1.3	1.2	A
2c	CH_2CH_3	phenyl	6-OCH_3	0.052	0.014	0.024	1.45	1.15	A
2d	CH_2CH_3	$COOCH_3$	6-OCH_3	0.83	0.23	0.38	0.93	1.02	A
2e	CH_2CH_3	phenyl	H	82	23	38	0.95	0.98	A
2f	CH_2CH_3	H	5-OCH_3	5400	1500	2450	0.29	0.4	PA
2g	CH_2CH_3	I	5-OCH_3	360	99	165	0.75	0.8	A−PA
2h	cyclopropyl	H	H	3200	880	1450	0.14	0.2	AN−PA
2i	cyclobutyl	H	H	18000	5000	8180	0.16	0.25	AN−PA
2j	cyclobutyl	phenyl	H	10000	2800	4550	0.18	0.25	AN−PA
2k	CF_3	H	6-OCH_3	35	9.6	16	0.97	0.99	A
2l	cyclopropyl	H	6-OCH_3	89	24	40	0.32	0.44	PA
2m	$CH_2CH_2CH_3$	H	6-OCH_3	11	3.0	5	1.1	1	A
2n	CH_2CH_3	H	6-OCH_3	7.1	1.9	3.2	0.98	0.96	A
6				4.8	1.3	2.2	1.05	1.06	A
7				7100	1900	3230	0.08	0	AN
2-Br-melatonin				0.13	0.036	0.059	1.02	1.15	A
2-Ph-melatonin				0.062	0.017	0.028	0.82	0.78	A−PA
6-Cl-melatonin				3.6	0.99	1.6	0.91	1.04	A

[a] IC_{50} and K_i values are expressed in nM and are the means of three to 20 independent determinations, derived from nonlinear fitting strategies. The SEM values were below 15% of the mean. [b] Relative affinity = (IC_{50} compound/IC_{50} aMT) determined in parallel, in the same experiment. [c] GTPγS index = [(IC_{50} with GTPγS)/(IC_{50} without GTPγS)] compound/[(IC_{50} with GTPγS)/(IC_{50} without GTPγS)] aMT. [d] cAMP index = (percent inhibition compound)/(percent inhibition aMT) determined in parallel in the same experiment. [e] A, agonist; PA, partial agonist; AN, antagonist.

Scheme 1[a]

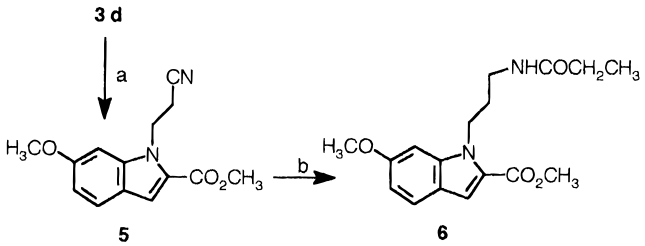


3a-h **4a-h** **2a-n**

[a] Reagents: (a) NaH, $ClCH_2CN$, DMF, room temperature, 16 h (method A); (b) Raney nickel, H_2, 4 atm, $(RCO)_2O$, THF, 50 °C, 6 h (method B); (c) Raney nickel, H_2, 4 atm, EtOH−NH_3, THF, 50 °C, 6 h; (d) $(CF_3CO)_2O$ or cyclopropanecarbonyl or cyclobutanecarbonyl chloride, THF, TEA, room temperature.

of indoles **3a**−**h** with sodium hydride and chloroacetonitrile in DMF gave the required 1-(cyanomethyl)indoles **4a**−**h** that were converted to the final compounds **2a**−**g**,**m**,**n** by hydrogenation over Raney nickel and concomitant *N*-acylation with a suitable anhydride (method A). Hydrogenation of the nitriles **4a**, **4e**, and **4h** over Raney nickel in THF and ammonia in ethanol provided the desired crude amines which were then acylated (cyclopropane or cyclobutanecarbonyl chloride/TEA) or trifluoroacylated $(CF_3CO)_2O$/TEA) to give the desired amides **2h**−**l** (method B). The 1-(2-cyanoethyl)indole derivative **5** was prepared by reacting **3d** with acrylonitrile at 50 °C in the presence of sodium methoxide (Scheme 2). Reduction (Raney nickel, H_2) of the resulting nitrile and concomitant *N*-acylation with propionic anhydride gave the desired *N*-propylpropionamido derivative **6**. The indole starting materials **3e**,**f**,**h** are commercially available (Aldrich Chemical Co). Indoles **3a**,[14] **3d**,[14] and **3g**[15] were prepared according to previously described procedures. 6-Methoxy-2-bromoindole (**3b**) was prepared according to a method previously described for related compounds[16] (Scheme 3). A modified Madelung synthesis[17] was adopted for the preparation of 6-methoxy-2-phenyl-1*H*-indole (**3c**)[18] (Scheme 4). The intermediate *N*-(2-methyl-5-methoxyphenyl)benzamide was prepared by condensation of the commercial 2-methyl-5-methoxyaniline with benzoyl chloride/TEA (toluene, reflux, 3 h, room temperature, 16 h; yield 83%, mp 123−125 °C). 1-(Cyanomethyl)indole (**4h**) was synthesized according to a literature method.[19] Compound **7**, *N*-[(2-phenyl-1*H*-indol-3-yl)ethyl]cyclobutane-

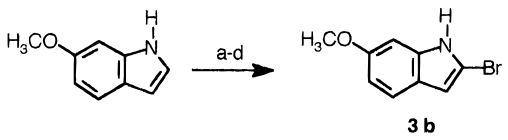
Scheme 2[a]



5 **6**

[a] Reagents: (a) $CH_2{=}CH_2CN$, $NaOCH_3$, 50 °C; (b) Raney nickel, H_2, 4 atm, $(EtCO)_2O$, THF, 50 °C, 6 h.

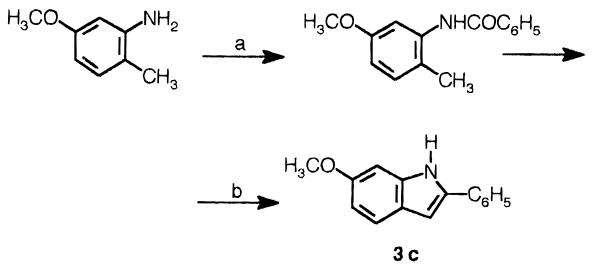
Scheme 3[a]



3b

[a] Reagents: (a) *n*-BuLi, −78 °C; (b) CO_2; (c) *t*-BuLi, −78 °C; (d) 1,2-dibromotetrachloroethane.

Scheme 4[a]



3c

[a] Reagents: (a) benzoyl chloride, TEA, toluene, reflux, 3 h, room temperature 16 h; (b) *n*-BuLi, THF, room temperature, 20 h.

carboxamide, used as reference antagonist, was synthesized as previously described.[20a]

Pharmacology

Binding Studies and Determination of Affinity. The affinity of the analogs at the melatonin binding site in the quail optic tecta was determined in competition binding analyses using 2-[^{125}I]iodomelatonin as a labeled ligand (100 pM). The IC_{50} values were determined and K_i values calculated by using nonlinear fitting strategies. The source of the animals, the characterization of the melatonin receptor, and the isolation of the crude membrane preparations have been described in detail elsewhere.[21,22]

Biological Activity Determination. Biological activity was evaluated using two methods: (i) effects on the forskolin-stimulated cAMP accumulation in quail optic tecta explants and (ii) effects of coincubation with GTPγS (10^{-4} M) on the IC_{50} values (GTPγS index).

(i) Effects on Forskolin-Stimulated cAMP Accumulation. The punching technique, the handling of tissue explants, and cAMP determinations have been described in detail elsewhere.[23] The concentrations of the compounds to be used were calculated on the basis of their affinity, as follows: the experimentally derived maximum effective dose of melatonin was around 10^{-7} M. Therefore, the relative affinity for each compound was determined as IC_{50} compound/IC_{50} aMT and the dose of the compound equivalent to that of melatonin (MED, melatonin equivalent dose) in terms of receptor occupancy was calculated (MED compound = relative affinity compound $\times$ 10^{-7}). Afterward, two doses of each compound were assayed: one that was equal (or closest) to the calculated MED and another greater by 1 order of magnitude. For example, a compound with a relative affinity of 0.025 has a MED value of 2.5×10^{-9}. Therefore the doses utilized were 10^{-9} and 10^{-8} M. This avoided the use of inadequately high or low doses in the analyses, as has been the common practice to date. In all cases the two concentrations employed gave similar results, thus confirming the validity of the theoretical assumption regarding the receptor occupancy. In only a few cases, the higher dose could not be used, due to problems related to the solubility of the compounds. This was the case with compounds **2i**, **2j**, and **7**. On the basis of the data obtained, a cAMP index, assigning a rank of potency for the compounds, was calculated as follows: percent inhibition compound/percent inhibition aMT.

(ii) Effects of Coincubation with GTPγS on the IC_{50} Values (GTPγS Index). The choice of introducing the GTPγS index was based on the knowledge that the aMT receptor is coupled to a regulatory G-protein in its signal-transduction pathway.[21,24] It is well-known that guanine nucleotides shift a significant part of the available receptors from the state of high-affinity conformation (Rh) to a state of lower affinity conformation (Rl).[25] Agonists possess higher affinity for the Rh form, while antagonists cannot distinguish between Rh and Rl or have a higher affinity for Rl.[25,26] This is also the mechanism by which the difference between the efficacy of agonists and antagonists is explained.[27] Assuming that under basal, unstimulated conditions there is an existing ratio of Rh/Rl < 1, the system is prevalently inactive. In that case the agonists, because of their higher affinity for the Rh, would create a shift in the equilibrium toward the Rh state, with a consequent activation of the signal-transduction pathway, while the antagonists would keep the basal conditions unaltered or at best change the ratio of Rh/Rl toward values less than basal values.[28] This type of interaction would allow prediction of the efficacy of a compound after having measured its affinity for both Rh and Rl.[29] The prediction was that, under conditions of competition analysis, coincubation with GTPγS would result in increased IC_{50} values for an agonist (giving a shift to the right of the curve), while the IC_{50} values for an antagonist would remain unchanged or decrease (the curve would shift to the left). The series of pilot studies using melatonin (agonist) and compound **7**[20a] (antagonist) completely confirmed the prediction of this model. In the presence of GTPγS, the aMT IC_{50} values increased by 3−6 times while the IC_{50} values for compound **7** showed a 2−3-fold decrease. These results completely justified the application of this new approach to assess the agonist/antagonist nature of all the compounds reported in this study.

Briefly, in each experiment aMT was assayed as a reference standard, GTPγS was used in a constant concentration of 10^{-4} M, and the labeled ligand was always 200 pM. The rest of the conditions of the experiment were as described elsewhere.[21,22] In order to compare these results with those of the cAMP analysis, a numerical index was introduced (GTPγS index), equal to: $[(IC_{50} + GTP\gamma S)/(IC_{50} - GTP\gamma S)]$-compound/$[(IC_{50} + GTP\gamma S)/(IC_{50} - GTP\gamma S)]$ aMT.

As seen in Table 1, the GTPγS index evaluation thoroughly corresponded to that obtained using the cAMP data. The final evaluation of the compounds (agonist, partial agonist, antagonist) was made taking into consideration both indices (GTPγS index and cAMP). Compounds with indices having numerical values >0.8 were considered full agonists; values ranging from 0.2 to 0.8 were indicative of partial agonists and values <0.2 were considered characteristic for the antagonists. In the few cases in which the values of both indices were close to, but not within, the theoretically determined limits of the range 0.2−0.8, a double denomination was adopted, i.e. if a compound had a

GTPγS index = 0.16 and cAMP index = 0.25, it was considered as antagonist−partial agonist.

Results and Discussion

The binding affinities for the melatonin receptor, as well as the biological activity of the new compounds **2a**−**n** and **6**, are reported in Table 1. In accordance with our working hypothesis, the highest binding affinity occurs when the side chain and the methoxy substituent are in the N-1 and C-6 positions of the indole, respectively. In these compounds (**2a**−**d** and **2k**−**n**) the distance between the methoxy group and the amido function is comparable to that of aMT. In our derivatives the importance of the methoxy substituent on the benzene nucleus of the indole for melatonin-like properties was investigated by deleting this group or by shifting its position. Both deletion of the methoxy substituent, as in compounds **2e**,**h**−**j**, and its displacement from the C-6 to the C-5 position, as in compound **2f**, led to a dramatic decrease in the binding affinity. Interestingly, the compound **2f** also exhibited a decreased biological activity. This indicates that **2f** behaves like a partial agonist. As in other series of melatonin analogues, structural variations at the *N*-alkanamido group were found to be important in adjusting the binding of the side chain to the receptor. Replacement of the acetyl group by propanoyl (**2n**) or butanoyl (**2m**) led to a sharp increase in the binding affinity, but a marked drop was observed with cyclopropanoyl (**2l**) substitution. A slight decrease in binding affinity also occurred when moving from the *N*-acetyl (**2a**) to the *N*-trifluoroacetyl (**2k**) derivative. While the compound with a shorter C-3 alkyl chain [*N*-[(5-methoxy-1*H*-indol-3-yl)methyl]propanamide][9] exhibited no affinity to the melatonin receptor ($IC_{50} > 10^{-5}$ M), compound **6**, with a longer *N*-alkyl chain [*N*-(3-alkanamidopropyl)], was slightly less potent than **2d** which has the natural ethyl alkanamido chain. Another modification concerned the introduction of a suitable substituent in the C-2 indole position. In fact, our previous studies[9,30,31] and the very high affinity of 2-iodomelatonin demonstrated the importance of this type of substitution for affinity to the melatonin receptor. As entries **2b**−**d** clearly illustrate, the binding affinity of the 2-bromo (**2b**), 2-phenyl (**2c**), and 2-carbomethoxy (**2d**) derivatives is much greater than that found for the corresponding compounds lacking the C-2 indole substituent. This effect is also evident for the 5-methoxy analogues, among which the 2-iodo derivative **2g** has a binding affinity 15 times greater than that of the C-2 unsubstituted compound **2f**, thus confirming our hypothesis regarding the presence of a secondary binding site around the C-2 indole position, in agreement with a previous study[11b] using different compounds.

The potential agonist or antagonist properties of the synthesized compounds were evaluated by using the GTPγS index (Figure 2) or by measuring their influence on the cAMP synthesis (Figure 3) (see Pharmacology section). The analogues bearing a 6-methoxy substituent and *N*-acylated with alkyl or trifluoromethyl groups (**2a**−**d**,**k**,**m**,**n**) exhibited evident agonistic activity. On the contrary, the 6-methoxy-*N*-cyclopropylamido derivative **2l** showed a decreased efficacy and appeared to be a partial agonist. All of the 2-substituted *N*-propanoyl derivatives (**2b**−**e**) behaved as full agonists. Compounds lacking the methoxy substituent and *N*-acylated with an alicyclic group (**2h**−**j**) showed a dramatic decrease in biological efficacy, behaving as partial agonists or antagonists. The indole C-2 phenyl substitution does not appear to influence the biological activity, as the C-2 substituted (**2j**) and C-2 unsubstituted (**2i**) analogues showed the same efficacy. Therefore, the biological activity of these new derivatives appears to be modulated by the presence and the position of the methoxy substituent and by the nature of the *N*-acyl group, and these data are in agreement with recently reported results,[20] using different compounds.

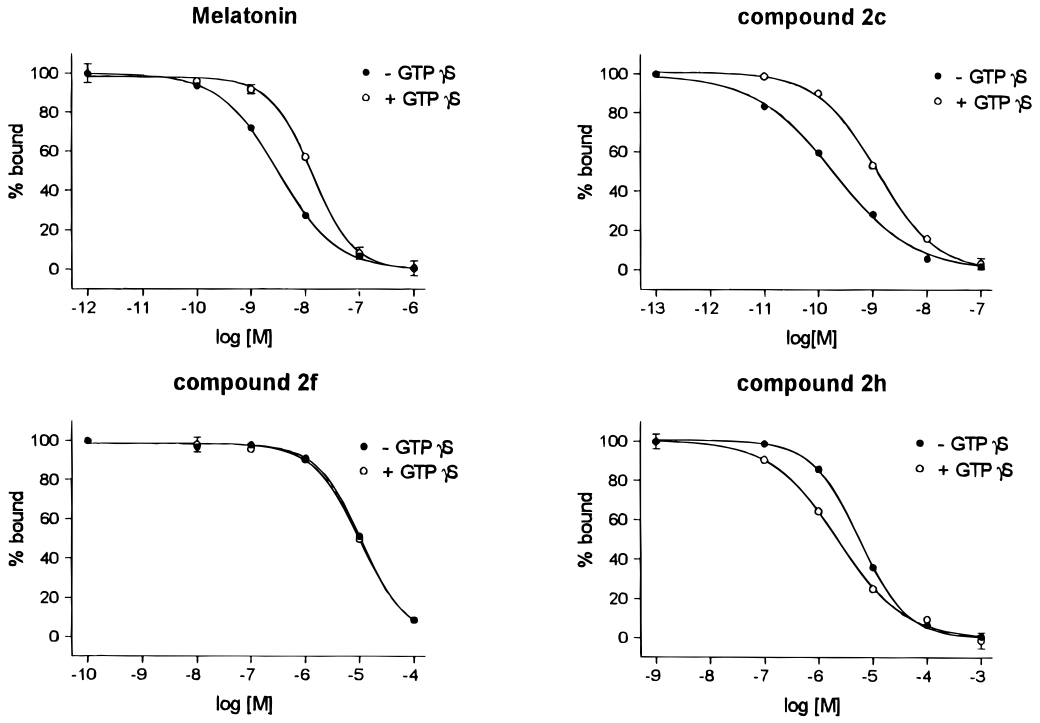
The results of this study are consistent with our initial hypothesis that it should be possible to shift the pharmacophoric groups of the aMT moiety to suitable positions on the indole nucleus, while still retaining high binding affinity and efficacy. This confirms that the proton-donor NH indole is not essential to the anchoring of a ligand to the aMT receptor and that the lower affinity of the *N*-methylmelatonin is not due to the lack of the hydrogen donor, but rather to steric hindrance of the alkyl substituent in the receptor ligand interaction. By comparing **2b** and **2c** with aMT it can be seen that when the rest of the molecule was optimized by means of the introduction of a C-2 bromine or a C-2 phenyl, we obtained compounds which, with ca. 40-fold greater affinity than aMT itself, represent some of the most potent melatonin agonists reported to date.

Another important contribution of this study was the introduction of the GTPγS index. This approach completely confirmed the cAMP data. Moreover, it allows rapid and reliable screening of a large number of aMT analogues for their efficacy, allowing the estimation of their potency and the assigning of the order of efficacy derived from structure−activity studies.

Experimental Section

Solvents and reagents were of the highest commercial grade available and were used without additional purification. Melting points were determined on a Büchi SMP-510 capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AC 200 spectrometer; chemical shifts are reported in ppm and given in δ units. EI-MS spectra (70 eV) were taken on a Fisons Trio 1000. Only molecular ions (M^+) and base peaks are given. Infrared spectra were obtained on a Bruker FT-48 spectrometer; absorbances are reported in ν (cm^{-1}). Elemental analyses were performed on a Carlo Erba analyzer.

2-Bromo-6-methoxyindole (3b). *n*-Butyllithium (4.2 mL of 2.5 M solution in hexane, 10.5 mmol) was added dropwise over 15 min to a solution of 6-methoxyindole (1.47 g, 10 mmol) in dry THF (20 mL) at −70 °C, under a nitrogen atmosphere. After 30 min of stirring at −68 °C, CO_2 gas was bubbled through the solution for 10 min, and the clear solution was allowed to reach room temperature. The excess of CO_2 was removed under vacuum (10 °C, 1 mmHg), and the solvent was concentrated to ca. 5 mL. An additional 15 mL of dry THF was added, and the suspension was cooled to −70 °C. *tert*-Butyllithium (6.2 mL of 1.7 M solution in pentane, 10.5 mmol) was then added dropwise over 10 min, and the mixture was stirred for 2 h at −68 °C. A solution of 1,2-dibromotetrachloroethane (3.26 g, 10 mmol) in dry THF (7 mL) was added dropwise, and the mixture was stirred at −68 °C for 1.5 h. The mixture was then poured into ice−water (30 g), and a saturated aqueous NH_4Cl solution was added to acidic pH. The aqueous solution was extracted with ether (3 × 30 mL), and the combined extracts were washed with brine, dried (Na_2SO_4), and evaporated *in vacuo* at room temperature. The semisolid residue was purified by flash chromatography (silica gel, cyclohexane/ethyl acetate, 9:1) and crystallization from $CHCl_3$/



	IC_{50}	IC_{50} + GTPγS	ratio	GTPγS index	Activity
Melatonin	2.98	13.1	4.39	1	A
2c	0.189	1.18	6.24	1.42	A
2h	5700	2250	0.39	0.09	AN
2f	10500	10400	1	0.23	PA

Figure 2. Examples of the evaluation of the biological activity of the compounds by using the GTPγS index: competition experiments performed with a fixed concentration of the labeled ligand (200 pM 2-[^{125}I]iodomelatonin) and varying concentrations of the competing drug, in the absence or presence of GTPγS (10^{-4} M). Ratio = IC_{50} + GTPγS/IC_{50}. GTPγS index = ratio compound/ratio aMT. IC_{50} values are expressed in nM. Activity: when GTPγS index <0.2 = AN (antagonist); between 0.2 and 0.8 = PA (partial agonist); >0.8 = A (agonist).

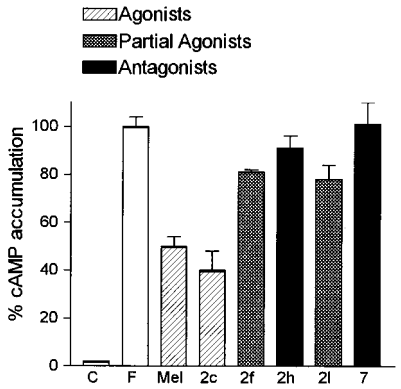


Figure 3. Examples of the evaluation of the biological activity of the compounds by measuring the effect on forskolin (10^{-5} M)-stimulated cAMP accumulation in explants from quail optic tecta. C, control; F, forskolin. Agonist: ratio of percent inhibition compound/percent inhibition aMT > 0.8. Partial agonist: ratio of percent inhibition compound/percent inhibition aMT is between 0.8 and 0.2. Antagonist: ratio of percent inhibition compound/percent inhibition aMT < 0.2.

hexane, yielding 0.68 g (30%) of the title compound: mp 69−71 °C dec; ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H), 6.46 (m, 1H), 6.77, 6.82 (dd, 1H, J = 2.24 Hz and J = 8.3 Hz), 6.81 (br s, 1H), 7.40 (dd, 1H, J = 0.64 Hz and J = 8.26 Hz), 7.95 (br s, 1H); IR 3370, 1440 cm^{-1}; MS (EI) m/z 225, 227 (M^+), 210, 212 (100).

6-Methoxy-2-phenylindole (3c). *n*-Butyllithium (13.75 mL of 1.6 M solution in hexane, 22 mmol) was added dropwise to an ice-cooled stirred solution of *N*-(2-methyl-5-methoxyphenyl)benzamide (2.41 g, 10 mmol) in dry THF (20 mL), under a nitrogen atmosphere. The stirred mixture was kept at room temperature for 20 h, then cooled to 0 °C, and treated dropwise with 2 N HCl (11 mL). The organic layer was separated and the aqueous layer extracted with ether (3 × 20 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to obtain a black residue which was then purified by flash chromatography (silica gel, toluene as eluent) and crystallization from toluene to give **3c** as a white solid (0.45 g, 20%): mp 177−178 °C (lit.[18] mp 168−170 °C); ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H), 6.76 (m, 1H), 6.79−7.66 (m, 8H), 6.26 (br s, 1H); MS (EI) m/z 223 (M^+), 208 (100).

General Procedure for the Synthesis of 1-(Cyanomethyl)indole Derivatives (4a−h). A solution of the appropriate indole **3a−h** (10 mmol) in dry DMF (10 mL) was added dropwise to a stirred ice-cooled suspension of sodium hydride (0.42 g of an 80% dispersion in mineral oil, 14 mmol) in dry DMF (30 mL) under a N_2 atmosphere. After the addition, the mixture was stirred at 0 °C for 30 min, then chloroacetonitrile (1.06 g, 14 mmol) was added dropwise, and the resulting mixture was stirred at room temperature for 16 h, then poured into ice−water (250 g), and extracted with ethyl acetate (3 × 70 mL). The organic phase was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to give a residue which was purified by flash column chromatography (silica gel; cyclohexane/ethyl acetate, 7:3) and crystallization.

6-Methoxy-1-(cyanomethyl)indole (4a): white solid, 0.56 g (30%); mp 104−5 °C (ether/hexane); ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H), 4.96 (s, 2H), 6.54 (dd, 1H, J = 0.86 Hz and J = 3.3 Hz), 6.82, 6.85 (dd, 1H, J = 8.57 Hz and J = 2.2 Hz), 6.89 (d, 1H, J = 2.2 Hz), 6.99 (d, 1H, J = 3.3 Hz), 7.53 (d, 1H, J = 8.57 Hz); IR (Nujol) 2249, 1626 cm^{-1}; MS (EI) m/z 186 (M^+), 171 (100).

2-Bromo-6-methoxy-1-(cyanomethy)lindole (4b): white solid, 1.99 g (75%); mp 68−70 °C dec (methanol); ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H), 5.04 (s, 2H), 6.61 (d, 1H, J = 0.64 Hz), 6.80 (br s, 1H), 6.83, 6.88 (dd, 1H, J = 8.58 Hz and J = 2.23 Hz), 7.42, 7.46 (dd, 1H, J = 8.58 Hz and J = 0.64 Hz); IR (Nujol) 2250, 1620 cm^{-1}; MS (EI) m/z 264, 266 (M^+), 249, 251 (100).

6-Methoxy-2-phenyl-1-(cyanomethyl)indole (4c): amorphous solid, 0.21 g (8%); ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H), 4.70 (s, 2H), 6.43 (br s, 1H), 6.60−6.90 (m, 3H), 7.10−7.60 (m, 5H); MS (EI) m/z 262 (M^+), 247 (100).

2-Carbomethoxy-6-methoxy-1-(cyanomethyl)indole (4d): 1.7 g (70%); mp 178−80 °C (methanol); ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H), 3.93 (s, 3H), 5.58 (s, 2H), 6.80 (br s, 1H), 6.89, 6.93 (dd, 1H, J = 8.88 Hz and J = 2.22 Hz), 7.33 (br s, 1H), 7.58 (d, 1H, J = 8.57 Hz); IR (Nujol) 1707, 1624 cm^{-1}; MS (EI) m/z 244 (M^+), 229 (100).

2-Phenyl-1-(cyanomethyl)indole (4e). This compound was used as a crude intermediate because it is difficult to separate from the starting unreacted 2-phenylindole.

5-Methoxy-1-(cyanomethyl)indole (4f): 0.62 g (33%); mp 97−98 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H), 4.95 (s, 2H), 6.53, 6.54 (dd, 1H, J = 0.94 Hz and J = 3.18 Hz), 6.95, 7.00 (dd, 1H, J = 8.9 Hz and J = 2.54 Hz), 7.06 (d, 1H, J = 3.18 Hz), 7.12 (d, 1H, J = 2.22 Hz), 7.27 (dd, 1H, J = 8.9 Hz and J = 2.2 Hz); IR (Nujol) 1611, 1577 cm^{-1}; MS (EI) m/z 186 (M^+), 171 (100).

2-Iodo-5-methoxy-1-(cyanomethyl)indole (4g): 2.18 g (70%); mp 126−127 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 5.04 (s, 2H), 6.79 (br s, 1H), 6.88, 6.93 (dd, 1H, J = 8.9 Hz and J = 2.23 Hz), 7.01 (d, 1H, J = 2.22 Hz), 7.26 (d, 1H, J = 8.9 Hz); IR (Nujol) 2255, 1621 cm^{-1}; MS (EI) m/z 312 (M^+), 76 (100).

2-Carbomethoxy-6-methoxy-1-(2-cyanoethyl)indole (5). Sodium methoxide (0.054 g, 1 mmol) was added to a 50 °C preheated solution of **3d** (0.205 g, 1 mmol) in acrylonitrile (3 mL). The reaction mixture was stirred for 0.5 h at room temperature, then poured into ice−water, and extracted (3×) with $CHCl_3$; the combined extracts were washed with brine and dried (Na_2SO_4). After removal of the solvent the crude residue was chromatographed over silica gel (cyclohexane/ethyl acetate, 8:2, as eluent) to give a white crystalline solid: yield, 0.23 g (88%). Crystallization from ethanol: mp 114−116 °C; ^{1}H NMR ($CDCl_3$) δ 2.92 (t, 2H), 3.91 (s, 3H), 3.92 (s, 3H), 4.81 (t, 2H), 6.84, 6.89 (dd, 1H, J = 8.3 Hz and J = 2.44 Hz), 7.27 (s, 1H), 7.30 (br s, 1H), 7.54, 7.58 (dd, 1H, J = 8.3 Hz and J = 0.98 Hz); IR (Nujol) 2249, 1696 cm^{-1}; MS (EI) m/z 258 (M^+), 218 (100).

General Procedure for the Synthesis of Acylamino-*N*-[2-(1*H*-indol-1-yl)ethyl] Derivatives (2a−g,m,n). Method A. A solution of the suitable 1-(cyanomethyl)indole **4a**−**g** (1 mmol) in THF (5 mL) and acetic, propionic, or butyric anhydride (2 mL) was hydrogenated over Raney nickel at 4 atm of H_2 for 6 h at 50 °C. The catalyst was filtered on Celite, and the filtrate was concentrated *in vacuo* and partitioned between ethyl acetate and 2 N NaOH. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give the crude title compounds (**2a**−**g**,**m**,**n**) which were purified by flash chromatography (silica gel; cyclohexane/ethyl acetate, 3:7) and crystallization.

***N*-[2-(6-Methoxy-1*H*-indol-1-yl)ethyl]acetamide (2a):** white crystalline solid, 0.17 g (73%); mp 118−119 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 3.61 (q, 2H), 3.87 (s, 3H), 4.24 (t, 2H), 5.50 (br s, 1H), 6.45 (d, 1H, J = 3.16 Hz), 6.78, 6.82 (dd, 1H, J = 8.37 Hz and J = 2.22 Hz), 6.83 (d, 1H, J = 2.22 Hz), 6.96 (d, 1H, J = 3.17 Hz), 7.50 (d, 1H, J = 8.35 Hz); IR (Nujol) 3303, 1639 cm^{-1}; MS (EI) m/z 232 (M^+), 160 (100). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

***N*-[2-(2-Bromo-6-methoxy-1*H*-indol-1-yl)ethyl]propanamide (2b):** white crystalline solid, 0.15 g (45%); mp 97−98 °C (ether); ^{1}H NMR ($CDCl_3$) δ 1.09 (t, 3H), 2.12 (q, 2H), 3.61 (q, 2H), 3.86 (s, 3H), 4.33 (t, 2H), 5.48 (br s, 1H), 6.52 (s, 1H), 6.75, 6.80 (dd, 1H, J = 8.37 Hz and J = 2.22 Hz), 6.84 (d, 1H, J = 2.22 Hz), 7.40 (d, 1H, J = 8.58 Hz); IR (Nujol) 3230, 1634 cm^{-1}; MS (EI) m/z 324, 326 (M^+), 189 (100). Anal. ($C_{14}H_{17}BrN_2O_2$) C, H, N.

***N*-[2-(6-Methoxy-2-phenyl-1*H*-indol-1-yl)ethyl]propanamide (2c):** white crystalline solid, 0.19 g (58%); mp 106−107 °C (ether/hexane); ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H), 1.93 (q, 2H), 3.41 (q, 2H) 3.91 (s, 3H), 4.35 (t, 2H), 5.20 (br s, 1H), 6.49 (d, 1H, J = 0.64 Hz), 6.80, 6.85 (dd, 1H, J = 8.57 Hz and J = 2.22 Hz), 6.97 (d, 1H, J = 2.22 Hz), 7.46−7.53 (m, 6H); IR (Nujol) 3190, 1678 cm^{-1}; MS (EI) m/z 322 (M^+), 236 (100). Anal. ($C_{20}H_{22}N_2O_2$) C, H, N.

***N*-[2-(2-Carbomethoxy-6-methoxy-1*H*-indol-1-yl)ethyl]-propanamide (2d):** white crystalline solid, 0.21 g (68%); mp 133 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 1.06 (t, 3H), 2.10 (q, 2H), 3.68 (q, 2H), 3.88 (s, 3H), 3.89 (s, 3H), 4.65 (t, 2H), 6.02 (br s, 1H), 6.80, 6.84 (dd, 1H, J = 8.37 Hz and J = 2.22 Hz), 6.87 (br s, 1H), 7.27 (s, 1H), 7.52 (d, 1H, J = 8.9 Hz); IR (Nujol) 3262, 1707, 1638 cm^{-1}; MS (EI) m/z 304 (M^+), 231 (100). Anal. ($C_{16}H_{20}N_2O_4$) C, H, N.

***N*-[2-(2-Phenyl-1*H*-indol-1-yl)ethyl]propanamide (2e):** oil, overall yield (starting from 2-phenylindole) 7%; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H), 1.92 (q, 2H), 3.42 (q, 2H), 4.40 (t, 2H), 5.19 (br s, 1H), 6.57 (s, 1H), 7.15−7.66 (m, 9H); IR (Nujol) 3155, 1683 cm^{-1}; MS (EI) m/z 292 (M^+), 206 (100). Anal. ($C_{19}H_{20}N_2O$) C, H; N: calcd, 9.58; found, 9.11.

***N*-[2-(5-Methoxy-1*H*-indol-1-yl)ethyl]propanamide (2f):** white crystalline solid, 0.14 g (55%); mp 80−81 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3H), 2.12 (q, 2H), 3.61 (q, 2H) 3.85 (s, 3H), 4.26 (t, 2H), 5.50 (br s, 1H), 6.43 (dd, 1H, J = 3.16 Hz and J = 0.65), 6.86, 6.90 (dd, 1H, J = 8.9 Hz and J = 2.22 Hz), 7.03 (d, 1H, J = 3.18 Hz), 7.10 (d, 1H, J = 2.22 Hz), 7.25 (dd, 1H, J = 8.9 Hz and J = 0.64 Hz); IR (Nujol) 3312, 1647 cm^{-1}; MS (EI) m/z 246 (M^+), 160 (100). Anal. ($C_{14}H_{18}N_2O_2$) C, H, N.

***N*-[2-(2-Iodo-5-methoxy-1*H*-indol-1-yl)ethyl]propanamide (2g):** white crystalline solid, 0.093 g (25%); mp 129−130 °C (ether/hexane); ^{1}H NMR ($CDCl_3$) δ 1.08 (t, 3H), 2.09 (q, 2H), 3.55 (q, 2H), 3.82 (s, 3H), 4.28 (t, 2H), 5.65 (br s, 1H), 6.69 (s, 1H), 6.76, 6.81 (dd, 1H, J = 8.9 Hz and J = 2.55 Hz), 6.96 (d, 1H, J = 2.54 Hz), 7.24 (d, 1H, J = 8.9 Hz); IR ($CDCl_3$) 3445, 1668 cm^{-1}; MS (EI) m/z 372 (M^+), 245 (100). Anal. ($C_{14}H_{17}IN_2O_2$) C, H, N.

***N*-[2-(6-Methoxy-1*H*-indol-1-yl)ethyl]butanamide (2m):** white crystalline solid, 0.19 g (72%); mp 93−94 °C (ether/hexane); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H), 1.60 (m, 2H), 2.08 (t, 2H), 3.63 (q, 2H), 3.87 (s, 3H), 4.24 (t, 2H), 5.43 (br s, 1H), 6.44, 6.45 (dd, 1H, J = 3.18 Hz and J = 0.63 Hz), 6.77 (d, 1H, J = 2.23 Hz), 6.82 (br s, 1H), 6.95 (d, 1H, J = 3.18 Hz), 7.48, 7.52 (dd, 1H, J = 8.26 Hz and J = 0.95 Hz); IR (Nujol) 3252, 1640 cm^{-1}; MS (EI) m/z 260 (M^+), 173 (100). Anal. ($C_{15}H_{20}N_2O_2$) C, H, N.

***N*-[2-(6-Methoxy-1*H*-indol-1-yl)ethyl]propanamide (2n):** white crystalline solid, 0.14 g (55%); mp 105−106 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3H), 2.13 (q, 2H), 3.62 (q, 2H), 3.87 (s, 3H), 4.24 (t, 2H), 5.43 (br s, 1H), 6.45 (d, 1H, J = 3.16 Hz), 6.78, 6.82 (dd, 1H, J = 8.34 Hz and J = 2.22 Hz), 6.83 (d, 1H, J = 2.22 Hz), 6.95 (d, 1H, J = 3.16 Hz), 7.52 (d, 1H, J = 8.34 Hz); IR (Nujol) 3253, 1641 cm^{-1}; MS (EI) m/z 246 (M^+), 160 (100). Anal. ($C_{14}H_{18}N_2O_2$) C, H, N.

General Procedure for the Synthesis of Acylamino-*N*-[2-(1*H*-indol-1-yl)ethyl] Derivatives (2h−l). Method B. A solution of the suitable 1-(cyanomethyl)indole (**4a**,**e**,**h**) (1 mmol) in THF (10 mL) and an ammonia-saturated solution in ethanol (1 mL) was hydrogenated over Raney nickel at 4 atm of H_2 for 6 h at 50 °C. The catalyst was filtered on Celite and the filtrate concentrated *in vacuo* and dissolved in dry THF (3 mL). To this ice-cooled solution were added TEA (0.14 mL, 1 mmol) and trifluoroacetic anhydride (0.14 mL, 1 mmol) [or cyclopropanecarbonyl chloride or cyclobutanecarbonyl chloride (1 mmol)]. The ice bath was removed and the solution stirred for 1−3 h (until the amine disappeared in time course

TLC analysis, $CHCl_3$ ammonia saturated−MeOH, 95:5, as eluent). The solvent was evaporated *in vacuo*, and the residue was taken up in ethyl acetate, washed with a saturated aqueous solution of $NaHCO_3$ followed by brine, dried (Na_2SO_4), and evaporated again. Purification by flash chromatography (silica gel; cyclohexane/ethyl acetate, 4:6) and crystallization gave the required compounds **2h**−**l**.

***N*-[2-(1*H*-Indol-1-yl)ethyl]cyclopropanecarboxamide (2h):** white crystalline solid, 0.13 g (55%); mp 91−92 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 0.73 (m, 2H), 0.99 (m, 2H), 1.21 (m, 1H), 3.66 (q, 2H), 4.31 (t, 2H), 5.59 (br s, 1H), 6.54 (d, 1H, J = 3.1 Hz), 7.09−7.27 (m, 3H), 7.38 (d, 1H, J = 8.04 Hz), 7.66 (d, 1H, J = 7.93 Hz); IR (Nujol) 3304, 1640 cm^{-1}; MS (EI) m/z 228 (M^+), 143 (100). Anal. ($C_{14}H_{16}N_2O$) C, H, N.

***N*-[2-(1*H*-Indol-1-yl)ethyl]cyclobutanecarboxamide (2i):** white crystalline solid, 0.18 g (74%); mp 103 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 1.84−2.22 (m, 6H), 2.87 (m, 1H) 3.62 (q, 2H), 4.31 (t, 2H), 5.31 (br t, 1H), 6.51, 6.52 (dd, 1H, J = 2.93 Hz and J = 0.98 Hz), 7.05−7.27 (m, 3H), 7.34, 7.39 (dd, 1H, J = 8.3 Hz and J = 0.97 Hz), 7.65 (d, 1H); IR (Nujol) 3264, 1647 cm^{-1}; MS (EI) m/z 242 (M^+), 143 (100). Anal. ($C_{15}H_{18}N_2O$) C, H, N.

***N*-[2-(2-Phenyl-1*H*-indol-1-yl)ethyl]cyclobutanecarboxamide (2j):** white crystalline solid, 0.032 g, total yield (starting from 2-phenylindole) 10%; mp 118−119 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 1.70−2.11 (m, 6H), 2.70 (m, 1H), 3.42 (q, 2H), 4.39 (t, 2H), 5.12 (br t, 1H), 6.57 (s, 1H), 7.12−7.44 (m, 9H); IR (Nujol) 3258, 1643 cm^{-1}; MS (EI) m/z 318 (M^+), 206 (100). Anal. ($C_{21}H_{22}N_2O$) C, H, N.

***N*-[2-(6-Methoxy-1*H*-indol-1-yl)ethyl]trifluoroacetamide (2k):** white crystalline solid, 0.17 g (58%); mp 101−102 °C (ether/hexane); ^{1}H NMR ($CDCl_3$) δ 3.76 (q, 2H), 3.87 (s, 3H), 4.32 (t, 2H), 6.35 (br s, 1H), 6.48 (d, 1H, J = 3.05 Hz), 6.82 (d, 1H), 6.82, 6.84 (dd, 1H), 6.94 (d, 1H, J = 3.17 Hz), 7.52 (d, 1H, J = 8.39 Hz); IR (Nujol) 3310, 1705 cm^{-1}; MS (EI) m/z 286 (M^+), 160 (100). Anal. ($C_{13}H_{13}F_3N_2O_2$) C, H, N.

***N*-[2-(6-Methoxy-1*H*-indol-1-yl)ethyl]cyclopropanecarboxamide (2l):** white crystalline solid, 0.15 g (60%); mp 121−123 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 0.74 (m, 2H), 0.98 (m, 2H), 1.22 (m, 1H), 3.63 (q, 2H), 3.87 (s, 3H), 4.23 (t, 2H), 5.68 (br s, 1H), 6.46 (d, 1H, J = 2.44 Hz), 6.80 (m, 2H), 7.98 (dd, 1H, J = 2.92 Hz and J = 1.46 Hz), 7.51 (d, 1H, J = 8.3 Hz); IR (Nujol) 3309, 1637 cm^{-1}; MS (EI) m/z 258 (M^+), 41 (100). Anal. ($C_{15}H_{18}N_2O_2$) C, H, N.

***N*-[2-[2-(Carboxymethyl)-6-methoxy-1*H*-indol-1-yl]propyl]propanamide (6)** was prepared by method A, as described above, starting from 6-methoxy-2-carbomethoxy-1-(2-cyanoethyl)indole (**5**): white solid, 0.21 g (66%); mp 115−116 °C (ethanol); ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 2H), 2.09 (m, 3H), 2.24 (q, 2H), 3.26 (q, 2H), 3.90 (s, 6H), 4.59 (t, 2H), 6.34 (br t, 1H), 6.76 (d, 1H), 6.82, 6.86 (dd, 1H, J = 8.79 Hz and J = 1.95 Hz), 7.27 (d, 1H, J = 2.93 Hz), 7.55 (d, 1H, J = 8.79 Hz); IR (Nujol) 3307, 1701, 1646 cm^{-1}; MS (EI) m/z 318 (M^+), 174 (100). Anal. ($C_{17}H_{22}N_2O_4$) C, H, N.

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