Articles

Mapping the Melatonin Receptor. 6. Melatonin Agonists and Antagonists Derived from 6*H*-Isoindolo[2,1-*a*]indoles, 5,6-Dihydroindolo[2,1-*a*]isoquinolines, and 6,7-Dihydro-5*H*-benzo[*c*]azepino[2,1-*a*]indoles

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6*H*-Isoindolo[2,1-*a*]indoles (**5**, **7**, **10**, **13**), 5,6-dihydroindolo[2,1-*a*]isoquinolines (**20**, **21**), and 6,7-dihydro-5*H*-benzo[*c*]azepino[2,1-*a*]indoles (**23**, **25**, **27**, **30**) have been prepared as melatonin analogues to investigate the nature of the binding site of the melatonin receptor. The affinity of analogues was determined in a radioligand binding assay using cloned human mt_1 and MT_2 receptor subtypes expressed in NIH 3T3 cells. Agonist and antagonist potency was measured using the pigment aggregation response of a clonal line of *Xenopus laevis* melanophores. The 2-methoxyisoindolo[2,1-*a*]indoles (**7a**−**d**) showed much higher binding affinities than the parent isoindoles (**5a**−**e**), and whereas **7a**−**c** were agonists in the functional assay, **7d** and **5a**−**e** were antagonists. The 2-ethoxyisoindolo[2,1-*a*]indoles (**10a**−**d**) showed reduced binding affinities compared to their methoxy analogues, while the 5-chloro derivative **13** showed a considerable reduction in binding affinity and potency compared to **7a**. The 10-methoxy-5,6-dihydroindolo-[2,1-*a*]isoquinolines (**21a**−**c**) had higher binding affinities than the corresponding parent indoloisoquinolines (**20a**−**c**) in the human receptor subtypes, and the parent compounds were antagonists whereas the 10-methoxy derivatives were agonists in the functional assay. The *N*-cyclobutanecarbonyl derivatives of both the parent (**20d**) and 10-methoxyl (**21d**) series had similar binding affinities and were both antagonists with similar potencies. The 11-methoxy-6,7-5*H*-benzo[*c*]azepino[2,1-*a*]indoles (**25a**−**d**) had higher binding affinities than the corresponding parent compounds (**23a**−**d**) at the MT_2 receptor but similar affinities at the mt_1 site; all of the compounds were antagonists in the functional assay. Changing 11-methoxy for 11-ethoxy decreased the binding affinity slightly, and this was more evident at the MT_2 receptor. All of the derivatives investigated had either the same or a greater affinity for the human MT_2 receptor compared to the mt_1 receptor (range 1:1−1:132). This suggests that the mt_1 and MT_2 receptor pockets differ in their ability to accommodate alkyl groups in the indole nitrogen region of the melatonin molecule. Two compounds (**7c** and **25c**) were tested in functional assays on recombinant mt_1 and MT_2 melatonin receptors. Compound **7c** is a potent agonist with some selectivity (44-fold) for the MT_2 receptor, while **25c** is an MT_2-preferring antagonist. Increasing the carbon chain length between N-1 of indole and the 2-phenyl group from $n = 1$ through $n = 3$ leads to a fairly regular decrease in the binding affinity, but, remarkably, when $n = 3$, it converts the methoxy compounds from melatonin agonists to antagonists. The *Xenopus* melatonin receptor thus cannot accommodate an *N*-*n*-alkyl chain attached to a 2-phenyl substituent with $n > 2$ in the required orientation to induce or stabilize the active receptor conformation.

Introduction

Sleep problems are common, particularly among the elderly, with up to 50% of people over the age of 65 reporting trouble sleeping some or part of the time.[1,2] Night time insomnia is associated with increased daytime sleepiness, reduced motor and cognitive performance, and reduced productivity in the workplace, and it is an important cause of industrial and road traffic

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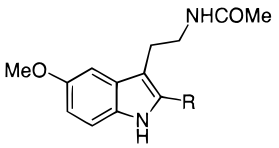
accidents. Current hypnotic drugs are recommended only for short-term treatment of insomnia, but concerns about "hangover" effects and problems upon withdrawal persist. Many people with occasional sleep problems resort to self-medication, and over-the-counter sales of medicines for sleep problems are increasing rapidly.[3] Melatonin (*N*-acetyl 5-methoxytryptamine, **1a**), a hormone synthesized and released from the pineal gland at night, has been shown to have a hypnotic action in animals[4] and humans,[5−7] and there has been considerable recent interest in the therapeutic potential of melatonin analogues as hypnotics and as agents for restoring circadian rhythms disturbed by jet-lag, shift-work, and aging.[8−10]



1a R = H
1b R = I

The physiological actions of melatonin in regulating seasonal and circadian rhythms are thought to be mediated through a family of specific, high affinity, G-protein-coupled cell membrane receptors.[11] Radioligand binding studies using 2-[^{125}I]iodomelatonin **1b** have revealed a widespread, heterogeneous distribution of binding sites throughout the central nervous system.[12] Binding sites are particularly abundant in tissues known to respond to melatonin, including the retina, the suprachiasmatic nuclei of the hypothalamus (the site of the biological clock in mammals), the pars tuberalis of the pituitary, and cerebral and tail arteries.[13] Two receptor subtypes (mt_1 and MT_2) have been cloned in mammals[14,15] which, when expressed in host cells, show the general pharmacological characteristics of native melatonin receptors. A third subtype (termed Mel_{1c}), not detected in mammals, has been cloned from chicken, *Xenopus*, and zebra fish.[16]

Notwithstanding the renewed interest in melatonin and its actions in recent years, the pharmacology of melatonin is in its infancy. In a series of studies we have sought to understand how melatonin binds to and activates its receptor[17−21] and to use the knowledge gained to design subtype specific receptor agonists and antagonists which will be useful tools for defining the full physiological and pathophysiological role of this hormone. We and others[22] have shown that the 5-methoxyl group of melatonin is an important site for binding to the receptor, but it is not an essential requirement for agonist activity.[17,21] The active conformation of the 3-ethanamide side chain has also been established from studies with conformationally restricted indole[18,21,23] and non-indole[24−28] analogues. Models for the binding of melatonin to its receptor have been formulated on the basis of structure−activity data, analogies with other G-protein-coupled receptors for small molecular weight ligands (such as the catecholamines and serotonin), and comparative molecular field analysis.[19,21,29−31] Recent site-directed mutagenesis studies on the ovine mt_1 subtype[32] have given experimental support to aspects of this model. We have now examined the role of the indole nitrogen region of the molecule on radioligand binding at the recombinant mt_1 and MT_2 subtypes and receptor activation using the pigment aggregation assay on a clonal *Xenopus* melanophore cell line[33] and, for two specific compounds, have investigated the agonist and antagonist behavior in a functional assay on recombinant mt_1 and MT_2 melatonin receptors expressed in the NIH 3T3 cell line. These new analogues use a 2-phenyl substituent annelated to the indole nitrogen by an alkyl chain of one to three carbons to retain the 3-ethanamide side chain in an optimum conformation.

Chemistry

The isoindolo[2,1-*a*]indoles and benzo[*c*]azepeno[2,1-*a*] indoles were prepared by the method of Kozikowski et al.[34,35] The appropriate *N*-acetyl tryptamine was alkylated on the indole nitrogen with 2-bromobenzyl bromide (**3a**) or 3-(2-bromophenyl)-1-bromopropane (**3b**), and the derived *N*-alkyl indole was then cyclized with $Pd(PPh_3)_4$ to give the desired product (Scheme 1).

Attempts to apply the same methodology to the synthesis of the indolo[2,1-*a*]isoquinolines were unsuccessful owing to a preferred elimination of the 2-(2-bromophenyl)bromoethane to 2-bromostyrene. Substitution of the tosylate **14** for the bromide led to the desired alkylation of the indole nitrogen with 3-formylindoles **15** to give the *N*-alkyl indoles **16**.[36] Cyclization of the *N*-2-(2-bromophenyl)ethylindoles **16** with $Pd(PPh_3)_4$ gave the tetracyclic indolo[2,1-*a*]isoquinoline derivatives **17**, and the sequence of the Henry reaction followed by reduction gave the amines **19** which were not purified but were immediately acylated with the appropriate reagent to give the desired indolo[2,1-*a*]-isoquinolines **20** and **21** (Scheme 2).

Pharmacology

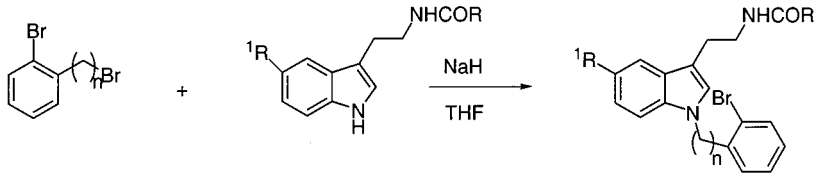
The affinity of the analogues was determined in competition radioligand binding assays using 2-[^{121}I]-iodomelatonin (specific activity 2200 Ci/mol, DuPont, Stevenage, U.K.) as described previously[37] on the recombinant human mt_1 and MT_2 subtypes expressed in NIH 3T3 cells,[14,15] kindly provided by Dr. S. M. Reppert (Harvard Medical School, Boston, MA).The biological activity of the analogues was assessed in a well-established, specific model of melatonin action, the pigment aggregation response of *Xenopus laevis* melanophores.[38,39] In these cells many thousands of black pigment granules are normally distributed throughout the cell, and addition of melatonin induces their rapid movement toward the center of the cell. This response is termed pigment aggregation and can be quantified by measuring the change in light absorbance of the cells as the pigment concentrates near the cell center. In the present study, a clonal melanophore cell line, generously provided by Dr. Michael Lerner (Department of Dermatology, University of Texas), was used.

The agonist and antagonist potency of two analogues (**7c** and **25c**) was also determined on mt_1 and MT_2 receptors by measuring their ability to alter the concentration of intracellular cyclic AMP.

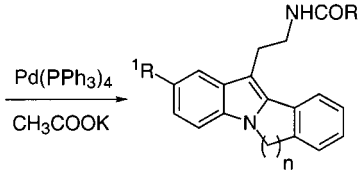
Results and Discussion

We have previously examined the limiting structural requirements, the optimal *N*-alkanoyl group, the preferred conformation of the 3-amidoethane side chain,

Scheme 1



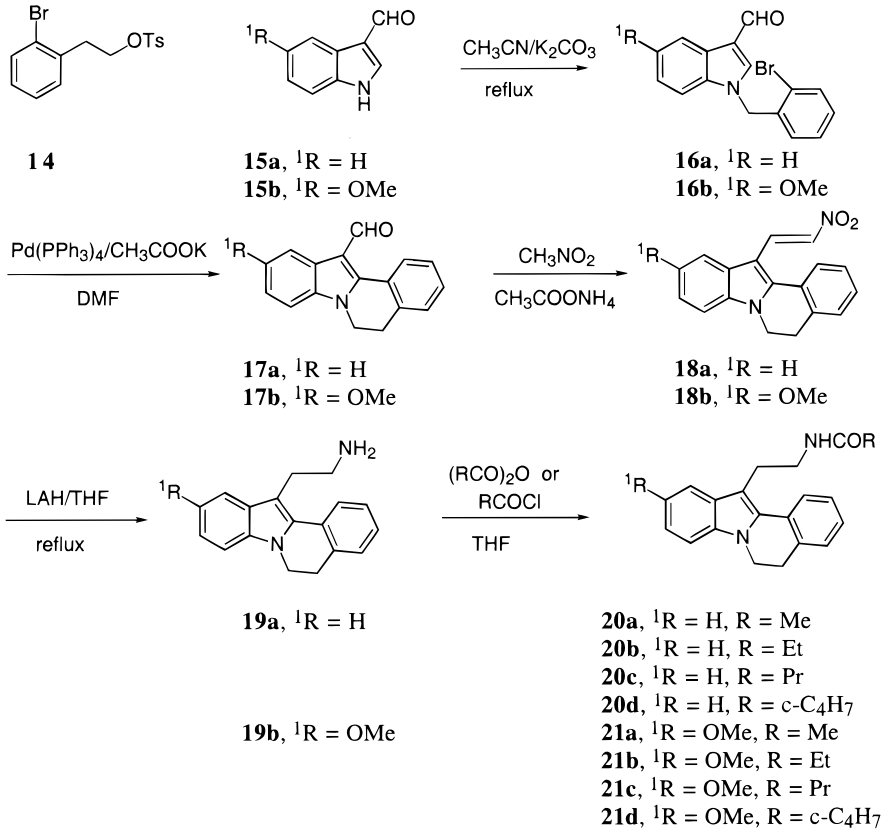
3a, n = 1	**2a**, ^{1}R = H, R = Me	**4a**, n = 1, ^{1}R = H, R = Me
	2b, ^{1}R = H, R = Et	**4b**, n = 1, ^{1}R = H, R = Et
	2c, ^{1}R = H, R = Pr	**4c**, n = 1, ^{1}R = H, R = Pr
	2d, ^{1}R = H, R = c-C_3H_5	**4d**, n = 1, ^{1}R = H, R = c-C_3H_5
	2e, ^{1}R = H, R = c-C_4H_7	**4e**, n = 1, ^{1}R = H, R = c-C_4H_7
	1a, ^{1}R = OMe, R = Me	**6a**, n = 1, ^{1}R = OMe, R = Me
	1c, ^{1}R = OMe, R = Et	**6b**, n = 1, ^{1}R = OMe, R = Et
	1d, ^{1}R = OMe, R = Pr	**6c**, n = 1, ^{1}R = OMe, R = Pr
	1e, ^{1}R = OMe, R = c-C_3H_5	**6d**, n = 1, ^{1}R = OMe, R = c-C_3H_5
	8a, ^{1}R = OEt, R = Me	**9a**, n = 1, ^{1}R = OEt, R = Me
	8b, ^{1}R = OEt, R = Et	**9b**, n = 1, ^{1}R = OEt, R = Et
	8c, ^{1}R = OEt, R = Pr	**9c**, n = 1, ^{1}R = OEt, R = Pr
	8d, ^{1}R = OEt, R = c-C_3H_5	**9d**, n = 1, ^{1}R = OEt, R = c-C_3H_5
	11, ^{1}R = Cl, R = Me	**12**, n = 1, ^{1}R = Cl, R = Me
3b, n = 3	**2a**, ^{1}R = H, R = Me	**22a**, n = 3, ^{1}R = H, R = Me
	2b, ^{1}R = H, R = Et	**22b**, n = 3, ^{1}R = H, R = Et
	2c,^{1}R = H, R = Pr	**22c**, n = 3, ^{1}R = H, R = Pr
	2d, ^{1}R = H, R = c-C_3H_5	**22d**, n = 3, ^{1}R = H, R = c-C_3H_5
	2e, ^{1}R = H, R = c-C_4H_7	**22e**, n = 3, ^{1}R = H, R = c-C_4H_7
	1a, ^{1}R = OMe, R = Me	**24a**, n = 3, ^{1}R = OMe, R = Me
	1c, ^{1}R = OMe, R = Et	**24b**, n = 3, ^{1}R = OMe, R = Et
	1d, ^{1}R = OMe, R = Pr	**24c**, n = 3, ^{1}R = OMe, R = Pr
	1e, ^{1}R = OMe, R = c-C_3H_5	**24d**, n = 3, ^{1}R = OMe, R = c-C_3H_5
	1f, ^{1}R = OMe, R = c-C_4H_7	**24e**, n = 3, ^{1}R = OMe, R = c-C_4H_7
	8a, ^{1}R = OEt, R = Me	**26a**, n = 3 ^{1}R = OEt, R = Me
	8b, ^{1}R = OEt, R = Et	**26b**, n = 3, ^{1}R = OEt, R = Et
	8c, ^{1}R = OEt, R = Pr	**26c**, n = 3, ^{1}R = OEt, R = Pr
	8d, ^{1}R = OEt, R = c-C_3H_5	**26d**, n = 3, ^{1}R = OEt, R = c-C_3H_5
	28, ^{1}R = Cl, R = Me	**29**, n = 3, ^{1}R = Cl, R = Me



5a, n = 1, ^{1}R = H, R = Me	**23a**, n = 3, ^{1}R = H, R = Me
5b, n = 1, ^{1}R = H, R = Et	**23b**, n = 3, ^{1}R = H, R = Et
5c, n = 1, ^{1}R = H, R = Pr	**23c**, n = 3, ^{1}R = H, R = Pr
5d, n = 1, ^{1}R = H, R = c-C_3H_5	**23d**, n = 3, ^{1}R = H, R = c-C_3H_5
5e, n = 1, ^{1}R = H, R = c-C_4H_7	**23e**, n = 3, ^{1}R = H, R = c-C_4H_7
7a, n = 1, ^{1}R = OMe, R = Me	**25a**, n = 3, ^{1}R = OMe, R = Me
7b, n = 1, ^{1}R = OMe, R = Et	**25b**, n = 3, ^{1}R = OMe, R = Et
7c, n = 1, ^{1}R = OMe, R = Pr	**25c**, n = 3, ^{1}R = OMe, R = Pr
7d, n = 1, ^{1}R = OMe, R = c-C_3H_5	**25d**, n = 3, ^{1}R = OMe, R = c-C_3H_5
	25e, n = 3, ^{1}R = OMe, R = c-C_4H_7
10a, n = 1, ^{1}R = OEt, R = Me	**27a**, n = 3, ^{1}R = OEt, R = Me
10b, n = 1, ^{1}R = OEt, R = Et	**27b**, n = 3, ^{1}R = OEt, R = Et
10c, n = 1, ^{1}R = OEt, R = Pr	**27c**, n = 3, ^{1}R = OEt, R = Pr
10d, n = 1, ^{1}R = OEt, R = c-C_3H_5	**27d**, n = 3, ^{1}R = OEt, R = c-C_3H_5
13, n = 1, ^{1}R = Cl, R = Me	**30**, n = 3, ^{1}R = Cl, R = Me

and the range of C-5 substituents tolerated in melatonin agonists. In continuing our attempts to define the characteristics of the melatonin receptor, we have turned our attention to the indole nitrogen region of the molecule. It has previously been shown that a methyl substituent is reasonably well tolerated on the indole nitrogen but that longer alkyl groups or the benzyl group markedly decreased binding affinity.[18,19,40,41] Following the observation that 2-iodomelatonin was a more potent agonist than melatonin itself, a number of groups

Scheme 2



14

15a, ^{1}R = H
15b, ^{1}R = OMe

16a, ^{1}R = H
16b, ^{1}R = OMe

17a, ^{1}R = H
17b, ^{1}R = OMe

18a, ^{1}R = H
18b, ^{1}R = OMe

19a, ^{1}R = H

19b, ^{1}R = OMe

20a, ^{1}R = H, R = Me
20b, ^{1}R = H, R = Et
20c, ^{1}R = H, R = Pr
20d, ^{1}R = H, R = c-C_4H_7
21a, ^{1}R = OMe, R = Me
21b, ^{1}R = OMe, R = Et
21c, ^{1}R = OMe, R = Pr
21d, ^{1}R = OMe, R = c-C_4H_7

have prepared 2-substituted melatonin analogues and have shown that these can be more potent than melatonin.[19,22,42,43] This increase in potency has been ascribed to both the increased population of the active conformation for binding to the melatonin receptor and to the presence of a C-2 binding site. To take advantage of this increase in binding while examining the steric requirements at the indole nitrogen, we decided to synthesize a series of indoles in which a 2-phenyl substituent was attached at the ortho position to the indole nitrogen by *n* methylene groups ($n = 1-3$).

The cloning of the melatonin receptor[14,44] and the identification of high affinity melatonin subtypes[11,15] have allowed the comparison of agonists at the two sites, and for all compounds examined, the binding affinity at the MT_2 receptor is equal to or greater than that at the mt_1 receptor. As part of our study we have therefore examined the binding affinities of a series of new compounds at both the mt_1 and MT_2 receptor in order to determine if there are marked differences in the binding site of the receptors for this region of the melatonin molecule.

The compounds prepared were characterized by standard methods as described in the Experimental Section. The results of the binding and melanophore assays for 6*H*-isoindolo[2,1-*a*]indoles, 5,6-dihydroindolo[2,1-*a*]isoquinolines and 6,7-dihydro-5*H*-benzo[*c*]-azepino-[2,1-*a*]-indoles are shown in Tables 1, 2, and 3, respectively.

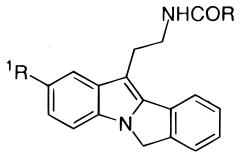
The melatonin receptor subtype mediating pigment aggregation has not been formally defined. *Xenopus leavis* melanophores do express a Mel_{1c} subtype, as these cells were the source of the cDNA used for the first melatonin receptor cloning,[44] but it is not known if *Xenopus* mt_1 or MT_2 subtypes are expressed in these cells. Only partial sequences of *Xenopus* mt_1 and MT_2 receptors subtypes have been cloned.[15] A correlation of potency (IC_{50}) in the pigment aggregation assay with binding affinity at human mt_1 or MT_2 receptor subtypes for the 28 melatonin antagonists in Tables 1, 2, and 3 was not significant (potency vs mt_1 affinity $r^2 = 0.002$, $p = 0.80$; potency vs MT_2 affinity $r^2 = 0.063$, $p = 0.20$). This lack of correlation suggests that melatonin-induced pigment aggregation is not mediated by either mt_1 or MT_2 receptor subtypes.

The 6*H*-isoindolo[2,1-*a*]indoles **5a**−**e** with a proton at R^1 exhibit little change in binding affinity on going from acetyl to butanoyl as the acylating group as is shown by *N*-acyl 5-methoxytryptamines analogues **45** (Table 1), but cyclic *N*-acyl groups reduce affinity. The series has higher binding affinities at the MT_2 receptor and the MT_2/mt_1 selectivities for the alkanoyl systems **5a**−**c** were all around 10-fold. All of these compounds were weak antagonists in the *Xenopus* assay. Introduction of a methoxyl group at R^1 led to the expected increase in binding affinity, the alkanoyl systems **7a**−**c** being comparable to melatonin but with a much greater selectivity for MT_2 over mt_1. These compounds were highly potent agonists in the *Xenopus* assay (Table 1, Figure 1).

One analogue (**7c**), which was 89-fold selective for the MT_2 receptor (compared to the mt_1 receptor, Table 1) in radioligand binding assays, was also shown to have potent agonist activity on the human mt_1 and MT_2 receptors expressed in NIH 3T3 cells (Figure 2).

Compound **7c**, like melatonin, inhibited forskolin stimulation of cyclic AMP (EC_{50}, mean ± SEM, $n = 4$: melatonin, mt_1 0.08 ± 0.04 nM, MT_2 0.15 ± 0.02 nM; **7c**, mt_1 2.19 ± 0.8 nM, MT_2 0.05 ± 0.01 nM). In this

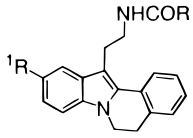
Table 1



compound	R^1	R	receptor binding (K_i, nM) human mt_1	receptor binding (K_i, nM) human MT_2	selectivity MT_2/mt_1	*Xenopus* melanophores agonist (EC_{50}, nM)	*Xenopus* melanophores antagonist (IC_{50}, nM)
melatonin			0.66 ± 0.34	0.33 ± 0.14	2	0.08 ± 0.005	NA
luzindole[a]			603 ± 37.9	44.7 ± 21.8	13	NA	2460 ± 460
N-CBCPT[b]						NA	3670
5a	H	Me	195 ± 70	17.0 ± 6.3	11	NA	24600 ± 7600
5b	H	Et	204 ± 49	17.4 ± 4.8	12	NA	4571 ± 1260
5c	H	Pr	174 ± 45	12.0 ± 3.3	14	NA	2340 ± 650
5d	H	c-C_3H_5	776 ± 228	224 ± 6.7	3	NA	4170 ± 190
5e	H	c-C_4H_7	1200 ± 406	1120 ± 229	1	NA	3160 ± 70
7a	OMe	Me	1.82 ± 0.44	0.06 ± 0.03	30	2.29 ± 1.0	NA
7b	OMe	Et	4.37 ± 0.72	0.17 ± 0.06	26	21.9 ± 0.98	NA
7c	OMe	Pr	4.47 ± 1.10	0.05 ± 0.10	89	5.75 ± 0.38	NA
7d	OMe	c-C_3H_5	46.8 ± 9.7	0.95 ± 0.42	49	NA	7410 ± 490
10a	OEt	Me	18.2 ± 3.5	0.21 ± 0.08	87	316 ± 70.8	NA
10b	OEt	Et	14.5 ± 3.1	0.26 ± 0.05	56	178 ± 8.0	NA
10c	OEt	Pr	8.91 ± 1.60	0.16 ± 0.02	56	204 ± 4.65	NA
10d	OEt	c-C_3H_5	234 ± 60	5.50 ± 0.98	42	NA	28200 ± 6800
13	Cl	Me	282 ± 54	33.9 ± 4.6	8	2090	NA

[a] See ref 58. [b] *N*-(Cyclobutanecarbonyl)-2-phenyltryptamine, ref 19. NA = no agonist or antagonist effect detected at 100 mM. mt_1 and MT_2 data are the mean of quadruplicate determinations. Agonist and antagonist data on melanophores are the mean of triplicate experiments.

Table 2



compound	R^1	R	receptor binding (K_i, nM) human mt_1	receptor binding (K_i, nM) human MT_2	selectivity (MT_2/mt_1	*Xenopus* melanophores agonist (EC_{50}, nM)	*Xenopus* melanophores antagonist (IC_{50}, nM)
melatonin			0.66 ± 0.34	0.33 ± 0.14	2	0.08 ± 0.005	NA
luzindole[a]			603 ± 37.9	44.7 ± 21.8	13	NA	2460 ± 460
N-CBCPT[b]						NA	3670
20a	H	Me	389 ± 196	36.3 ± 21.2	11	NA	8910 ± 3290
20b	H	Et	148 ± 130	9.33 ± 5.3	16	NA	2290 ± 250
20c	H	Pr	70.8 ± 43.1	3.7 ± 1.2	19	NA	447 ± 67
20d	H	c-C_4H_7	2040 ± 630	355 ± 196	6	NA	3720 ± 250
21a	OMe	Me	7.24 ± 0.43	0.51 ± 0.26	14	12.6 ± 2.12	<100000
21b	OMe	Et	5.89 ± 0.34	0.12 ± 0.21	49	6.03 ± 0.66	79400 ± 3600
21c	OMe	Pr	4.07 ± 0.24	0.20 ± 0.018	20	9.33 ± 1.75	42700 ± 8000
21d	OMe	c-C_4H_7	437 ± 27	324 ± 179	1	NA	3720 ± 1020

[a] See ref 58. [b] *N*-(Cyclobutanecarbonyl)-2-phenyltryptamine, ref 19. NA = no agonist or antagonist effect detected at 100 mM. See footnotes, Table 1, for methods.

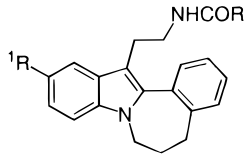
biological assay **7c** was considerably more potent (44-fold) at the MT_2 receptor subtype than the mt_1 subtype and is the most selective agonist yet described.

The cyclopropanecarbonyl derivative **7d** had a high binding affinity at both subtypes but was a weak antagonist in the *Xenopus* assay. Changing R^1 from methoxy to ethoxy led to some diminution in binding affinity and melanophore potency, but otherwise the compounds mirrored the methoxy derivatives. The trends for the 6*H*-isoindolo[2,1-*a*]indoles series of compounds are very similar to those found for the corresponding 2-phenyltryptamine derivatives except that the parent *N*-alkanoyl 2-phenyltryptamines were agonists in the *Xenopus* assay.[19]

The 5,6-dihydroindolo[2,1-*a*]isoquinolines **20a**−**c** had similar binding affinities to the isoindoloindoles and had relatively similar selectivities for the MT_2 receptor (Table 2). These derivatives were also antagonists in the *Xenopus* assay, and **20c** (R = Pr) was reasonably potent. Introducing the methoxyl group at R^1 (**21a**−**c**) substantially increased the binding affinity, and the compounds were fairly potent agonists in the *Xenopus* assay. When examined as antagonists, however, these compounds also showed very weak antagonist potency. The *N*-cyclobutanecarbonyl derivatives **20d** (R^1 = H) and **21d** (R^1 = OMe) both exhibited similar low binding affinity at the MT_2 receptor and the same antagonist potency, suggesting that the methoxyl group is not operating as an important binding site in **21d** at MT_2 or melanophore receptors.

The 6,7-dihydro-5*H*-benzo[*c*]azepino[2,1-*a*]indoles showed a profile of binding affinities similar to the other two series. Introducing the methoxyl group at R^1 increased the binding affinity substantially at the MT_2

Table 3



compound	R^1	R	receptor binding (K_i, nM) human mt_1	receptor binding (K_i, nM) human MT_2	selectivity (MT_2/mt_1)	Xenopus melanophores agonist (EC_{50}, nM)	Xenopus melanophores antagonist (IC_{50}, nM)
melatonin			0.66 ± 0.34	0.33 ± 0.14	2	0.08 ± 0.005	NA
luzindole[a]			603 ± 37.9	44.7 ± 21.8	13	NA	2460 ± 460
N-CBCPT[b]						NA	3670
23a	H	Me	240 ± 76	224 ± 55	1	NA	3470 ± 520
23b	H	Et	115 ± 30	45.7 ± 15	2	NA	1410 ± 1240
23c	H	Pr	79.4 ± 23	63.1 ± 18	1	NA	1860 ± 120
23d	H	c-C_3H_5	117 ± 46	52.5 ± 37	2	NA	1180 ± 30
23e	H	c-C_4H_7	251 ± 111	309 ± 92	1	NA	3470 ± 1330
25a	OMe	Me	275 ± 50	6.31 ± 1.94	44	NA	2400 ± 620
25b	OMe	Et	52.5 ± 16	1.41 ± 0.45	37	NA	1100 ± 360
25c	OMe	Pr	66.1 ± 18	0.50 ± 0.14	132	NA	525 ± 216
25d	OMe	c-C_3H_5	501 ± 123	12.0 ± 5.3	42	NA	550 ± 103
25e	OMe	c-C_4H_7	759 ± 165	31.6 ± 10.7	24	NA	3470 ± 1590
27a	OEt	Me	513 ± 152	117 ± 18.4	4	NA	37100 ± 14200
27b	OEt	Et	269 ± 77	21.9 ± 3.4	12	NA	3980 ± 590
27c	OEt	Pr	257 ± 70	6.46 ± 1.24	40	NA	380 ± 90
27d	OEt	c-C_3H_5	2950 ± 845	85.1 ± 14	35	NA	224 ± 33
30	Cl	Me	288 ± 110	5.01 ± 3.98	57	NA	282 ± 63

[a] See ref 58. [b] *N*-(Cyclobutanecarbonyl)-2-phenyltryptamine, ref 19. NA = no agonist or antagonist effect detected at 100 mM. See footnotes, Table 1, for methods.

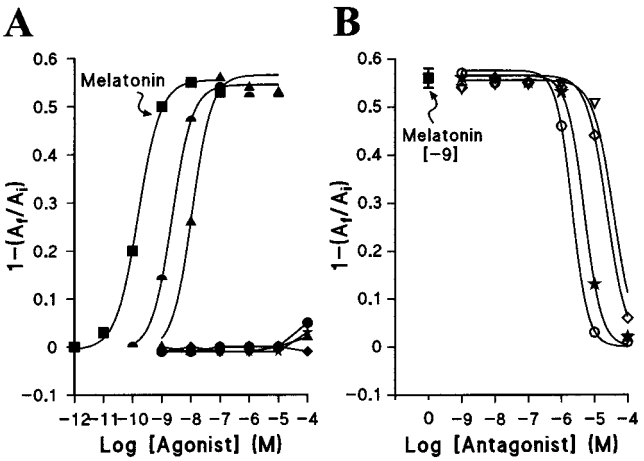


Figure 1. Changes in pigment distribution in *Xenopus leavis* melanophores. (A) Aggregation of pigment in response to varying concentrations of melatonin, **7a** (solid semicircle), **21a** (▲), **20a** (◆), **5a** (▼), **23a** (★), **25a** (●). The change in pigment distribution was determined by measuring cell absorbance before (A_i) and 60 min after (A_f) addition of analogues. (B) Antagonism of melatonin-induced (10^{-9} M) pigment aggregation by varying concentrations of **20a** (◇), **5a** (▽), **23a** (★), and **25a** (○). Each point is the mean ± SEM of quadruplicate wells. Error bars are omitted when the SEM was less than 0.02.

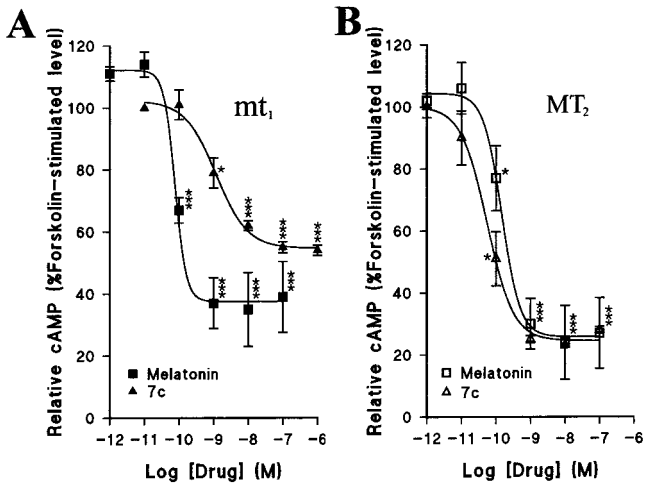


Figure 2. Inhibition of cyclic AMP synthesis induced by forskolin by melatonin and **7c** in NIH 3T3 cells expressing human mt_1 (A) or MT_2 (B) melatonin receptor subtypes. Cells were treated with forskolin (10^{-5} M, 10 min) in the absence or presence of varying concentrations of agonists. For each cell sample cyclic AMP was measured in duplicate by radioimmunoassay. Data are expressed as the percentage inhibition of the response to forskolin alone. Each point is the mean ± SEM of quadruplicate cell samples. Error bars are omitted when the SEM was less than the area covered by the symbol. *p < 0.05, ***p < 0.001 using Student's t-test compared to forskolin-stimulated cAMP level.

receptor, and substitution of ethoxy for methoxy led to a decrease in binding affinity. In the *Xenopus* assay, however, no agonist activity was detected in any of these compounds up to 10^{-4} M, and all the compounds, including the methoxy and ethoxy derivatives, were antagonists. The methoxy derivative **25c** (R = Pr) showed the largest selectivity for MT_2 over mt_1 (132-fold) of all the derivatives investigated in this study. Furthermore, **25c** was shown to act as a melatonin receptor antagonist not only on *Xenopus* melanophores but also on the mt_1 and MT_2 receptor subtypes expressed in NIH 3T3 cells (Figure 3). Compound **25c** antagonized melatonin inhibition of forskolin stimulation of cyclic AMP, but it did not have any agonist activity at these receptor subtypes as no significant inhibition of forskolin stimulation of cyclic AMP was detected at up to 10 mM (data not shown). The antagonist potency of **25c** (IC_{50}, concentration producing half-maximal antagonism of the melatonin response, mean ± SEM, n = 3−4) was 7.7 ± 1.5 nM at the mt_1 subtype

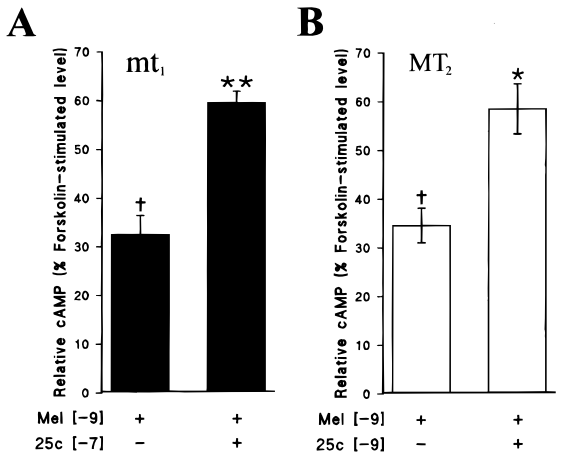


Figure 3. Antagonism of melatonin inhibition of cyclic AMP synthesis stimulated by forskolin in NIH 3T3 cells expressing human mt_1 (A) and MT_2 (B) receptor subtypes. Cells were pretreated (1 h) with vehicle or **25c** (10^{-7} M for mt_1 cells or 10^{-9} M for MT_2 cells) before addition of forskolin (10^{-5} M) plus melatonin (10^{-9} M) for 10 min. These concentrations were close to the K_i values for **25c** at each receptor subtype (Table 3). Each bar represents the mean ± SEM of quadruplicate cell samples. Significantly different from forskolin plus melatonin alone, $^*p < 0.05$, $^\dagger p < 0.001$ compared to forskolin-treated cells, $^{**}p < 0.01$ compared to melatonin-treated cells using Student's *t*-test.

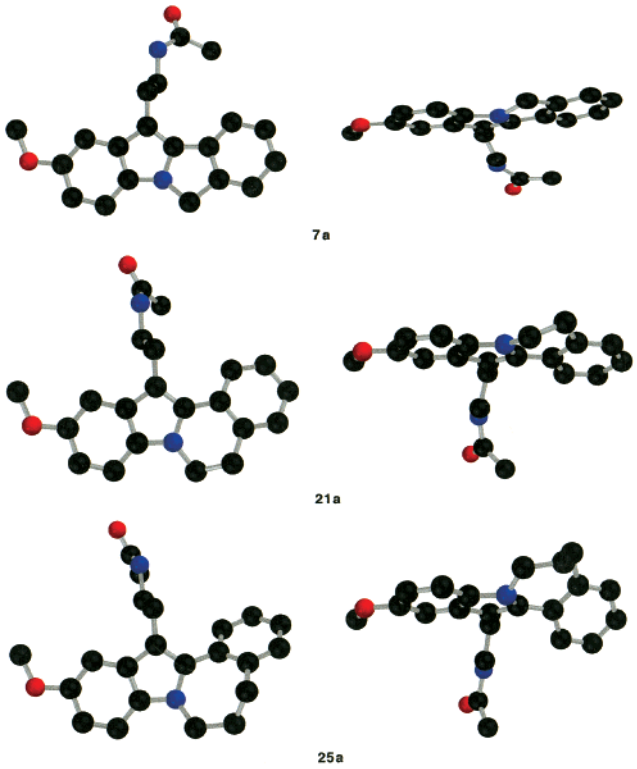


Figure 4. PM3 minimized energy structures for compounds **7a**, **21a**, and **25a** showing similar disposition of the methoxyl with the ethanamide side chains.

and 0.14 ± 0.05 nM at the MT_2 subtype). Thus **25c** has some selectivity (55-fold) for the MT_2 receptor subtype.

An interesting difference can be noted for the substitution of chlorine for methoxyl between the isoindoloindole and benzazepinoindole series. Substitution of chlorine in the isoindoloindole series (**13**) greatly reduces the binding affinity for both the mt_1 and MT_2 receptors, **13** showing a lower binding affinity than the parent compound **5a**. It is, however, unlike **5a** in being a weak agonist rather than a weak antagonist (Table 1). Substituting a chlorine in the benzazepinoindole series gives a compound (**30**) with binding affinities very similar to those of the methoxyl derivative **25a**. The binding affinities of **25a** and **30** are similar to the parent compound **23a** at the mt_1 receptor, but both methoxy and chloro compounds have much higher binding affinities at the MT_2 receptor. The chloro compound **30** is, however, 8-fold more potent as an antagonist than **25a**, which is itself little more potent than **23a** (Table 3).

In the present work, the three series of compounds differ mainly in the number of atoms bridging the indole to phenyl ring, although the changes in ring size that this engenders results in a conformational change between the phenyl ring and the indole portion of the molecule. This is illustrated in Figure 4, which shows energy minimized PM3 structures for **7a**, **21a**, and **25a**. In all three compounds, however, the methoxyl and ethanamide side chain have a similar relationship to each other. While introduction of a 2-phenyl group gave agonists with improved binding affinity compared to melatonin,[19] a 2-phenyl ring bridged ortho to the indole reduces affinity. A single carbon is more easily accommodated than two, which in turn is better tolerated than three carbons. The requirements at the binding pocket of the MT_2 subtype appear less stringent than those at the mt_1 receptor, resulting in several analogues with considerable MT_2 subtype specificity. These included compounds which mimicked the action of melatonin on *Xenopus* melanophores (agonists) and others which acted as competitive antagonists. Recent studies with agonist analogues[45] have also indicated that the MT_2 subtype tolerates changes at the 5-position more readily than the mt_1 subtype, while changes to the *N*-acyl group have a similar effect at both receptor subtypes.

The finding that many of the present series of melatonin analogues acted as melatonin receptor agonists or antagonists in the *Xenopus* melanophore model system does not necessarily imply that they will have the same activity at native or recombinant mt_1 and MT_2 receptor subtypes. However, two representative compounds (**7c** and **25c**) have been demonstrated to have the same action (agonist and antagonist, respectively) on human mt_1 and MT_2 receptor subtypes expressed in NIH 3T3 cells (Figures 2 and 3). It will be interesting to examine their activity at native melatonin receptors in other mammalian model systems.

It was initially suggested that the mt_1 subtype mediated melatonin's ability to entrain free-running circadian rhythms because of the high level of expression of its mRNA within the primary pacemaker of the brain, the suprachiasmatic nuclei (SCN) of the hypothalamus.[14] However, melatonin retains its phase-shifting ability in mice in which the mt_1 subtype has been deleted,[46] and receptor antagonists having MT_2 subtype selectivity have recently been shown to reduce melatonin-induced phase advances,[47] indicating the importance of the MT_2 receptor subtype in the actions of melatonin on circadian rhythms. It follows that potent, selective MT_2 receptor subtype agonists might be useful in treating various circadian rhythm disorders.[8−10] Disturbances of circadian physiology are not simply an

inconvenience to transatlantic travellers; chronic rhythm disturbance experienced by many shift workers is associated not only with sleep problems but also with gastrointestinal disturbances[48] and increased incidence of cardiovascular disease[49] and may contribute to increased risk of breast cancer.[50] It has been suggested that melatonin's sleep promoting action is related to its ability to influence the circadian sleep timing mechanisms by acting on the SCN,[51] although acute sleep-inducing effects of melatonin have been reported which may be mediated by actions on other brain regions.[4−7] Selective and potent MT_2 agonists and antagonists such as **7c** and **25c**, and compounds that can now be designed with them as leads, will make possible experiments to test the role of the receptor subtypes in sleep timing mechanisms and in the acute hypnotic action of melatonin. Continuing attempts to define the characteristics of melatonin required for ligand binding, subtype selectivity, and receptor activation are needed to provide the tools necessary to define the full range of its physiological actions and to realize the therapeutic potential of this molecule.

Experimental Section

Melting points were determined on a Reichert melting point apparatus or in glass capillary tubes on an Electrothermal 9100 apparatus and are uncorrected. EI mass spectra were recorded on a VG ZAB-2F mass spectrometer, CI mass spectra on a VG 12-250 mass spectrometer, and FAB mass spectra on a M550 mass spectrometer. Only molecular ions (M^+) or $M^+ + 1$ ions, base peaks, and the next two peaks due to ions of maximum abundance are given. IR spectra were recorded on Perkin-Elmer 883, PE-983, or 1650 FTIR spectrometers, using KBr pellets unless otherwise stated. NMR spectra were taken in $CDCl_3$ unless otherwise stated. ^{1}H NMR spectra were taken on either a Varian VXR-400 or at 75 MHz on a Bruker AC300 MHz spectrometer, and the spectra are reported in δ. ^{13}C NMR spectra were recorded at 100 MHz on a Varian VXR-400 spectrometer and are reported in δ.

DC-Alufolien plates (Kieselgel 60 F_{254}, Schichtdicke 0.2 mm, Merck) were used for analytical TLC and were visualized with ultraviolet light or developed with *p*-anisaldehyde, iodine, or ninhydrin. Flash chromatography was performed using Sorbsil c60-A silica as the stationary phase. Microanalyses were carried out by either Service Central de Microanalyses of CNRS in Vernaison, France, or Microanalytical Section, Dept. of Chemistry, UCL.

NIH 3T3 cells expressing recombinant human mt_1 or MT_2 receptor subtypes were grown in complete Dulbecco's Eagle medium (DMEM) containing 100 i.u./mL penicillin, 0.1 mg/mL streptomycin, 4 mM L-glutamine, 10% fetal bovine serum (Imperial Laboratories, England, U.K.), and Geneticin (G418; 1 mg/mL; GIBCO/BRL) in humidified 5% CO_2/95% air at 37 °C. The affinity constant (K_d) of the mt_1 receptor subtype was 51 ± 7.8 pM, and the maximal binding density (B_{max}) was 165.54.7 fmol/mg protein; the K_d of the MT_2 receptor subtype was 73.4 ± 9.9 pM, and the B_{max} was 70.1 ± 0.6 fmol/mg protein.

Melanophore cells were grown in 96-well tissue culture plates, and growth medium[33,52] was replaced with 0.7 × L-15 culture medium 18 h before analogues were tested. Initial absorbance of cells (A_i, 630 nm) was measured in each well using a Bio-Tek microtiter plate reader (model EL3115, Anachem, U.K.), then cells were treated with the concentrations of the analogues indicated. All experiments used quadruplicate wells at six concentrations of analogue. The final absorbance (A_f) was measured after 60 min, and the fractional change in absorbance ($1 - A_f/A_i$) was calculated. Vehicle did not alter pigment granule distribution itself or inhibit responses to melatonin. The concentration of analogue producing 50% of the maximum agonist response (EC_{50}) was determined using a curve-fitting program.[53] For evaluation of antagonist potency, cells were treated with vehicle (1% DMSO or methanol) or varying concentrations (10^{-4}−10^{-9} M) of the analogues for 60 min before melatonin (10^{-9} M) was added. The concentration of analogue reducing melatonin-induced pigment aggregation by 50% (IC_{50}) was determined.

NIH 3T3 cells expressing human mt_1 or MT_2 receptor subtypes were seeded into 24-well plates and cultured for 48 h until they reached confluence (3−4 10^4 cells/well). After they were washed twice with DMEM, 3-isobutyl-1-methylxanthine (IBMX, 2.5×10^{-4} M) was added for 10 min at 37 °C before the addition of the adenylate cyclase activator forskolin (10^{-5} M) in the absence or presence of melatonin or **7c** to inhibit stimulation of cyclic AMP. All drugs were diluted in DMEM and contained IBMX as described above. After a 10 min incubation at 37 °C, the medium in each well was aspirated and 300 mL of ice-cold acetic acid (50 mM) was added, and cells were scraped from the plate. The lysate was then collected and boiled for 3 min. Antagonist action was determined by treating cells with forskolin (10^{-5} M) and melatonin (10^{-9} M) in the presence or absence of **25c**. All samples were kept at −20 °C until cyclic AMP was determined by RIA following acetylation as described previously,[54,55] using cyclic AMP 2′-*O*-succinyl [^{125}I]-tyrosine methyl ester (DuPont, Stevenage, U.K.).

Physical data for one of each type of analogue is given, and the data for the remaining analogues can be found in the Supporting Information.

General Preparation for the *N*-Alkyl Derivatives 4a−e, 6a−d, 9a−d, 12, 22a−e, 24a−e, 26a−d, and 29. NaH (80%, 0.10 g, 2.3 mmol) was added to a stirred solution of the required *N*-acylated 2-(indol-3-yl)ethylamine (0.9 mmol) in dry THF (3 mL) at 0 °C, and a solution of the required 2-bromoalkylbromide (1.9 mmol) in dry THF (3 mL) was then added slowly. The resulting mixture was stirred at room temperature for 3 h, and saturated aqueous ammonium chloride was then added. The mixture was extracted with ethyl acetate (2 × 50 mL), the organic layers were combined, washed with brine, and dried ($MgSO_4$). The *N*-acyl 2-(*N*-alkylindol-3-yl)ethylamines were isolated by flash chromatography and, if solids, recrystallized from ethyl acetate/petroleum ether 60−80.

General Preparation for the Tetracyclic Derivatives 5a−e, 7a−d, 10a−d, 13, 23a−d, 25a−e, 27a−d, and 30. The required *N*-acyl 2-(*N*-alkylindol-3-yl)ethylamine (0.5 mmol), prepared by the above method, was added to tetrakis(triphenylphosphine)palladium(0) (ca. 30 mg) and potassium acetate (ca. 50 mg) in dimethylamine (8 mL), and the mixture was stirred at 160 °C under N_2 for 16 h. The solvent was then removed under reduced pressure and ethyl acetate (50 mL) added to the residue. The mixture was washed with water (50 mL) and the aqueous phase extracted with ethyl acetate (3 × 50 mL). The organic phases were combined, washed with water (80 mL), brine, and dried ($MgSO_4$). The tetracyclic ethanamides were isolated by flash chromatography and, if solids, recrystallized from ethyl acetate/petroleum ether 60−80.

***N*-Acetyl 2-[1-(2-bromobenzyl)indol-3-yl]ethanamine (4a):** white solid, 60%, mp 110−115 °C; ^{1}H NMR δ 1.9 (s, 3H), 2.97 (t, J = 6.7 Hz, 2H), 3.57 (q, J = 6.8 Hz, 2H), 5.32 (s, 2H), 5.68 (br s, 1H), 6.56 (dd, J = 5.7, 3.5 Hz, 1H), 7.13 (m, 3H), 7.18 (m, 1H), 7.23 (d, J = 8.4 Hz, 1H), 7.58 (dd, J = 5.7, 3.7 Hz, 1H), 7.62 (d, J = 7.5 Hz, 1H); ^{13}C NMR δ 23.3, 25.2, 39.8, 50.0, 109.7, 112.5, 119.0, 119.4, 122.1, 122.3, 126.1, 127.8, 127.9, 128.1, 129.1, 132.8, 136.5, 136.7, 170.0; IR 3293, 2929, 1652, 1556, 1466, 1441, 1025, 740 cm^{-1}; MS *m/e* 373, 372, 371, 370 (100), 314, 313, 312, 311, 300, 298, 171, 169. $C_{19}H_{19}N_2O$-^{79}Br requires 370.2719; Found: 370.2735.

***N*-Propanoyl 2-[1-(2-bromobenzyl)indol-3-yl]ethanamine (4b):** yellow solid, 91%, mp 82−84 °C (lit.[56] 83−84 °C).

***N*-Butanoyl 2-[1-(2-bromobenzyl)indol-3-yl]ethanamine (4c):** yellow solid, 85%, mp 79−80 °C.

***N*-Cyclopropanecarbonyl 2-[1-(2-bromobenzyl)indol-3-yl]ethanamine (4d):** yellow solid, 80%, mp 117.5−118.5 °C.

***N*-Cyclobutanecarbonyl 2-[1-(2-bromobenzyl)indol-3-yl]ethanamine (4e):** white solid, 67%, mp 107−108 °C.

***N*-Propanoyl 2-[1-(2-bromobenzyl)-5-methoxyindol-3-yl]ethanamine (6a):** yellow oil, 91%; ^{1}H NMR δ 1.08 (t, J = 7.6 Hz, 3H), 2.16 (q, J = 7.6 Hz, 2H), 2.93 (t, J = 6.7 Hz, 2H), 3.58 (q, J = 6.5 Hz, 2H), 3.84 (s, 3H), 5.28 (s, 2H), 5.54 (br s, 1H), 6.55 (m, 1H), 6.83 (dd, J = 8.8, 2.5 Hz, 1H), 7.04 (d, J = 2.3 Hz, 1H), 7.11 (m, 3H), 7.57 (m, 1H); ^{13}C NMR δ 9.9, 25.4, 29.9, 39.6, 50.2, 56.0, 100.8, 110.6, 112.1, 112.4, 122.3, 126.7, 127.8, 128.2, 128.4, 129.2, 131.1, 132.8, 135.0, 154.2, 174.0; IR 3298, 2929, 1641, 1454, 1228, 1043, 796 cm^{-1}; MS *m*/*e* 417, 416, 415 (100), 414, 344, 343, 342, 341, 330, 328, 171, 169. $C_{21}H_{24}N_2O_2{}^{79}Br$ (M^+ + H) requires 415.1010; Found: 415.1024.

***N*-Butanoyl2-[1-(2-bromobenzyl)-5-methoxyindol-3-yl]-ethanamine (6b):** yellow solid, 89%, mp 75−76 °C.

***N*-Acetyl 2-(6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (5a):** pale yellow solid, 70%, mp 155−175 °C; ^{1}H NMR δ 1.79 (s, 3H), 3.23 (t, J = 6.5 Hz, 2H), 3.60 (m, 2H), 5.40 (s, 2H), 5.56 (br s, 1H), 7.11 (m, 1H), 7.20 (m, 1H), 7.29 (m, 1H), 7.33 (d, J = 7.9 Hz, 1H), 7.40 (m, 1H), 7.46 (d, J = 7.4 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H); ^{13}C NMR δ 23.3, 25.2, 39.8, 49.8, 103.5, 109.3, 119.3, 119.5, 121.1, 121.8, 123.7, 126.9, 128.3, 128.4, 132.3, 133.1, 133.8, 141.7, 175.0; IR 3290, 3050, 2926, 2854, 1644, 1557, 1443, 1362, 1300, 1189, 734 cm^{-1}; MS *m*/*e* 291, 290, 232, 231, 218, 204; $C_{19}H_{19}N_2O$ (M^+ + H) requires 291.1480: Found: 291.1497. Anal. ($C_{19}H_{18}N_2O$) C, H, N.

***N*-Propanoyl 2-(6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (5b):** yellow solid, 76%, mp 97−98 °C.

***N*-Butanoyl 2-(6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (5c):** yellow crystals, 66%, mp 200−201 °C.

***N*-Cyclopropanecarbonyl 2-(6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (5d):** white solid, 46%, mp 189−192 °C.

***N*-Cyclobutanecarbonyl 2-(6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (5e):** yellow solid, 37%, mp 190−191 °C.

***N*-Acetyl 2-(9-methoxy-6*H*-isoindolo[2,1-*a*]indol-11-yl)-ethanamine (7a):** white solid, 88%, mp 159−161 °C; ^{1}H NMR δ 1.75 (s, 3H), 3.08 (t, J = 7.2 Hz, 2H), 3.29 (q, J = 7.2 Hz, 2H), 3.79 (s, 3H), 5.11 (s, 2H), 6.78 (dd, J = 8.7, 2.4 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 7.34 (m, 1H), 7.43 (t, J = 7.5 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 7.84 (d, J = 7.5 Hz, 1H), 8.00 (br t, 1H); ^{13}C NMR δ 22.7, 24.3, 48.2, 55.4, 101.1, 103.2, 110.4, 111.2, 120.3, 123.9, 126.5, 127.9, 128.7, 132.7, 140.8, 142.0, 153.4, 169.2; IR 3308, 3074, 2928, 2860, 1643, 1559, 1486, 1440, 1372, 1300, 1238, 1225, 1164, 1127, 1106, 1042, 846, 762, 716 cm^{-1}; MS *m*/*e* 321, 320, 262, 261, 248, 234, 43. Anal. ($C_{20}H_{20}N_2O_2$) C, H, N

***N*-Propanoyl 2-(9-methoxy-6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (7b):** pale yellow solid, 56%, mp 165−166 °C.

***N*-Butanoyl 2-(9-methoxy-6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (7c):** pale yellow solid, 68%, mp 154−155 °C.

***N*-Cyclopropanecarbonyl 2-(9-methoxy-6*H*-isoindolo-[2,1-*a*]indol-11-yl)ethanamine (7d):** white solid, 81%, mp 154−156 °C.

***N*-Acetyl 2-(9-ethoxy-6*H*-isoindolo[2,1-*a*]indol-11-yl)-ethanamine (10a):** pale yellow solid, 37%, mp 166−170 °C; ^{1}H NMR δ 1.46 (t, J = 7.0 Hz, 3H), 1.83 (s, 3H), 3.21 (m, 2H), 3.62 (m, 2H), 4.11 (q, J = 7.0 Hz, 2H), 5.03 (s, 2H), 5.6 (br s, 1H), 6.89 (dd, J = 8.7, 2.3 Hz, 1H), 7.07 (d, J = 2.3 Hz, 1H), 7.24 (d, J = 8.7 Hz, 1H), 7.29 (m, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.47 (d, J = 7.4 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H); ^{13}C NMR δ 15.1, 23.5, 24.6, 40.2, 48.4, 64.4, 102.5, 103.0, 110.2, 112.5, 120.7, 123.6, 126.7, 128.3, 129.2, 132.7, 133.3, 141.8, 153.3, 170.3; IR 2983−2865, 1650, 1559,1442, 1221, 765, 723 cm^{-1}; MS *m*/*e* 335, 334, 276, 275, 262 (100). $C_{21}H_{23}N_2O_2$ (M^+ + H) requires 335.1750; Found: 335.1760. Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

***N*-Propanoyl 2-(9-ethoxy-6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (10b):** white solid, 50%, mp 160−164 °C.

***N*-Butanoyl 2-(9-ethoxy-6*H*-isoindolo[2,1-*a*]indol-11-yl)ethanamine (10c):** yellow solid, 66%, mp 153−155 °C.

***N*-Cyclopropanecarbonyl 2-(9-ethoxy-6*H*-isoindolo-[2,1-*a*]indol-11-yl)ethanamine (10d):** yellow solid, 43%, mp 200−201 °C.

***N*-Acetyl 2-(9-chloro-6*H*-isoindololo[2,1-*a*]indol-11-yl)-ethanamine (13):** yellow solid, 62%, mp 186−189 °C; ^{1}H NMR δ 1.85 (s, 3H), 3.21 (m, 2H), 3.60 (m, 2H), 5.05 (s, 2H), 5.6 (br s, 1H), 7.16 (m, 1H), 7.25 (m, 1H), 7.33 (m, 1H), 7.44 (m, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.08 (m, 1H), 7.82 (d, J = 7.7 Hz, 1H); ^{13}C NMR δ 23.6, 24.4, 40.3, 48.4, 102.5, 103.2, 110.0, 112.4, 120.9, 123.5, 126.6, 128.3, 129.3, 132.5, 133.3, 141.7, 153.3, 173.5; IR 3296, 2922, 1652, 1446, 1361, 1060, 788, 763 cm^{-1}; MS *m*/*e* 327, 326, 325 (100), 324, 268, 267, 266, 265, 254, 252. $C_{19}H_{18}N_2O^{35}Cl$ (M^+ + H) requires 325.1108; Found: 325.1097. Anal. ($C_{19}H_{17}ClN_2O$) C, H, N, Cl.

Toluene-4-sulfonic Acid 2-(2-Bromophenyl)ethyl Ester (14). $LiAlH_4$ (1.77 g, 46.64 mmol) was added in one portion at 0 °C to a stirred solution of 2-bromophenylacetic acid (2.50 g, 11.6 mmol) in tetrahydrofuran (150 mL). The resulting suspension was allowed to reach ambient temperature and then refluxed for 18 h. The mixture was then chilled (0 °C) and quenched by the careful dropwise addition of H_2O (12 mL). The resulting white suspension was allowed to reach room temperature and stirred for 15 min. The precipitated salts were removed by filtration, and the filtrate was taken up in ethyl acetate (150 mL). The organic layer was washed with H_2O (2 × 25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo to give 2-bromophenylethyl alcohol as a colorless oil (2.00 g, 9.98 mmol, 86%) which was used without any further purification in the next step. A solution of 2-bromophenylethyl alcohol (2.16 g, 10.75 mmol) in dichloromethane (10 mL) was added dropwise at 0 °C to a stirred solution of *p*-toluenesulfonyl chloride (5.74 g, 30.11 mmol) in pyridine (10 mL) and dichloromethane (4 mL). After the solution stood overnight at −18 °C, the TLC (dichloromethane/ethyl acetate) (90:10) R_f = 0.22, showed that the reaction was complete. The solution was poured into ice-H_2O (100 mL), the mixture stirred for 30 min, the layers separated, and the aqueous phase was extracted with dichloromethane (3 × 25 mL). The combined organic phase was washed with 2 N HCl (5 mL), 5% $NaHCO_3$ (5 mL), and brine (2 × 25 mL) and dried (Na_2SO_4). The solvent was then removed under vacuum to give **14** as a colorless, viscous oil (3.32 g, 9.4 mmol, 87%): ^{1}H NMR δ 2.42 (s, 3H), 3.07 (t, 2H, J = 6.8 Hz), 4.22 (t, 2H, J = 6.8 Hz), 7.27−7.07 (m, 5H), 7.44 (d, 1H, J = 7.9 Hz),7.67 (d, 2H, J = 8.2 Hz).

1-[2-(2-Bromophenyl)ethyl]-1*H*-indole-3-carboxaldehyde (16a). A solution of **14** (3.09 g, 8.70 mmol) in acetonitrile (5 mL)was added dropwise to a reluxing, stirred suspension of indole-3-carboxaldehyde (**15a**) (1.26 g, 8.68 mmol) and potassium carbonate (2.41 g, 17.41 mmol) in acetonitrile (30 mL). The mixture was refluxed for 4.5 h, cooled to room temperature, and then poured into ice-H_2O (100 mL). The solution was stirred for 30 min, ethyl acetate (150 mL) was added, and the mixture separated. The organic layer was washed with H_2O (2 × 50 mL) and brine (25 mL) and dried (Na_2SO_4). The solvent was removed under vacuum, and the residue was purified by flash chromatography (silica gel; petroleum ether 40−60 °C/ethyl acetate) (70:30) to give **16a** as a pale yellow solid: 1.85 g, 5.66 mmol, 65%, mp 96−98 °C (lit.[36] 98−100 °C).

1-[2-(2-Bromophenyl)ethyl]-5-methoxy-1*H*-indole-3-carboxaldehyde (16b). Compound **16b** was obtained in the same manner as a beige solid: 65%, mp 95−97 °C (lit.36 96−98 °C).

5,6-Dihydroindolo[2,1-*a*]isoquinoline-12-carboxaldehyde (17a). Potassium acetate (0.69 g, 7.07 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.27 g, 0.24 mmol) were added sequentially to a solution of **16a** (1.47 g, 4.48 mmol) in dimethylformamide (65 mL). The resulting suspension was stirred at 110 °C overnight. The solvent was evaporated under reduced pressure, and the resulting dark suspension was chromatographed (flash column, petroleum ether 40−60 °C/ethyl acetate, 90:10) to give **17a** as a beige powder: 0.97 g, 3.94 mmol, 88%, mp 123−125 °C (lit.[36] 124−126 °C).

5,6-Dihydro-10-methoxyindolo[2,1-*a*]isoquinoline-12-carboxaldehyde (17b). Compound **17b** was obtained in the

same manner as a beige solid: 77%, mp 136−138 °C (lit.[36] 135−137 °C).

2-(5,6-Dihydroindolo[2,1-*a*]isoquinolin-12-yl)-1-nitroethene (18a). A solution of **17a** (0.16 g, 0.65 mmol) and ammonium acetate (0.032 g, 0.42 mmol) in nitromethane (2 mL) was refluxed for 2 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in dichloromethane (3 mL) and H_2O (3 mL) was added. The aqueous layer was washed with dichloromethane (2 × 10 mL), and the combined organic layers were washed with H_2O (10 mL) and brine (10 mL) and dried (Na_2SO_4). Removal of the solvent under vacuo gave **18a** as an orange powder: 0.183 g, 0.63 mmol, 97%, mp 109−111 °C; ^{1}H NMR δ 3.17 (t, 2H, J = 6.4 Hz), 4.26 (t, 2H, J = 6.4 Hz), 7.49−7.32 (m, 6H), 7.84−7.80 (m, 2H), 7.95 (d, 1H, J = 13.3 Hz), 8.75 (d, 1H, J = 13.3 Hz); ^{13}C NMR δ 36.2, 48.8, 108.0, 110.6, 120.8, 122.5, 123.9, 125.7, 127.2, 128.6, 128.85, 132.1, 133.3, 135.9, 137.4, 137.5. Anal. ($C_{18}H_{14}N_2O_2$) C, H, N.

10-Methoxy-12-[(*E*)-2-nitro-1-ethenyl]-5,6-dihydroindolo-[2,1-*a*]isoquinoline (18b). Compound **18b** was obtained in the same manner as a bright yellow fluorescent solid: 44%, mp 208−209 °C.

2-(5,6-Dihydroindolo[2,1-*a*]isoquinolin-12-yl)-1-ethanamine (19a). A solution of **18a** (0.19 g, 0.66 mmol) in tetrahydrofuran (15 mL) was added dropwise at 0 °C to a stirred suspension of $LiAlH_4$ (0.25 g, 6.59 mmol) in THF (6 mL). After completion of addition, the mixture was refluxed overnight and then allowed to reach ambient temperature. After cooling to 0 °C, water (17 mL) was added. The mixture was filtered, and the filtrate was taken up in ethyl acetate (50 mL), washed with H_2O (2 × 25 mL) and brine (25 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure to give crude amine **19a** which was used without further purification.

2-(10-Methoxy-5,6-dihydroindolo[2,1-*a*]isoquinolin-12-yl)-1-ethanamine (19b). Compound **19b** was obtained in the same manner as a clear, pale yellow oil, which was then used without further purification.

General Procedure for the Preparation of Amides 20, 21. To a cooled solution (0 °C) of 2-(5,6-dihydroindolo[2,1-*a*]-isoquinolin-12-yl)-1-ethanamine (**19a**) or 2-(10-methoxy-5,6-dihydroindolo[2,1-*a*]isoquinolin-12-yl)-1-ethanamine (**19b**) (0.32 mmol) in tetrahydrofuran (3 mL) were added triethylamine (0.15 mL) and the appropriate anhydride (0.10 mL). The ice bath was removed and the solution stirred for 3 h. The solvent was evaporated in vacuo, and the residue was taken up in ethyl acetate (50 mL) and washed with H_2O (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give a brown oil. Trituration of the oil with ethyl acetate (2 mL) afforded the desired amide.

***N*-Acetyl 2-(5,6-dihydroindolo[2,1-*a*]isoquinolin-12-yl)-ethanamine (20a):** beige amorphous solid, 42%, mp 188−190 °C; ^{1}H NMR δ 1.86 (s, 3H), 3.13 (t, 2H, J = 6.0 Hz), 3.34 (t, 2H, J = 6.7 Hz),3.66 (dt, 2H, J = 6.5, 6.5 Hz), 4.24 (t, 2H, J = 5.9 Hz), 5.59 (bs, 1H), 7.38−7.12 (m, 6H), 7.63 (d, 1H, J = 7.8 Hz), 7.92 (d, 1H, J = 7.7 Hz); ^{13}C NMR δ 23.3, 25.15, 30.1, 40.0, 40.1, 108.5, 108.8, 118.7, 119.5, 122.2, 125.2, 127.2, 127.5, 128.5, 129.0, 129.6, 131.4, 133.7, 135.4, 170.2. $C_{20}H_{20}N_2O$ requires 305.1640; Found: 305.1654.

***N*-Propanoyl 2-(5,6-dihydroindolo[2,1-*a*]isoquinolin-12-yl)ethanamine (20b):** white amorphous solid, 38%, mp 183−185 °C.

***N*-Butanoyl 2-(5,6-dihydroindolo[2,1-*a*]isoquinolin-12-yl)ethanamine (20c):** beige amorphous solid, 38%, mp 165−167 °C.

***N*-Cyclobutanecarbonyl 2-(5,6-dihydroindolo[2,1-*a*]-isoquinolin-12-yl)ethanamine (20d):** white powder, 34%, mp 94−96 °C.

***N*-Acetyl 2-(10-methoxy-5,6-dihydroindolo[2,1-*a*]isoquinolin-12-yl)ethanamine (21a):** beige amorphous solid, 68%, mp 153−154 °C; ^{1}H NMR δ 1.87 (s, 3H), 3.11 (t, 2H, J = 6.30 Hz), 3.30 (t, 2H, J = 6.95 Hz), 3.64 (q, 2H, J = 6.6 Hz), 3.88 (s, 3H), 4.19 (t, 2H, J = 6.3 Hz), 5.72 (bs, 1H), 6.89 (dd, 1H, J = 2.3, 8.9 Hz),7.39−7.07 (m, 5H), 7.89 (d, 1H, J = 7.8 Hz); ^{13}C NMR δ 23.3, 25.2, 30.0, 39.9, 40.1, 55.9, 100.2, 109.5, 112.6, 124.9, 127.0, 127.4, 128.5, 129.2, 129.6, 130.7, 131.8, 133.4, 154.1, 170.2. Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

***N*-Propanoyl 2-(10-methoxy-5,6-dihydroindolo[2,1-*a*]-isoquinolin-12-yl)-ethanamine (21b):** white amorphous solid, 64.5%, mp 159−160 °C.

***N*-Butanoyl 2-(10-methoxy-5,6-dihydroindolo[2,1-*a*]-isoquinolin-12-yl)ethanamine (21c):** 59%, mp 134−135 °C.

***N*-Cyclobutanecarbonyl 2-(10-methoxy-5,6-dihydroindolo[2,1-*a*]isoquinolin-12-yl)ethanamine (21d):** pale yellow oil, 21%.

***N*-Acetyl 2-(5,6,7-trihydrobenzo[*c*]cyclohept[2,1-*a*]-indol-13-yl)ethanamine (23a):** white foam, 68%; ^{1}H NMR δ 1.73 (s, 3H), 2.23 (br s, 2H), 2.60 (t, J = 6.9 Hz, 2H), 3.10 (t, J = 6.7 Hz, 2H), 3.52 (br d, 2H), 4.07 (br s, 2H), 5.4 (br s, 1H), 7.12 m, 1H), 7.24 (m, 1H), 7.30 (dd, J = 7.2, 1.6 Hz, 1H), 7.30 (dd, J = 7.2, 1.6 Hz, 1H), 7.34 (m, 2H), 7.37 (m, 1H), 7.49 (dd, J = 7.1, 1.6 Hz, 1H), 7.67 (d, J = 7.9 Hz, 1H); ^{13}C NMR δ 23.2, 24.4, 30.7, 31.1, 40.4, 108.6, 119.1, 121.9, 127.1, 127.7, 128.6, 129.2, 129.4, 132.2, 135.6, 137.7, 138.9, 169.8; IR 3294, 3074, 2937, 1693, 1560, 1489, 1437, 1364, 1239, 1222, 1169, 722, 696, 542 cm^{-1}; MS *m/e* 319, 318, 260, 259, 246 (100). $C_{21}H_{23}N_2O$ (M^+ + H) requires 319.1800; Found: 319.1810.

***N*-Propanoyl2-(5,6,7-trihydrobenzo[*c*]cyclohept[2,1-*a*]-indol-13-yl)ethanamine (23b):** white foam, 95%.

***N*-Butanoyl 2-(5,6,7-trihydrobenzo[*c*]cyclohept[2,1-*a*]-indol-13-yl)ethanamine (23c):** white foam, 61%.

***N*-Cyclopropanecarbonyl 2-(5,6,7-trihydrobenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (23d):** brown solid, 50%, mp 142−144 °C.

***N*-Cyclobutanecarbonyl 2-(5,6,7-trihydrobenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (23e):** white solid, 75%, mp 138−139 °C.

***N*-Acetyl 2-(5,6,7-trihydro-11-methoxybenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (25a):** white foam, 97%; ^{1}H NMR δ 1.74 (s, 3H), 2.23 (br s, 2H), 2.59 (m, 2H), 3.07 (t, J = 6.8 Hz, 2H), 3.51 (m, 2H), 3.89 (s, 3H), 4.10 (br s, 2H), 5.40 (br s, 1H), 6.90 (dd, J = 8.8, 2.5 Hz, 1H), 7.12 (d, J = 2.3 Hz, 1H), 7.23 (d, J = 8.8 Hz, 1H), 7.31 (dd, J = 6.9, 2.1 Hz, 1H), 7.34 (m, 1H), 7.37 (m, 1H), 7.50 (d, J = 7.1 Hz, 1H); ^{13}C NMR δ 23.5, 24.4, 30.9, 31.1, 40.3, 40.6, 56.1, 100.7, 108.2, 109.4, 112.2, 127.1, 127.9, 128.5, 129.1, 129.4, 130.9, 132.3, 138.2, 140.3, 153.5, 174.0; IR 3275, 2935, 1652, 1558, 1486, 1438, 1222, 1168, 1119, 722 cm^{-1}; MS *m/e* 349, 348, 290, 289, 276 (100). $C_{22}H_{25}N_2O_2$ (M^+ + H) requires 349.1900; Found: 349.1916. Anal. ($C_{22}H_{24}N_2O_2$) C, H, N.

***N*-Propanoyl 2-(5,6,7-trihydro-11-methoxybenzo[*c*]cyclohept[2,1-*a*]-indol-13-yl)ethanamine (25b):** white foam, 82%.

***N*-Butanoyl 2-(5,6,7-trihydro-11-methoxybenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (25c):** white foam, 72%.

***N*-Cyclopropanecarbonyl 2-(5,6,7-trihydro-11-methoxybenzo[*c*]cyclohept[2,1-*a*]-indol-13-yl)ethanamine (25d):** white foam, 72%.

***N*-Cyclobutanecarbonyl 2-(5,6,7-trihydro-11-methoxybenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (25e):** white foam, 76%.

***N*-Acetyl 2-(5,6,7-trihydro-11-ethoxybenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (27a):** white foam, 67%; ^{1}H NMR δ 1.48 (t, J = 7.0 Hz, 3H), 1.77 (s, 3H), 2.25 (br s, 2H), 2.63 (t, J = 6.8 Hz, 2H), 3.09 (t, J = 6.7 Hz, 2H), 3.52 (m, 2H), 4.02 (br s, 2H), 4.14 (m, 2H), 5.50 (br s, 1H), 6.93 (dd, J = 8.9, 2.4 Hz, 1H), 7.16 (d, J = 2.3 Hz, 1H),7.25 (d, J = 8.8 Hz, 1H), 7.32 (m, 1H), 7.35 (m, 1H), 7.38 (m, 1H), 7.51 (dd, J = 6.9, 1.6 Hz, 1H); ^{13}C NMR δ 15.2, 23.2, 24.4, 31.1, 40.3, 40.6, 64.5,102.0, 108.1, 109,4, 112.7, 127.1, 127.9, 128.5, 129.1, 129.4, 131.0, 132.3, 138.2, 138.9, 153.1, 169.9; IR 3282, 2932, 1652, 1553, 1471, 1364, 1180, 789, 762 cm^{-1}; MS *m/e* 363, 362, 304, 259, 290 (100). $C_{23}H_{27}N_2O_2$ (M^+ + H) requires 363.2060; Found: 363.2073. Anal. ($C_{23}H_{26}N_2O_2$) C, H, N.

***N*-Propanoyl 2-(5,6,7-trihydro-11-ethoxybenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (27b):** white foam, 76%.

***N*-Butanoyl 2-(5,6,7-trihydro-11-ethoxybenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (27c):** white foam, 89%.

***N*-Cyclopropanecarbonyl 2-(5,6,7-trihydro-11-ethoxybenzo[*c*]cyclohept[2,1-*a*]indol-13-yl)ethanamine (27d):** white foam, 77%.

Modeling. Molecular modeling on compounds **7a**, **21a**, and **25a** was performed using the semiempirical PM3 parameter set[57] as implemented in MacSpartan Plus, Version 1.1.7, available from Wave function Inc., 18401 Von Karman, Suite 370, Irvine, CA 92612, mounted on a Power Macintosh G3. No geometry restrictions were applied, and no global minimum search was performed.

Supporting Information Available: Full physical data, in the same format as for the representative examples, are provided for all analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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