Brief Articles

Design, Synthesis, and Melatoninergic Activity of New Azido- and Isothiocyanato-Substituted Indoles

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To develop irreversibly binding ligands for the melatonin receptor(s) as tools for tracing the primary melatonin binding site, we report on the design and synthesis of new melatoninergic azido- and isothiocyanato-substituted indoles. All active compounds were partial agonists or antagonists in the *Xenopus* melanophore assay, the most potent being the 5-OMe C3-substituted azido **45** and isothiocyanato **46** analogues.

Introduction

Melatonin (*N*-acetyl 5-methoxytryptamine, Figure 1) is the principal hormone of the vertebrate pineal gland and is secreted mainly during darkness. It is well recognized that it regulates seasonal breeding in photoperiodic species and can entrain circadian rhythms in mammals including man.[1] Melatonin has a hypnotic action in animals and humans,[2] and ramelteon, a potent melatonin MT_1[a] and MT_2 receptor agonist, has recently been granted approval for the treatment of insomnia associated with sleep onset in the U.S.[3] Melatonin has also been reported to have antioxidant[4] and antiproliferative activity.[5]

A number of these effects are mediated through a family of high-affinity G-protein-coupled cell-membrane receptors MT_1, MT_2, and Mel_{1c},[6a–c] which are particularly abundant in tissues that are known to respond to melatonin (e.g., the body's biological clock in the suprachiasmatic nuclei of the hypothalamus and the retina). Melatonin receptors have been subjected to a number of modeling studies based on the amino acid sequence and pharmacophore models, and a number of active conformations have been proposed.[7a–e]

During the past decade, we sought to understand how melatonin interacts with its receptors. A number of structure−affinity relationships have been identified, and recently we have reported a variety of synthetic molecular probes for the melatonin binding site.[8,9a–c]

In our ongoing effort to probe the stereoelectronic requirements for optimal activity, we report herein on a series of N1 (**5**–**8**, Scheme 1), C2 (**23**–**26**, Scheme 2), and C3 (**30**, **31**, Scheme 3; **39**, **40** Scheme 4; and **45**, **46** Scheme 5) suitably substituted indoles as potential irreversibly binding ligands to the melatonin receptor(s). These agents may provide the basis, in the future, for tools for tracing the primary melatonin binding site. The design of these probes was based on the attachment

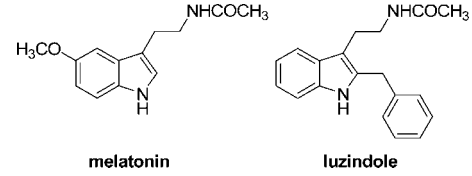
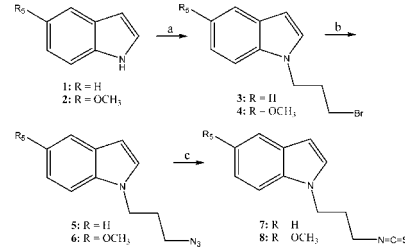


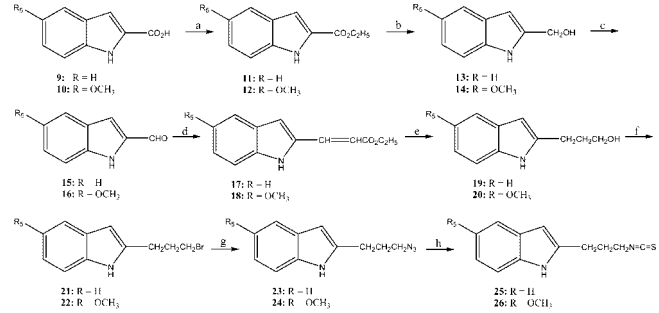
Figure 1. Structures of melatonin and luzindole.

Scheme 1. Synthesis of the N1-Substituted Analogues[a]



[a] Reagents and conditions: (a) 1,3-dibromopropane, KOH, DMF, room temp; (b) NaN_3, DMF, 45 °C; (c) CS_2, Ph_3P, THF, room temp.

Scheme 2. Synthesis of the C2-Substituted Analogues[a]



[a] Reagents and conditions: (a) $SOCl_2$, EtOH, 70 °C; (b) $LiAlH_4$, THF, room temp; (c) MnO_2, CH_2Cl_2, 35 °C; (d) $Ph_3P{=}CHCO_2C_2H_5$, benzene, 65 °C; (e) $LiAlH_4$, THF, room temp; (f) PBr_3, Et_2O, 25 °C; (g) NaN_3, DMF, 45 °C; (h) CS_2, Ph_3P, THF, room temp. The synthesis of the non-OMe C3-substituted analogues **30** and **31** is shown in Scheme 3.

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[a] Abbreviations: MT_1, melatonin receptor 1; MT_2, melatonin receptor 2; Mel_{1c}, melatonin receptor 1c; NMDA, *N*-methyl D-aspartate; PCP, phencyclidine (1-(1-phenylcyclohexyl)piperidine); *p*-TsOH, *p*-toluenesulfonic acid; *p*-TsCl, *p*-toluenesulfonyl chloride; pIC_{50}, the concentration of analogue reducing melatonin-induced pigment aggregation by 50%; pEC_{50}, the concentration of analogue producing 50% of the maximum agonist response.

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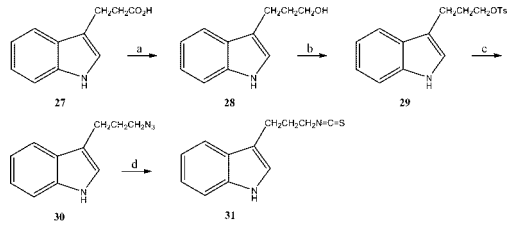
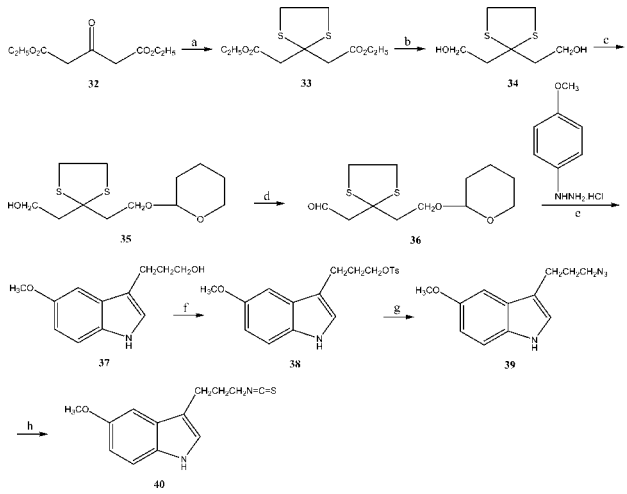
Scheme 3. Synthesis of the Non-OMe, C3-Substituted Analogues[a]



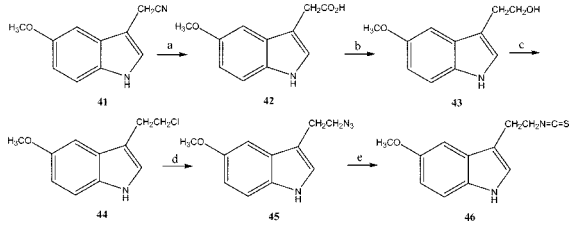
[a] Reagents and conditions: (a) $LiAlH_4$, Et_2O, reflux; (b) *p*-TsCl, pyridine, 4 °C; (c) NaN_3, DMF, 45 °C; (d) CS_2, Ph_3P, THF, room temp.

Scheme 4. Synthesis of the C5-OMe, C3-Substituted Analogues **39** and **40**[a]



[a] Reagents and conditions: (a) $HSCH_2CH_2SH$, $BF_3.OEt_2$, CH_2Cl_2, room temp; (b) $LiAlH_4$, THF, room temp; (c) 3,4-dihydro-2*H*-pyran, *p*-TsOH, THF, 50 °C; (d) pyridine, CrO_3, CH_2Cl_2, room temp; (e) concentrated HCl, THF, 50 °C; (f) *p*-TsCl, pyridine, 4 °C; (g) NaN_3, DMF, 45 °C; (h) CS_2, Ph_3P, THF, room temp.

Scheme 5. Synthesis of the C5-OMe, C3-Substituted Analogues **45** and **46**[a]



[a] Reagents and conditions: (a) (i) aqueous KOH, 100 °C, (ii) HCl (10 N), 0 °C; (b) $LiAlH_4$, THF, room temp; (c) *p*-TsCl, pyridine, 4 °C; (d) NaN_3, DMF, 45 °C; (e) CS_2, Ph_3P, THF, room temp.

of azido and isothiocyanato groups to the ω-position of the *N*1-, C2-, and C3-alkyl side chains of the new indolic analogues. The isothiocyanate group was chosen because it is inert in water but capable of nucleophilic reactions with amino, imidazoyl, and sulfhydryl functionalities on biological macromolecules under physiological conditions.[10] Moreover, isothiocyanate-containing probes have been extensively used as tools for the study and characterization of various receptors including benzodiazepine,[11] NMDA,[12] σ,[13] opioid,[14,15] and cannabinoid receptors.[16] On the other hand, the azido group was introduced in the skeletons of **5**, **6**, **23**, **24**, **30**, **39**, and **45**, since it is known to serve as a photoaffinity label, covalently attached to reactive residues at or in the vicinity of the binding site, after equilibration and photoirradiation. Photoaffinity labeling has been used to study a number of receptors including those for PCP,[17] muscarine,[18] catecholamines,[19,20] serotonin,[21] cannabinoids,[22] and retinal[23] and has provided useful information on their distribution and molecular weights or amino acid residues at or near the active site. The only drawback of this method is the generation of nitrenes after photoirradiation, which could undergo intramolecular rearrangement to the respective imine.[24] The situation being so, the affinity of the azido compound for the receptor should be high enough to allow for a fast reaction with the active site.[22]

The methoxy group and the ethylamide side chain of melatonin are critical for high receptor affinity. Previous studies have shown that changing the side chain of melatonin from C3 to *N*1 resulted in mainly agonist analogues, with highest affinity when the methoxy group was shifted from C5 to C6 to maintain the optimum spacing of functional groups.[25] Moving the amido side chain to the C2 indole position was shown to lead to partial agonist molecules when the methoxy was shifted to C4 to again maintain optimum spacing but gave antagonists when methoxy was retained at C5, though with dramatically reduced affinity.[26] In the present study, the pharmacophoric side chain has been transposed from *N*1 to C2 to C3, and an azido or isothiocyanato group was incorporated as a first step to developing covelent probes that may be useful new tools for studying the receptors.

Chemistry

The synthetic pathway followed for the preparation of analogues **5**–**8** is depicted in Scheme 1. Thus, commercially available indole (**1**) and 5-methoxyindole (**2**) were reacted with 1,3-dibromopropane in the presence of KOH in DMF to give the *N*1-alkylated indolic derivatives **3** and **4**,[27] respectively. These were in turn treated with an aqueous solution of sodium azide in DMF to afford azides **5** and **6**. Isothiocyanates **7** and **8** were obtained by reacting the latter with a mixture of carbon disulfide and triphenylphosphine in THF.

The synthesis of the C2-substituted indolic derivatives **23**–**26** (Scheme 2) was effected by esterification of 2-indolecarboxylic acid (**9**) and its 5-methoxy congener **10** with thionyl chloride and ethanol, followed by reduction of esters **11**[28] and **12**[26] with $LiAlH_4$ in THF to the respective alcohols **13**[29] and **14**.[26] These compounds were then oxidized to the carboxaldehydes **15**[30] and **16**,[26] which were then converted to **17**[31] and **18** via a Wittig reaction. Simultaneous reduction of the double bond and the ester groups led to alcohols **19**[32] and **20**, which were brominated with PBr_3 in ether to give the bromides **21** and **22**. These were treated with sodium azide in DMF, and the resulting azides **23** and **24** were reacted with CS_2 and triphenylphosphine in THF to give the isothiocyanates **25** and **26**.

Commercially available 3-indolepropanoic acid (**27**) was reduced with $LiAlH_4$ in diethyl ether to the alcohol **28**,[33] which was tosylated with tosyl chloride in the presence of pyridine to give **29**.[34] Treatment of **29** with an aqueous solution of sodium azide in DMF gave **30**, which was then reacted with carbon disulfide and triphenylphosphine in THF to give the isothiocyanate **31**. However, this route proved to be unsuccessful for preparing the analogous ligands **39** and **40**, and the alternative route shown in Scheme 4 was used to obtain these systems.

The keto group of diethyl 1,3-acetonedicarboxylate (**32**) was protected with 1,2-ethanedithiol in the presence of a catalytic amount of boron trifluoride−diethyl etherate to give the 1,3-dithiolano derivative **33**,[35] which was reduced with $LiAlH_4$ in THF to the diol **34**.[35] Monoprotection of the latter with dihydropyran in the presence of *p*-TsOH led to the formation of alcohol **35**,[36] which was oxidized under Sarett conditions to

Table 1. Melatoninergic Activity of Compounds **5**, **7**, **23**, **25**, **30**, and **31** in the *Xenopus laevis* Melanophore Assay[a]

compd	R_5	agonist pEC_{50}	antagonist pIC_{50}
melatonin		10.07	NA
luzindole		NA	5.61 ± 0.08
5	H	NA	4.82 ± 0.01
7	H	PA (28%)	4.04 ± 0.01
23	H	NA	4.72 ± 0.01
25	H	PA (38%)	4.35 ± 0.01
30	H	NA	4.79 ± 0.02
31	H	PA (31%)	3.92 ± 0.01

[a] Agonist and antagonist data are the mean of triplicate experiments ± SEM. NA = no agonist effect detected at 100 μM. PA = partial agonist at 100 μM. Number in parentheses indicates maximal agonist action as a percentage of that seen with 1 nM melatonin.

Table 2. Melatoninergic Activity of Compounds **6**, **8**, **24**, **26** **39**, and **40** in the *Xenopus laevis* Melanophore Assay[a]

compd	R_5	agonist pEC_{50}	antagonist pIC_{50}
melatonin		10.07	NA
luzindole		NA	5.61 ± 0.08
6	OCH_3	NA	4.81 ± 0.01
8	OCH_3	NA	4.91 ± 0.01
24	OCH_3	NA	4.15 ± 0.03
26	OCH_3	PA (41%)	4.47 ± 0.01
39	OCH_3	NA	5.03 ± 0.02
40	OCH_3	NA	4.49 ± 0.02

[a] Agonist and antagonist data are the mean of triplicate experiments ± SEM. NA = no agonist effect detected at 100 μM. PA = partial agonist at 100 μM. Number in parentheses indicates maximal agonist action as a percentage of that seen with 1 nM melatonin.

Table 3. Melatoninergic Activity of Compounds **45** and **46** in the *Xenopus laevis* Melanophore Assay[a]

compd	R_5	agonist pEC_{50}	antagonist pIC_{50}
melatonin		10.07	NA
luzindole		NA	5.61 ± 0.08
45	OCH_3	NA	5.09 ± 0.03
46	OCH_3	NA	5.47 ± 0.11

[a] Agonist and antagonist data are the mean of triplicate experiments ± SEM. NA = no agonist effect detected at 100 μM.

the aldehyde **36**.[36] Treatment of **36** with an acidic solution of *p*-methoxyphenylhydrazine hydrochloride in THF led to the Fischer indole product **37**,[37] which was first tosylated and then converted to the desired azide **39**. Isothiocyanate **40** was obtained by reacting **39** with carbon disulfide and triphenylphosphine in THF.

Since it had previously been shown that the number of methylene units between the aromatic ring and the acetamido group was critical,[9b] we prepared **45** and **46** with two methylene groups to compare with **39** and **40**, which bear a 3-CH_2 spacer in their side chain. Thus, 5-methoxy-1*H*-3-indolacetonitrile (**41**) was hydrolyzed to the acid **42**,[38] which was then reduced with $LiAlH_4$ in THF to the alcohol **43**.[39] This alcohol was then converted to the chloride **44** by treatment with *p*-TsCl in pyridine and the chloride converted to the azide **45** by heating with sodium azide in aqueous DMF. Reaction of **45** with CS_2 and triphenylphosphine in THF gave the desired isothiocyanate **46**.

Results and Discussion

It is apparent from the data presented in Tables 1 and 2 that with the exception of the isothiocyanate **7**, which exhibited a small partial agonist activity (28% of maximal) only at the highest concentration tested (100 μM), the new *N*1-substituted analogues **5**, **6**, and **8** are antagonists in the melanophore assay. This is not unexpected for **5** and **7** because the loss of the critical C5 methoxy group is known to favor an antagonist profile. Evidence from site-directed mutagenesis and structure–activity studies suggests that the C5 methoxy forms a hydrogen bond with His211 in the putative transmembrane domain 5 of the receptor.[9b,40a,b] Though **6** and **8** have a C5 methoxy, they are not likely to be well accommodated in the receptor binding site because of the unfavorable relative positions of the alkyl side chain on *N*1 and the C5 methoxy group and because of the lack of an acetamide group.

Similar behavior was also noticed in the case of the C2-substituted analogues **23**–**26** (Tables 1 and 2) with weak antagonist activity apparent, though again a small (~40% of maximal) partial agonist action was observed with 100 μM **25** and **26**. Moving the side chain of melatonin from C3 to C2 was previously shown to lead to compounds with MT_1 antagonist and partial agonist properties.[26] An earlier study reported that the C2 analogue of melatonin in which the methoxy group was positioned at C5[26] exhibited a low binding affinity ($pK_i = 4.79$), quite similar to the antagonist potency of **26**.

For the analogues with the side chain positioned at C3, potency remained relatively weak probably because the spacing between the C5 methoxy and the C3 side chain, which had a three-methylene spacer, was not optimal. Compounds **30**, **39**, and **40** antagonized melatonin's action, though a small (31%) partial agonist response was seen with one compound in the series, **31** (100 μM). Interestingly, the most active analogue of this series, the azido compound **39** ($pIC_{50} = 5.03$), is almost equipotent to luzindole (Figure 1), a commonly used melatonin receptor antagonist ($pIC_{50} = 5.61$), suggesting that a modest improvement in affinity may result in a useful melatonin receptor probe.

Analogues **7**, **25**, **26**, and **31** showed some agonist action, albeit at the very highest concentration tested (100 μM) and less than maximal activity (28–41%). This is surprising given that these compounds lack the C5 methoxy often considered necessary for functional agonist activity and lack any ability to form hydrogen bonds at their ω-substituent. Furthermore, this partial agonist action was apparent irrespective of the position of the melatonin side chain (*N*1, C2, C3). Because all four were isothiocyanate derivatives, substituted at different positions on the indole ring, and because isothiocyanate can undergo nucleophilic reactions with biological macromolecules under physiological conditions, a nonspecific interaction with melanophore proteins at the high concentration used must be considered. Such an interaction may have altered intracellular pigment position or cell morphology, giving the appearance of receptor-mediated aggregation rather than a genuine receptor-activated translocation of pigment.

In contrast to the three-methylene spacer analogue **40** ($pIC_{50} = 4.49$), the two-methylene congener **46** exhibits a noteworthy improvement in antagonistic activity ($pIC_{50} = 5.47$) (Table 3). The shorter spacer of **46** may occupy the pocket, while the methoxy group at C5 interacts with His211, while the longer spacer of **40** may make hydrogen bonding of the C5 methoxy unfavorable even if the longer side chain can be accommodated. Radioligand binding studies on melatonin receptors and computational assessment may allow this idea to be evaluated.

Conclusion

From the results presented, it becomes evident that one of the main factors influencing antagonist potency is the location of the side chain rather than the nature of its ω-substituent. The information gained in the present work can be used to develop congeners of the azido compounds as photoactivity labels and of the isothiocyanato compounds as electrophilic probes, in order to produce adducts covalently linked to key amino acid residues

of the melatonin receptor subtypes. This will reveal important stereoelectronic characteristics of the hormone's receptor and should lead to the development of high-affinity selective ligands.

Experimental Section

General Procedure 1 for the Preparation of Azides 5, 6, 23, 24, 30, 39, and 45. A solution of sodium azide (0.42 g, 7.63 mmol) in H_2O (2 mL) was added dropwise to a stirred solution of the appropriate bromide or chloride (for the synthesis of **45**) (3.36 mmol) in DMF (6 mL) at room temperature. The resulting mixture was then heated to 45 °C and stirred at this temperature for 3 h. Upon completion of the reaction, the mixture was poured onto crushed ice and extracted with AcOEt. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue obtained was purified by flash column chromatography to give the title azides as pale-yellow oils.

General Procedure 2 for the Preparation of Isothiocyanates 7, 8, 25, 26, 31, 40, and 46. Carbon disulfide (4.36 g, 3.4 mL, 57.4 mmol) and triphenylphosphine (0.89 g, 3.06 mmol) were sequentially added to a solution of the above azides (2.05 mmol) in THF (15 mL). The suspension formed was stirred for 20 h at room temperature, and upon completion of the reaction, the solvent was removed in vacuo. The residue obtained was purified by flash column chromatography to give the desired isothiocyanates as yellowish oils.

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Supporting Information Available: Experimental details on the synthesis of the compounds in this paper, spectral data for all compounds, elemental analysis data for key target compounds, and pharmacological assay. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bartness, T. J.; Powers, J. B.; Hastings, M. H.; Bittman, E. L.; Goldman, B. D. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses. *J. Pineal Res.* **1993**, *15*, 161–190.

(2) Garfinkel, D.; Laudon, M.; Nof, D.; Zisapel, N. Improvement of sleep quality in elderly people by controlled-release melatonin. *Lancet* **1995**, *346*, 541–544.

(3) Wurtman, R. Ramelteon: a novel treatment for the treatment of insomnia. *Expert Rev. Neurother.* **2006**, *6*, 957–964.

(4) Iakovou, K.; Varvaresou, A.; Kourounakis, A. P.; Stead, K.; Sugden, D. Design, synthesis and biological evaluation of novel β-substituted indol-3-yl ethylamido melatoninergic analogues. *J. Pharm. Pharmacol.* **2002**, *54*, 147–156.

(5) Molis, T. M.; Spriggs, L. L.; Jupiter, Y.; Hill, S. M. Melatonin modulation of estrogen-regulated proteins, growth factors, and proto-oncogenes in human breast cancer. *J. Pineal Res.* **1995**, *18*, 93–103.

(6) (a) Reppert, S. M.; Weaver, D. R.; Ebisawa, T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. *Neuron* **1994**, *13*, 1177–1185. (b) Reppert, S. M.; Godson, C.; Mahle, C. D.; Weaver, D. R.; Slaugenhaupt, S. A. Molecular characterisation of a second melatonin receptor expressed in human retina and brain: the Mel_{1b} melatonin receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8734–8738. (c) Ebisawa, T.; Karne, S.; Lerner, M. R.; Reppert, S. M. Expression cloning of a high-affinity melatonin receptor from *Xenopus* dermal melanophores. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6133–6137.

(7) (a) Mor, M.; Plazzi, P. V.; Spadoni, G.; Tarzia, G. Melatonin. *Curr. Med. Chem.* **1999**, *6*, 501–518. (b) Zlotos, D. P. Recent advances in melatonin receptor ligands. *Arch. Pharm. (Weinheim, Ger.)* **2005**, *338*, 229–247. (c) Voronkov, A. E.; Ivanov, A. A.; Baskin, I. I.; Palyulin, V. A.; Zefirov, N. S. Molecular modeling study of the mechanism of ligand binding to human melatonin receptors. *Dokl. Biochem. Biophys.* **2005**, *403*, 284–288. (d) Uchikawa, O.; Fukatsu, K.; Tokunoh, R.; Kawada, M.; Matsumoto, K. Synthesis of a novel series of tricyclic indan derivatives as melatonin receptor agonists. *J. Med. Chem.* **2002**, *12*, 4222–4239. (e) Rivara, S.; Lorenzi, S.; Mor, M.; Plazzi, P. V.; Spadoni, G. Analysis of structure−activity relationships for MT_2 selective antagonists by melatonin MT_1 and MT_2 receptor models. *J. Med. Chem.* **2005**, *48*, 4049–4060.

(8) Faust, R.; Garratt, P. J.; Jones, R.; Yeh, L.-K.; Tsotinis, A. 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. *J. Med. Chem.* **2000**, *43*, 1050–1061.

(9) (a) Tsotinis, A.; Vlachou, M.; Papahatjis, D. P.; Calogeropoulou, T.; Nikas, S. P. Mapping the melatonin receptor. 7. Subtype selective ligands based on β-substituted *N*-acyl-5-methoxytryptamines and beta-substituted *N*-acyl-5-methoxy-1-methyltryptamines. *J. Med. Chem.* **2006**, *49*, 3509–3519. (b) Garratt, P. J.; Travard, S.; Vonhoff, S.; Tsotinis, A.; Sugden, D. Mapping the melatonin receptor. 4. Comparison of the binding affinities of a series of substituted phenylalkylamides. *J. Med. Chem.* **1996**, *39*, 1797–1805. (c) Garratt, P. J.; Tsotinis, A. Synthesis of compounds as melatonin agonists and antagonists. *Mini Rev. Med. Chem.* **2007**, *7*, 1075–1088.

(10) Assony, S. J. In *Organic Sulfur Compounds*; Kharasch, N., Ed.; Pergamon Press: New York, 1961; p 326.

(11) Allen, M. S.; Hagen, T. J.; Trudell, M. L.; Codding, P. W.; Skolnick, P. Synthesis of novel 3-substituted β-carbolines as benzodiazepine receptor ligands: probing the benzodiazepine receptor pharmacophore. *J. Med. Chem.* **1988**, *31*, 1854–1861.

(12) Linders, J. T.; Monn, J. A.; Mattson, M. V.; George, C.; Jacobson, A. E. Synthesis and binding properties of MK-801 isothiocyanates; (+)-3-isothiocyanato-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrochloride: a new, potent and selective electrophilic affinity ligand for the NMDA receptor-coupled phencyclidine binding site. *J. Med. Chem.* **1993**, *36*, 2499–2507.

(13) Adams, J. T.; Teal, P. M.; Sonders, A. S.; Tester, B.; Esherick, J. S. Synthesis and characterization of an affinity label for brain receptors to psychotomimetic benzomorphans: differentiation of σ-type and phencyclidine receptors. *Eur. J. Pharmacol.* **1987**, *142*, 61–71.

(14) Rice, K. C.; Jacobson, A. E.; Burke, T. R., Jr.; Bajwa, B. S.; Streaty, R. A. Irreversible ligands with high selectivity toward δ- and μ-opiate receptors. *Science* **1983**, *220*, 314–316.

(15) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Naltrindole 5′-isothiocyanate: a non-equilibrium, highly selective δ-opioid receptor antagonist. *J. Med. Chem.* **1990**, *33*, 1547–1548.

(16) Guo, Y.; Abadji, V.; Morse, K. L.; Fournier, D. J.; Li, X. (−)-11-Hydroxy-7′-isothiocyanato-1′,1′-dimethylheptyl-Δ^8-THC: a novel, high-affinity irreversible probe for the cannabinoid receptor in the brain. *J. Med. Chem.* **1994**, *37*, 3867–3870.

(17) Haring, R.; Kloog, Y.; Sokolovsky, M. Identification of polypeptides of the phencyclidine receptor of rat hippocampus by photoaffinity labeling with [^{3}H]azidophencyclidine. *Biochemistry* **1986**, *25*, 612–620.

(18) Sokolovsky, M. Photoaffinity labeling of muscarinic receptors. *Pharmacol. Ther.* **1987**, *32*, 285–292.

(19) Leeb-Lundberg, L. M.; Cotecchia, S.; DeBlasi, A.; Caron, M. G.; Lefkowitz, R. J. Regulation of adrenergic receptor function by phosphorylation. I. Agonist-promoted desensitization and phosphorylation of alpha 1-adrenergic receptors coupled to inositol phospholipid metabolism in DDT1 MF-2 smooth muscle cells. *J. Biol. Chem.* **1987**, *262*, 3098–3105.

(20) Kanety, H.; Fuchs, S. Immuno-photoaffinity labeling of the D2-dopamine receptor. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 930–936.

(21) Raymond, J. R.; Fargin, A.; Lohse, J. M.; Regan, J. W.; Senogles, S. E. Identification of the ligand-binding subunit of the human 5-hydroxytryptamine 1A receptor with *N*-(*p*-azido-*m*-[^{125}I]iodophenethyl)spiperone, a high affinity radioiodinated photoaffinity probe. *Mol. Pharmacol.* **1989**, *36*, 15–21.

(22) Charalambous, A.; Yan, G.; Houston, D. B.; Howlett, A. C.; Compton, D. R. 5′-Azido-Δ^8-THC: a novel photoaffinity label for the cannabinoid receptor. *J. Med. Chem.* **1992**, *35*, 3076–3079.

(23) Nakayama, T. A.; Khorana, H. G. Orientation of retinal in bovine rhodopsin determined by cross-linking using a photoactivatable analogue of 11-cis-retinal. *J. Biol. Chem.* **1990**, *265*, 15762–15769.

(24) Kyba, E. P. Alkyl Azides and Nitrenes. In *Azides and Nitrenes*; Scriven, E. F. V., Ed.; Academic Press, Inc.: Orlando, FL, 1971; pp 23–28.

(25) Tarzia, G.; Diamantini, G.; Di Giacomo, B.; Spadoni, G.; Esposti, D. 1-(2-Alkanamidoethyl)-6-methoxyindole derivatives: a new class of potent indole melatonin analogues. *J. Med. Chem.* **1997**, *40*, 2003–2010.

(26) Spadoni, G.; Balsamini, C.; Bedini, A.; Diamantini, G.; Di Giacomo, B. 2-[*N*-Acylamino(C1−C3)alkyl]indoles as MT_1 melatonin receptor partial agonists, antagonists, and putative inverse agonists. *J. Med. Chem.* **1998**, *41*, 3624–3634.

(27) Dehaen, W.; Hassner, A. Cycloadditions. Annulation of heterocycles via intramolecular nitrile oxide−heterocycle cycloaddition reaction. *J. Org. Chem.* **1991**, *56*, 896–900.
(28) Wadia, M. S.; Mali, R. S.; Tilve, S. G.; Yadav, V. J. Facile syntheses of ethyl 2-alkylindole-3-carboxylates: reinvestigation of an earlier synthesis of ethyl 3-methylindole-2-carboxylates. *Synthesis* **1987**, *4*, 401–404.
(29) Mahanty, J. S.; De, M.; Das, P.; Kundu, N. G. Palladium-catalyzed heteroannulation with acetylenic carbinols as synthons—synthesis of quinolines and 2,3-dihydro-4(1*H*)-quinolones. *Tetrahedron* **1997**, *53*, 13397–13418.
(30) Pérez-Serrano, L.; Casarrubios, L.; Dominguez, G.; Gonzáles-Pérez, P.; Pérez-Castells, J. Synthesis of enynoindoles via vinyl and ethynyl indoles. *Synthesis* **2002**, *13*, 1810–1812.
(31) Bit, R. A.; Davis, P. D.; Hill, C. H.; Keech, E.; Vesey, D. R. A. Dieckmann/ring expansion approach to tetrahydropyrido- and tetrahydroazepino-[1,2-*a*]indoles. *Tetrahedron* **1991**, *47*, 4645–4664.
(32) Smith, A. B.; Visnick, M.; Haseltine, J. N.; Sprengeler, P. A. Organometallic reagents in synthesis: a new protocol for construction of the indole nucleus. *Tetrahedron* **1986**, *42*, 2957–2969.
(33) Benghiat, E.; Crooks, P. A. Multisubstrate adducts as potential inhibitors of *S*-adenosylmethionine dependent methylases: inhibition of indole *N*-methyltransferase by (5′-deoxyadenosyl)[3-(3-indolyl)prop-1-yl]methylsulfonium and (5′-deoxyadenosyl)[4-(3-indolyl)but-1-yl]-methylsulfonium salts. *J. Med. Chem.* **1983**, *26*, 1470–1477.
(34) Beck, L.; Mascal, M.; Moody, C. J.; Coates, W. J. Synthesis of 3,4-bridged indoles by photocyclisation reactions. Part 2. Photocyclisation of halogenoacetyl tryptophol derivatives and α-chloro indol-3-ylalkanoate esters. *J. Chem. Soc., Perkin Trans. 1* **1992**, 813–821.
(35) Berglund, R. A.; Fuchs, P. L. Synthesis of fluorinated dienylic stannanes via the Wadsworth−Emmons reaction. *Synth. Commun.* **1989**, *19*, 1965–1980.
(36) Zepeda, L. G.; Cervantes, H.; Morales-Rios, M. S.; Joseph-Nathan, P. Synthesis of 3,3-dimethylenedithio-5-oxyaldehydes. *Synth. Commun.* **1991**, *21*, 1369–1381.
(37) Zepeda, L. G.; Morales-Rios, M. S.; Joseph-Nathan, P. Fischer indole synthesis of (*β*-oxo)indol-3-yl ketones using protected 1,3-dicarbonyl compounds. *Synth. Commun.* **1992**, *22*, 3243–3256.
(38) Morales-Rios, M. S.; Santos-Sánchez, N. F.; Suárez-Castillo, O. R.; Joseph-Nathan, P. Aromatic bromination versus oxidation of indolylmalonates by bromine. *J. Org. Chem.* **2003**, *68*, 305–311.
(39) Chou, S.-Y. A novel substitution reaction of tetrahydropyrano[3,4-*b*]indole derivative—chain extension and structural correlation. *Heterocycles* **2003**, *60*, 1095–1110.
(40) (a) Davies, D. J.; Faust, R.; Garratt, P. J.; Marivingt-Mounir, C.; Davidson, K. Binding affinity and biological activity of oxygen and sulfur isosteres at melatonin receptors as a function of their hydrogen bonding capacity. *Bioorg. Chem.* **2004**, *32*, 1–12. (b) Barrett, P.; Conway, S.; Jockers, R.; Strosberg, A. D.; Guardiola-Lemaitre, B. Cloning and functional analysis of a polymorphic variant of the ovine Mel 1a melatonin receptor. *Biochem. Biophys. Acta* **1997**, *1356*, 299–307.

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