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MAPPING THE MELATONIN RECEPTOR. 2. SYNTHESIS AND BIOLOGICAL ACTIVITY OF INDOLE DERIVED MELATONIN ANALOGUES WITH RESTRICTED CONFORMATIONS OF THE C-3 AMIDOETHANE SIDE CHAIN

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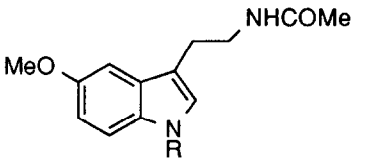
Abstract: A number of indole derived melatonin analogues have been prepared with the C-3 amidoethane side chain partially constrained by incorporation in a ring. The biological activity has been correlated with the conformation of the "side chain", the nature of the N-acylating group, and the spatial distance between the methoxyl and amide functions.

The pineal hormone melatonin (**1a**) plays a major role in the regulation of seasonal cycles and the control of circadian rhythms[1,2] and has been the focus of considerable clinical interest.[3] As part of a programme to map the melatonin receptor by examining the spatial and electronic restrictions that it imposes on melatonin analogues for them still to act as agonists,[4] we have synthesised a number of indoles annelated on the [b] face of the pyrrole moiety. Such compounds have constrained or partially constrained conformations of the C-3 amidoethane side chain. We chose indole as the basis for our model system in order to minimise deviations from the natural melatonin structure, since we were also concerned with the relative spatial arrangement of this side chain and the methoxyl group at a position equivalent to C-5 in melatonin We now report the preparation and biological activity of a number of N-acyl-3-amino-1,2,3,4-tetrahydrocarbazoles and N-acyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazoles.[5]

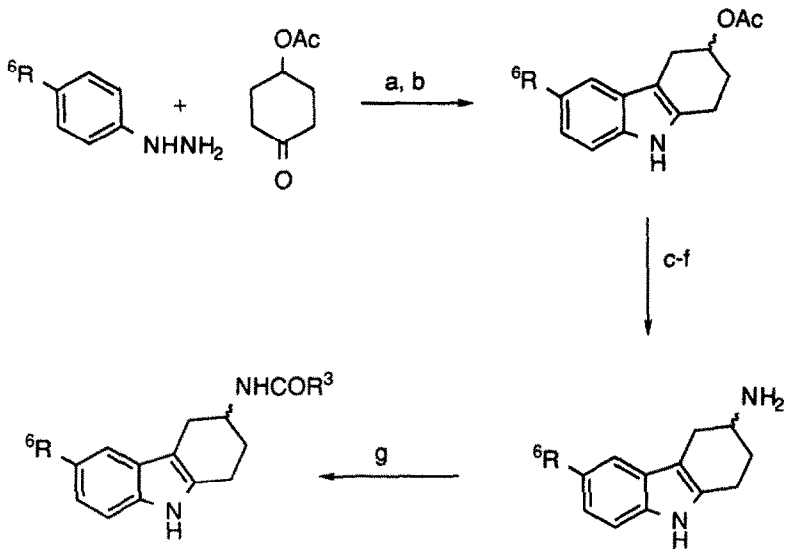


1a R = H

1b R = Me

The tetrahydrocarbazoles were synthesised as shown in the schemes. The N-acyl-3-amino-1,2,3,4-tetrahydrocarbazoles (Scheme 1) were prepared by Fischer synthesis, reacting phenylhydrazine or *p*-methoxyphenylhydrazine with 4-acetoxycyclohexanone.[6,7] The resulting 3-O-acetyltetrahydrocarbazole was saponified and the alcohol converted to the mesylate which was then treated with sodium azide. LAH reduction of the

Scheme 1 N-Acyl-3-amino-1,2,3,4-tetrahydrocarbazoles

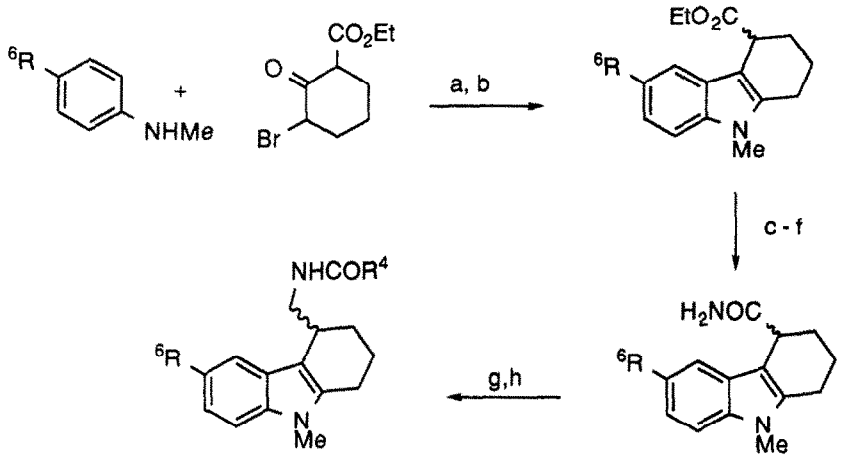


2 R^6 = H

3 R^6 = OMe

Reagents a AcOH, EtOH, Δ, 20 min; b AcOH, Δ, 4 h; c NaOH, EtOH, H_2O, Δ, 6 h; d $MeSO_2Cl$, Pyr, 20 °C, 24 h; e NaN_3, EtOH, H_2O, Δ, 12 h; f LAH, THF, 20 °C, 24 h; g R^3COCl or $(R^3CO)_2O$, Et_3N, CH_2Cl_2, 20 °C, 4 h

Scheme 2 N-Acyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazoles



4 R^6 = H

5 R^6 = OMe

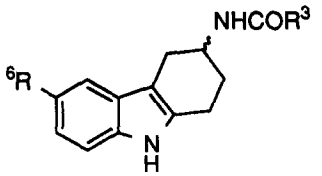
Reagents a propan-2-ol, Δ, 3 h; b $ZnCl_2$, propan-2-ol, Δ, 16 h; c NaOH, H_2O, EtOH, Δ, 6 h; d Et_3N, CH_2Cl_2, 0 °C,10 min; e $ClCO_2Me$, CH_2Cl_2, 0 °C, 4 h; f NH_3, 20 °C, 16 h; g THF, BH_3-THF, Δ, 4 h; h R^4COCl or $(R^4CO)_2O$, Et_3N, CH_2Cl_2, 20 °C, 4 h

resulting azide gave the corresponding amine which was acylated with a variety of acid halides or anhydrides to give the desired amides.

The N-acyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazoles (Scheme 2) were prepared by the Bischler synthesis, reacting N-methylaniline or *p*-methoxy-N-methylaniline with 2-bromo-6-carboethoxycyclohexanone[8] in the presence of $ZnCl_2$ to give regioselectively the 4-substituted compounds.[9,10] The resulting 4-carboethoxy-tetrahydrocarbazoles were converted to the corresponding amides and these were then reduced to the amines and then acylated as above to the desired amides.

The binding affinity of these compounds was determined in a 2-[^{125}I]-iodomelatonin radioligand binding assay in chick brain membranes,[11,12] and the results are shown in Tables 1 and 2.

Table 1



Compound	R^6	R^3	Receptor binding[12] [K_i], nM	*Xenopus* melanophores[13,14]
2a	H	Me	5350±810	NT
2b	H	Et	436±105	partial agonist
2c	H	n-Pr	1060±160	inactive
2d	H	CF_3	4630±1080	NT
2e	H	CH_2Br	740±150	partial agonist
2g	H	$CHBrCH_2CH_3$	>10000	NT
3a	OMe	Me	219±50	agonist
3b	OMe	Et	41±6	NT
3c	OMe	n-Pr	560±110	agonist
3d	OMe	CF_3	102±22	NT
3e	OMe	CH_2Br	8.3±1.3	mixed
3f	OMe	cyclo-C_3H_5	570±112	agonist

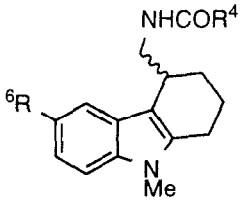
NT = not tested

Compared to melatonin (K_i 0.59 nM) the N-acyl-3-amino-1,2,3,4-tetrahydrocarbazoles (**2a-g**) show weak affinity for the chick brain melatonin receptor but exhibit the differential effect of changing the N-acylating group previously observed.[4,12] The propanoyl group gives the highest affinity for the receptor, duplicating the behaviour observed for 2-phenyltryptamine rather than the tryptamine and melatonin analogues.[4] Introduction of a methoxyl group at C-6 (**3a-f**), the equivalent position to C-5 in melatonin, leads, as expected, to a substantial increase in affinity. In this series the

propanoyl group again gives the highest affinity for the compounds with alkyl acyl substituents, but the bromoacetyl group has an even higher affinity.

The N-acyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazoles (**4a-f**) show a much higher affinity for the chick brain melatonin receptor than is shown by compounds **2a-g**, the acetyl (**4a**), propanoyl (**4b**) and butanoyl (**4c**) having essentially the same affinity, but there is a great decrease in affinity for the pentanoyl (**4d**) derivative. Introducing the 6-methoxyl group causes a substantial increase in affinity, with the butanoyl (**5c**) derivative having a somewhat greater affinity than the acetyl (**5a**) and propanoyl (**5b**) derivatives. Again the 6-methoxyl-4-aminomethyltetrahydrocarbazoles have enhanced affinity when compared to the corresponding 3-amino derivatives. If one takes the compounds of highest affinity in the two series, the butanoyl derivative **5c** binds one hundred fold more strongly than the propanoyl derivative **3b**. The N-methyl substituent on the 4-aminomethyltetrahydrocarbazole series probably lowers the affinity since N-methylmelatonin (**1b**) has an inhibition constant, K_i = 25.4 nM, compared to that of melatonin, K_i = 0.59 nM.

Table 2



Compound	R^6	R^4	Receptor binding[12] [K_i], nM
4a	H	Me	227±39
4b	H	Et	204±34
4c	H	n-Pr	215±33
4d	H	n-Bu	>10000
4e	H	CF_3	766±156
4f	H	cyclo-C_3H_5	4460±710
5a	OMe	Me	0.97±0.2
5b	OMe	Et	1.44±0.18
5c	OMe	n-Pr	0.378±0.056
5d	OMe	n-Bu	82±11
5e	OMe	CF_3	1.98±0.38
5f	OMe	cyclo-C_3H_5	30±3.7
5g	OMe	cyclo-C_4H_7	271±9

The biological potency of a selection of these compounds was examined using a pigment aggregation response test involving isolated melanophores obtained from the neural crest of *Xenopus laevis* embryos.[12,13] For

the N-acyl-3-amino-1,2,3,4-tetrahydrocarbazoles, the compounds lacking the 6-OMe group showed little (**2b** and **2e** EC_{50} >>10 μM) or no(**2c**) agonist activity. This was despite the propanoyl (**2b**) and bromoacetyl (**2e**) derivatives having affinities for the chick brain receptor of < 1μM. By contrast, the compounds with the 6-OMe group tested (**3a**, **3c**, **3f**) gave significant pigment aggregation. The bromoacetyl derivative **3e** (K_i 8 nM) aggregated pigment at low concentration (10^{-9} - 10^{-7} M). Interestingly, however, this effect is reversed with high concentrations of **3e** (10^{-6} - 10^{-5} M) This unusual result may have a number of explanations.First, since this analogue is chiral one enantiomer may act as an agonist at the receptor site and the other as an antagonist. Second, the reversal of response may relate to the reactivity of the bromoacetyl side chain High concentrations of the compound may non-specifically alkylate intracellular melanophore proteins essential in physically transporting pigment granules, thus preventing further aggregation. Another melatonin analogue, N-bromoacetyl-2-[^{125}I]-iodotryptamine irreversibly labels numerous proteins.[15] As aggregation of pigment was actually reversed with **3e**, this may not be the case here. Furthermore, **2e** and **2b**, which have little agonist activity, significantly reversed the aggregating action of melatonin. It will be interesting to determine whether other analogues in the series which have a high affinity (for example, **3b**) also have antagonist activity at high concentrations. Comparison between the butanoyl derivative in the 6-OMe series (**3c**, EC_{50} 1 0 μM) and the propanoyl derivative in the 6-H series (**2b**) is illuminating: both have very similar binding affinities but only the 6-OMe derivative has agonist activity, in accord with the Heward and Hadley hypothesis,[16] but not with our findings for the 2-phenyl and 2,6-dibromotryptamine series.[4]

Currently, only a few of the N-acyl 4-aminomethyl -1,2,3,4-tetrahydrocarbazoles have been tested in the melanophore assay. Compound **5c**, which has a similar affinity (K_i 0.38 nM) to melatonin, is an agonist on melanophores with an EC_{50} ≈ 2.5 nM, and compounds **5a** and **5g** are also agonists.[14]

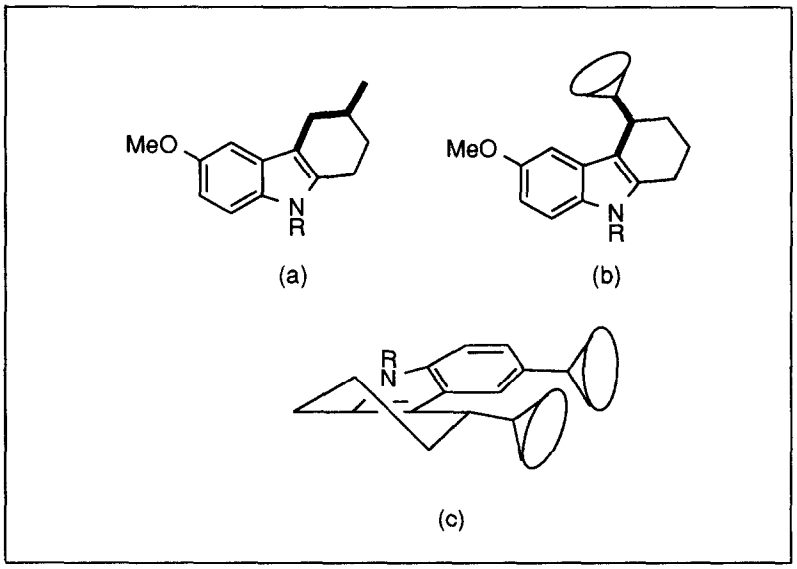


Figure Configurations of the 3-amino-tetrahydrocarbazole (a) and 4-aminomethyl-tetrahydrocarbazole (b) to indicate fixed relative position of O-Me and indole C-3 sidechain. Possible conformational structure of 4-aminomethyl derivatives is indicated in (c).

The structural and conformational differences in the two series of derivatives is illustrated in the figure. The 4-aminomethyl derivatives are more conformationally flexible but it is readily seen that the NHCOR and OMe groups can come into closer proximity. We are continuing our biological studies and are currently pursuing X-ray crystallographic, NMR and molecular modelling studies to determine the minimal energy structures and the barriers to conformational interchange in these and related melatonin agonists.[17]

The 6-methoxy-1,2,3,4-tetrahydrocarbazoles that we have described are melatonin agonists and the N-acyl-4-aminomethyl derivatives are comparable in affinity to melatonin. This gives considerable justification to the view that the higher activity of 2-halo and 2-phenyl derivatives of melatonin compared to melatonin itself arises from the C-3 side chain being restricted into favourable conformations for interaction with the receptor.

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(14) We define an agonist as a compound that produces a pigment area of < 30% the original; a partial agonist a compound that produces between 30 - 80% of the original pigment area; and an inactive compound that produces >80% of the original pigment area, all at the highest concentrations used (10μM or less).

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(17) Unlike melatonin, the derivatives described here are chiral. An investigation of the separate enantiomers of the biologically more active compounds is now in progress.

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