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Original article

Receptor-binding studies of 1-N-substituted melatonin analogues

Alfonso Lira-Rocha, Ofelia Espejo-González *, Elia B. Naranjo-Rodríguez

Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Cd. Universitaria, Coyoacán 04510, México, D.F.

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Abstract

In order to analyse the relevance of the indole electronic region in the binding of melatonin to its receptors, we prepared several analogues with *p*-H, *p*-NO_2, *p*-MeO, *p*-F and *p*-Me of benzyl, benzoyl and phenyl substituents at position 1 of the melatonin skeleton. The electronic properties of the analogues, as calculated with the semiempirical method AM1, were correlated with their affinity for the melatonin receptor from chicken brain membranes. Different trends were observed for each compound series. Compound **5c**, with a *p*-NO_2-benzoyl group, showed the best affinity indicating the importance of a polar bulky group in the receptor interaction.

Keywords: Melatonin; Binding affinity; Semiempirical method AM1; Melatonin receptor

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, MT, Fig. 1), one of the many endogenous body environment-sensors, has a number of important actions: it acts as a chemical mediator of photoperiodic information [1,2]; it is a maternal–fetal synchronizer [3]; it modulates endocrinological, neurophysiological and behavioral functions in vertebrates [4–7]; it is an inductor of sleep in humans [4]; it regulates retinal vertebral physiology. In addition, MT can influence the circadian rhythm in reptiles, birds and mammals including human. In the latter context, its exact function is not fully elucidated. The therapeutic relevance of MT, therefore, includes synchronization of disturbed circadian rhythms such as jet-lag [8], sleep-wake cycle [9], seasonal disorders [10] and winter depression [11].

Several MT receptors have been proposed (MT_1, MT_2, MT_3) [12]. However, specific receptors ligands have remained unknown. Neither have specific antagonists that can be used as pharmacological tools been found for MT receptors. To date, three kinds of agonist and/or antagonists have been synthesized, indole-based, 2-amidotetralines and naphthyl derivatives [13] and recently, chroman derivatives [14].

Lewis et al. [15] first proposed an MT receptor binding site based on electrostatic potential energy calculations of MT. The authors proposed that the molecule interacts with the molecular receptor through electrostatic forces, although these results do not provide any parameters that can be correlated with biological activity.

During the last years several molecular models of the binding site have been proposed. Some of them are based on the structure of bacteriorhodopsine [16,17], or on that of rhodopsin [18], and or CoMFA analysis [19–21].

All models agree on the hydrogen bonding of the 5-methoxy group as a donor group, the carbonyl as an acceptor group and the nitrogen atom of acetamido as a donor group [22]. In the case of indole nucleus a π–π stacking interaction between the indole ring and the aromatic amino acid residues has been proposed. The incorporation of a bulky group at position 2 has been considered as an additional pharmacophoric site.

The proposal of an interaction between the indole nucleus and the aromatic amino acid encouraged us to study the electronic properties of the indole ring. We focused our study on the substitution pattern on the nitrogen atom of the indole ring on the basis of the

* Correspondence and reprints
E-mail address: espejog@servidor.unam.mx (O. Espejo-González).

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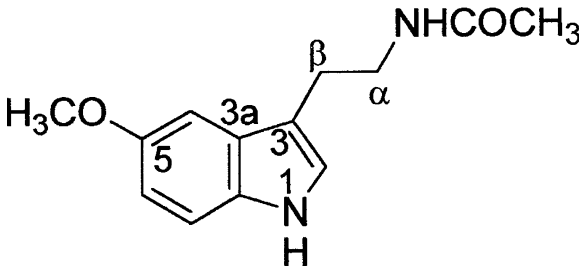


Fig. 1. Melatonin structure.

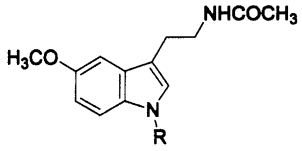
following arguments. First, this atom is far from the putative interaction zone, so that the possible steric interaction with substituents on this atom is minimized. Second, the nitrogen atom increases the electron density at 3 position of the indole ring, by the delocalization of its electronic pair. Therefore, if one alters this electronic distribution, one can modify the electronic properties of the molecular zone and alter biological activities. However, while this work was in progress, Mor et al. [20] reported that the incorporation of a phenyl or a benzyl group at 1 position of MT, respectively, diminishes the binding affinity for MT receptors. We also observed that this was the case in our preliminary studies. But we also observed that the incorporation of substituents in these rings affected the binding affinity. The present paper deals with these findings.

We selected three series of compounds: aralkyl, arylacyl, and aryl moieties with no-substitution or *p*-substituted (electron-releasing or -withdrawing groups). We present the synthesis, an analysis of the electronic properties and affinity binding data of MT and its derivatives.

2. Receptor-binding studies

Affinity constants for the substances were determined by NOVASCREEN Biosciences Corporation, Hanover, MD, USA, using a method already described [23]. In this method, chicken brain membrane is used as receptor source, 2-[^{125}I]-iodomelatonin as radioligand at a final concentration of 70 pM, with 2-iodomelatonin as non-specific determinant (1.0 μM). 2-Iodomelatonin is used as reference and positive control. Reactions are carried out in 50 mM Tris–HCl (pH 7.5) at 37 °C for 60 min. The reaction is terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters is determined and compared to control values in order to ascertain any interactions of test compound with the melatonin binding site. The results are shown in Table 1.

Table 1
Receptor binding affinities of **1a**–**4a**, **1b**–**5b**, **1c**–**5c**



Compunds	R	Receptor binding (Ki, nM)
MEL	H	0.82
1a	$-CH_2-C_6H_5$	1640
2a	$-CH_2-C_6H_4-p-OCH_3$	737
3a	$-CH_2-C_6H_4-p-CH_3$	738
4a	$-CH_2-C_6H_4-p-F$	3290
1b	$-C_6H_5$	60.4
2b	$-C_6H_5-p-OCH_3$	52.6
3b	$-C_6H_5-p-CH_3$	3020
4b	$-C_6H_5-p-F$	748
5b	$-C_6H_5-p-NO_2$	408
1c	$-CO-C_6H_5$	538
2c	$-CO-C_6H_5-p-OCH_3$	1090
3c	$-CO-C_6H_5-p-CH_3$	477
4c	$-CO-C_6H_5-p-F$	423
5c	$-CO-C_6H_5-p-NO_2$	82.4

3. Conformational analysis and calculation of electronic properties

Conformational analysis, geometry optimization and electronic structure calculations were performed with the Spartan [24] molecular modelling package, using a Silicon Graphics R4600 100 MHz 32 Mb RAM workstation. The conformational analysis was performed within Conformers Search option using Tripos force field and Systematic Method Search protocol.

The MT structure was built from standard fragments and minimized. According to previous published data [25], the methoxy group was oriented to the side chain. The MT side chain was analyzed conformationally. The rotation was performed around τ_1 (C3a–C3–Cβ–Cα), τ_2 (C3–Cβ–Cα–N) and τ_3 (Cβ–Cα–N–CO) (Fig. 1). The most stable conformer was optimized using AM1 method (Default options, MMK correction). The resultant structure was used as template for the construction of the MT analogues. After the introduction of the substituents in the MT template, a conformational analysis for each analogue was carried out.

The bonds between indole and phenyl rings were considered for the analysis, then, the geometry of the most stable conformer was optimized using the method outlined above. Electronic structure calculations and the resulting electronic distribution were used to generate molecular electrostatic potential (MEP) energies.

4. Chemistry

Table 1 shows the substitution pattern for the derivatives. The evaluated compounds were prepared by the general methods outlined in Fig. 2. The *N*-benzyl and *N*-benzoyl derivatives were synthesized by deprotonation of MT with KOH in DME and subsequent addition of chlorobenzyl or chlorobenzoyl reagents [26]. The *N*-phenyl derivatives were synthesized by reacting MT with iodophenyl reagent in the presence of CuI in DMF, Ullman arylation [27]. All compounds were characterized by melting point and spectroscopic data (IR, RMP, MS, elemental analysis).

5. Results

Conformational analysis of MT showed an antyperiplanar orientation between indole ring and acetamide group (Torsion angle = 177.6°) (Fig. 3). Both, the indole ring and acetamide group were oriented perpendicular to the side chain. The Cβ laid 0.29° above the indole ring plane. This conformation was not affected by the introduction of the substituents in the MT template.

For the analogue 'a' series, the methylene group was coplanar to the indole ring, whereas the phenyl group was oriented below the aromatic ring plane (torsion angle ca. −83.59°, C_2–N_1–CH_2–$C_{1'}$) (Fig. 4, compound **1a**). The phenyl ring orientation, in the 'b' series, showed a torsional angle ca. 38.62° (C_2–$N_{1'}$–$C_{1'}$–$C_{2'}$) (Fig. 5, compound **1b**).

The carbonyl group of the 'c' series was coplanar to the indole ring and the phenyl ring showed a torsional angle 54.33° (N_1–CO–$C_{1'}$–$C_{2'}$) with respect to carbonyl group (Fig. 6, compound **1c**).

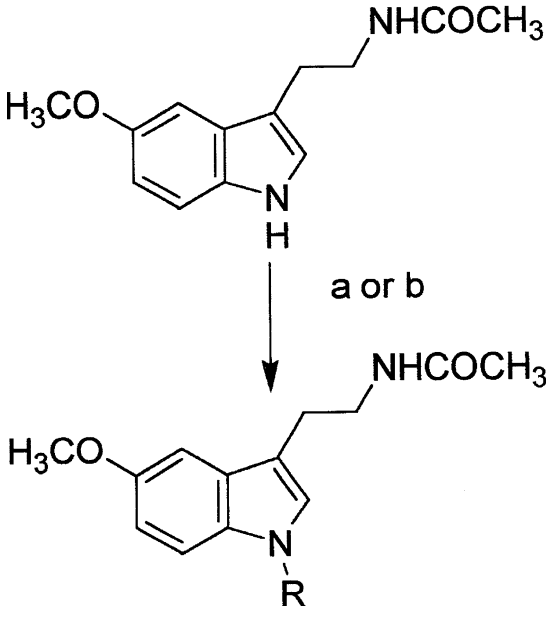


Fig. 2. (a) (i) KOH/DME; (ii) halobenzyl or halobenzoyl reagent; (b) iodophenyl reagent/K_2CO_3/CuI/DMF.

Taking into consideration these data, we assumed that the electronic properties in the 'northern' zone of the MT molecule were not affected by steric factors. In order to assess the influence of the substituents on these properties, we calculated the electronic properties of MT and their analogues. MEP's provide a highly informative means of establishing the electronic structure of molecules, particularly when biological recognition processes are involved.

MEP's volumes were calculated for all compounds (not showed). The isovalue showed (Figs. 7–10) −20.0 (range −20.25– −19.66), for all compounds. This volume corresponds to electrostatic potential which lays in a parallel plane 1.3 Å above the plane of the aromatic ring, just above of π-electron regions.

The isovalue volume of MT (Fig. 7) showed upper (region A) and lower (region B) lobules of similar proportions, above and below of the indole ring, respectively. These regions covered the N1, C3, C3a, C4 and C7a atoms. The remaining regions corresponded to the oxygen carbonyl, the nitrogen amide, and the oxygen methoxy groups.

6. Discussion

A comparison of MT A and B regions with the same regions of the compounds of 'a' series (Fig. 8) showed that the benzyl and *p*-methoxybenzyl substituents increased the volume of these regions.

The phenyl substituents failed to show a significant diminishing of regions A and B with the exception of *p*-nitrophenyl group (Fig. 9).

A significant diminishing of regions A and B by benzoyl group was observed by comparing MT regions (Fig. 10). Regions A and B completely disappeared with the *p*-nitrobenzoyl group.

As a rule, the nitro group always decreased the volume value in regions A–B for all the series.

On the other hand, a significant diminishing of regions A–B by benzoyl group was observed by comparing melatonin, 1-*N*-benzylmelatonin, 1-*N*-benzoylmelatonin and 1-*N*-phenylmelatonin.

In summary, the nitro derivatives decreased the electron density of the indole ring and in consequence one expects a lower binding affinity than MT.

With respect to the binding data, we observed that the incorporation of a bulky group at 1 position of the indole ring decreases the affinity. This is in concordance with data reported by Mor et al. [20]. However, the value of binding affinity in each series showed some differences.

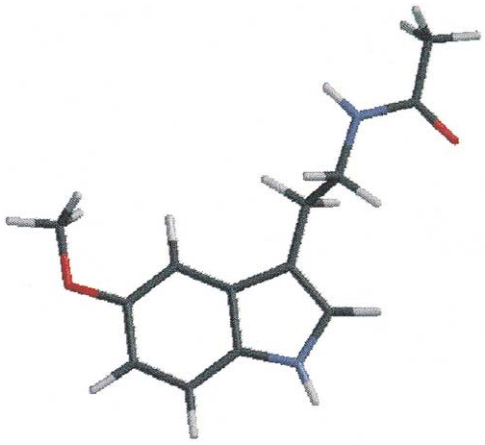


Fig. 3

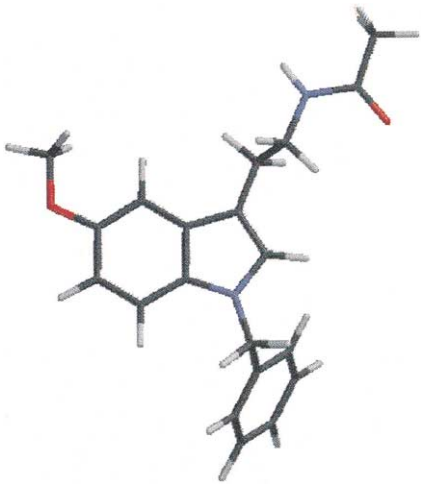


Fig. 4

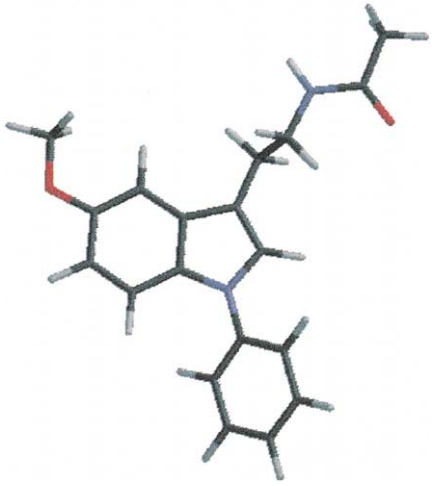


Fig. 5

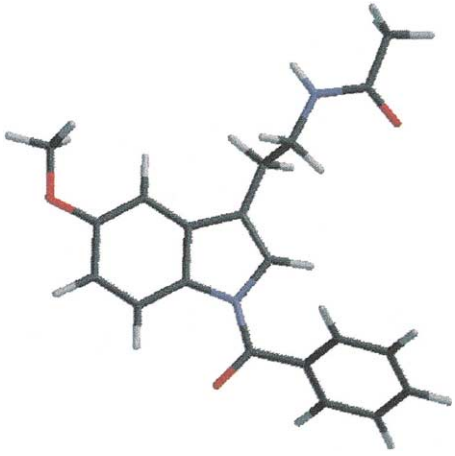


Fig. 6

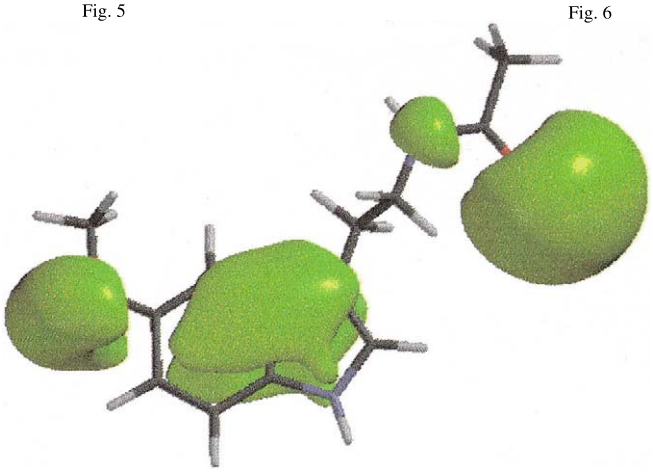


Fig. 7

Fig. 3. Framework view of MT. Fig. 4. Compound **1a**. Fig. 5. Compound **1b**. Fig. 6. Compound **1c**. Fig. 7. Isovalue of MT.

In the case of the series 'a', compounds with the presence of an electron-release group increased the affinity.

For the series 'b', we observed that the presence of a methoxy group showed the best affinity in this series. A comparison of the fluor and nitro derivatives data with that of the methyl derivative revealed that the compounds with polar groups have better affinity. We speculate that an electrostatic interaction occurs between the polar groups and the amino acid residues.

The binding affinity for the 'c' series showed a different trend. In this case the nitro derivative, an electron-withdrawing group, showed the best affinity value while the worst was the methoxy derivative.

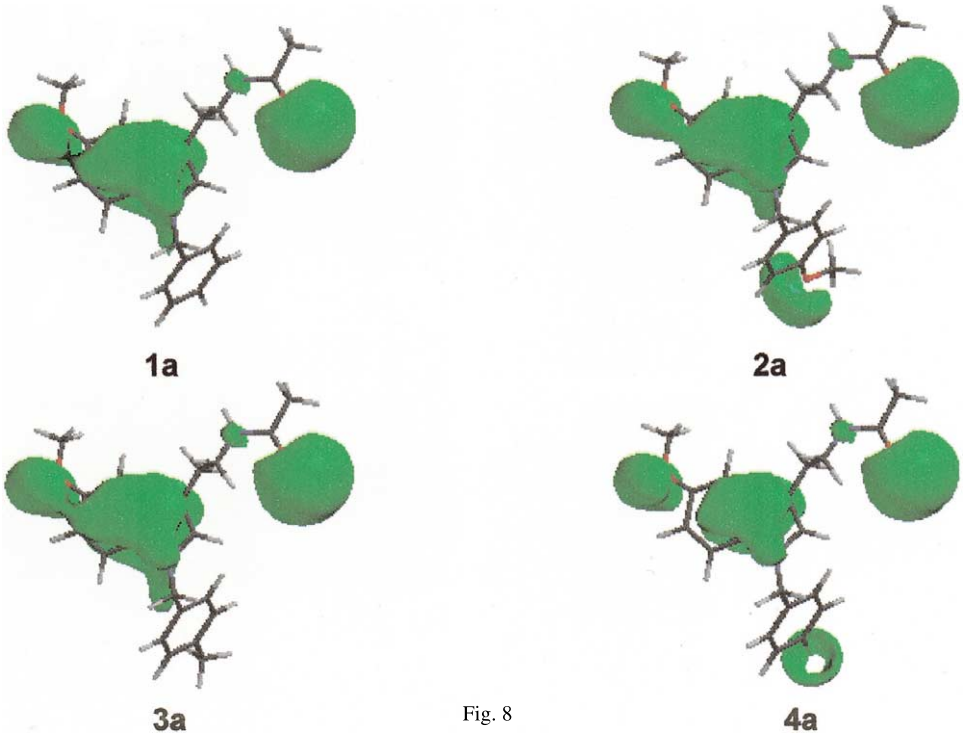


Fig. 8

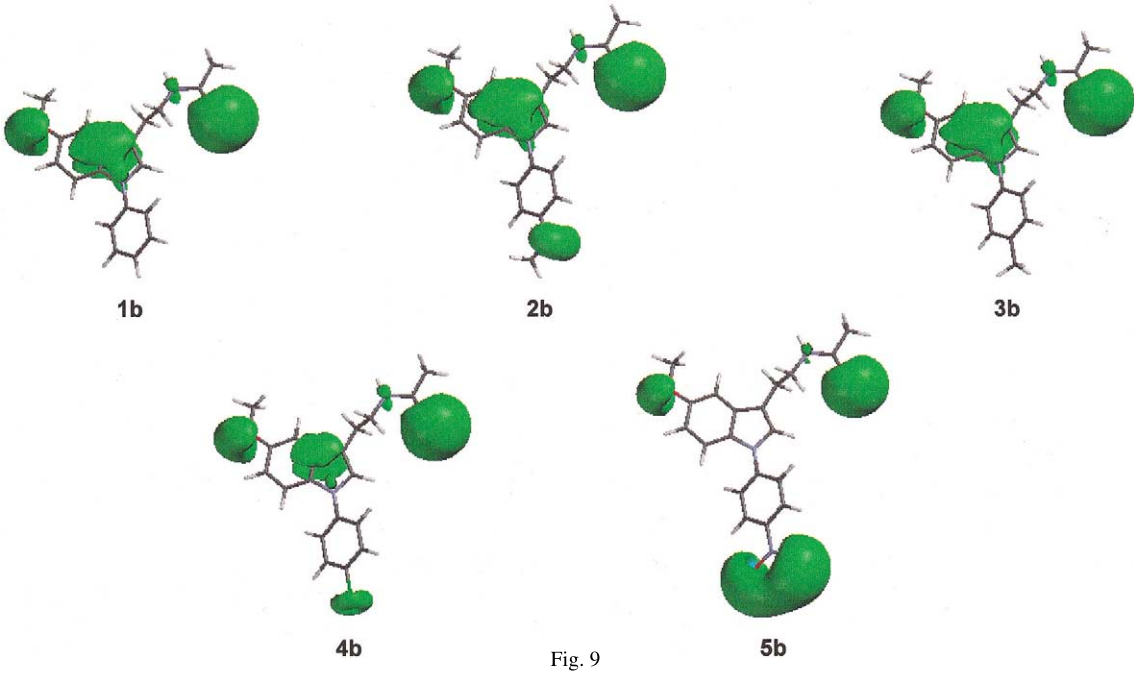


Fig. 9

Fig. 8. Isovalue for series 'a'. Fig. 9. Isovalue for series 'b'.

The analysis of the ^{1}H-NMR data for these compounds, outlined in Table 2, indicated that there is an interaction between the benzoyl aromatic ring and the β-methylene group. The shift of the last group is in the range of 2.84–2.87 ppm. This means an upperfield shift with respect to melatonin and the other series of compounds for the β-methylene group. The RMP data (Table 2) for 2-iodomelatonin indicate a similar shift for this group [28]. In the NOESY spectrum of compound **5c**, an interaction was observed between H-2 and H's β-methylene group, but not for the H's α-methylene group. Based on these findings, we assumed that a strong interaction occurs between the benzoyl group and the side chain.

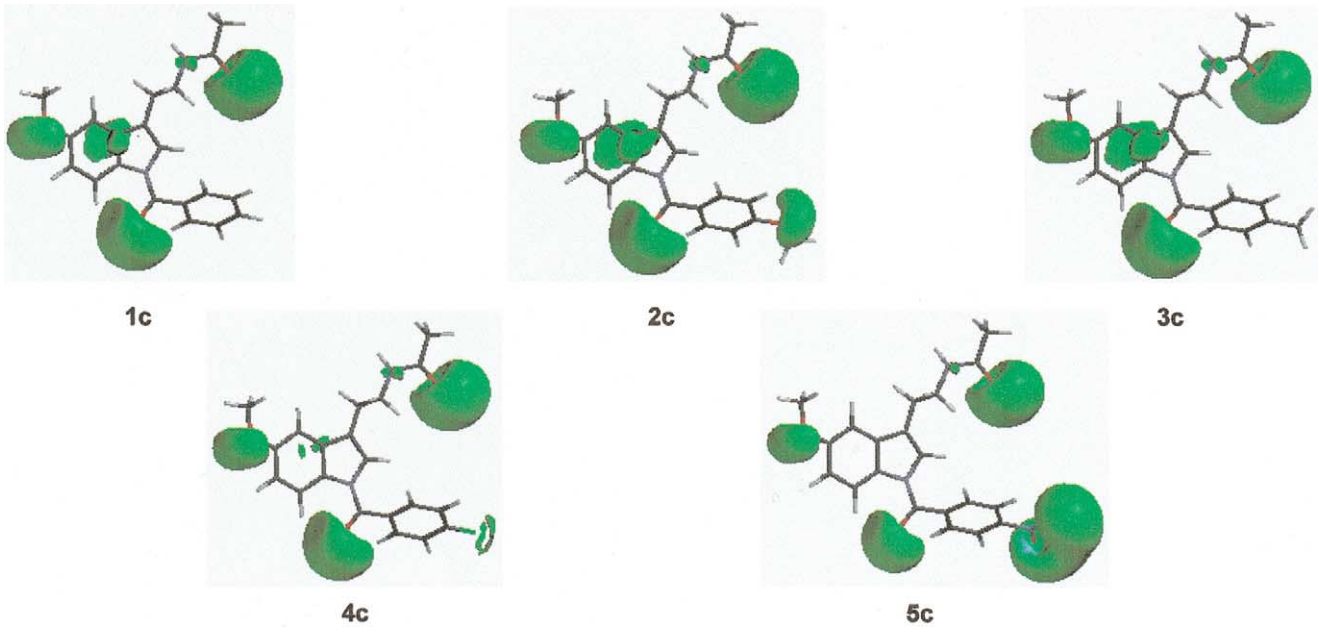


Fig. 10. Isovalue for series 'c'.

Table 2
^{1}H-NMR data of **1a**–**4a**, **1b**–**5b** and **1c**–**5c** (δ from TMS in $CDCl_3$, 300 MHz)

Compound	H-2	H-4	H-6	H-7	α H's	β H's	NH	CH_3O	CH_3CO
2-I-MT [a]	–	6.99	6.87	7.21	3.53	2.89	4.68	3.86	1.93
MT	7.365	7.03	6.87	7.27	3.6	2.95	6.01	3.86	1.96
1a	6.94	7.04	6.84	7.15	3.59	2.96	6.50	3.85	1.93
2a	–	–	–	–	3.54	2.90	5.50	3.83	1.89
3a	6.93	–	6.84	7.13	3.58	2.94	5.96	3.85	1.95
4a	6.92	7.03	6.84	7.16	3.57	2.95	6.15	3.85	1.97
1b	7.18	7.08	6.89	–	3.64	3.00	5.88	3.88	1.97
2b	7.16	7.02	6.83	7.46	3.50	2.88	6.28	3.79	1.87
3b	7.09	7.05	6.86	–	3.61	2.97	5.6	3.86	1.93
4b	7.12	7.08	6.89	7.37	3.67	3.03	5.80	3.92	2.01
5b	7.15	7.07	6.89	7.44	3.63	2.99	5.63	3.88	1.96
1c	7.07	–	6.97	8.27	3.52	2.84	5.5	3.87	1.92
2c	6.93	7.05	7.00	8.27	3.52	2.84	5.55	3.88	1.92
3c	7.16	–	–	8.24	3.56	2.88	5.59	3.89	1.94
4c	7.08	7.05	7.00	8.26	3.55	2.88	5.59	3.90	1.94
5c	7.12	7.03	6.99	8.27	3.54	2.87	5.59	3.89	1.94

[a] Ref. [28].

7. Conclusions

In Mor's model [20], the presence of a bulky group at position 2 of indole nucleus increases the affinity binding. Considering our findings, we conclude that the bulky group must have a higher charge density in order to get a good affinity. An iodine atom and a phenyl group are well tolerated in this position but not a cyclohexyl or a benzyl group [29,30]. On the other hand, it is noteworthy that in the series 'b', the methyl derivative showed the lowest binding value. A plausible explanation is that the presence of a polar group is necessary to yield a binding with high affinity.

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