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Synthesis and Preliminary Pharmacological Evaluation of Methoxilated Indoles with Possible Dopaminergic Central Action

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SUMMARY. Compounds **5-7** were synthesized from 4-tetralones with o-iodoanilines by a radical nucleophilic substitution or SRN1 reaction, and were pharmacologically evaluated in order to establish their possible antagonistic action on the central dopaminergic receptors. Behavioural parameters, such as stereotypy in rats were measured after intracerebroventricular administration of these compounds at doses of 10 μg/5 μL. Our results demonstrate that compounds **5-7** do not affect stereotypy behaviour. However, they inhibit the apomorphine-induced stereotypy behaviour, suggesting the involvement of the central dopaminergic system. Also we observe that there is a concordance between the behavioural profiles induced by our compounds and those reported for clozapine **8** and ziprasidone **9**. It is plausible to suggest that compounds **5-7** could be acting as potential atypical antipsychotic agents. Quantum calculations performed on the basis of a comparative conformational study of their structures indicate a stereoelectronic similarity between the basic nuclei of compounds **4** and **5-7**. In addition Molecular Dynamics (MD) simulations performed on compounds **5-7** at the binding site of dopamine D_2 receptor suggest that these compounds could interact with the human D_2 dopamine receptors.

INTRODUCTION

Dopamine (DA) **1** (Fig. 1) has been found to be an essential neurotransmitter. Dopaminergic neurotransmission has been shown to be critical for normal motor, motivational and reward-related functions. It is known that dopaminergic signalling is not only restricted to point-to-point synaptic contacts, but also involves volume transmission, which requires synaptic spillover of the released dopamine to reach distant target cells through extracellular diffusion.

Dopamine actions are mediated through its interaction with specific receptors, which are differentiated into two major types: the D_1-like receptor family, which includes the D_1 and D_5 receptors, and the D_2-like receptor family, which includes the D_2, D_3 and D_4 receptors [1,2]. Due to the absence of selective compounds for each receptor subtype, knowledge of the pharmacological and physiological roles of these receptors has not been fully elucidated.

Some studies have shown the localization of D_3 receptor in limbic and vestibule-cerebellar brain areas that affect locomotion and perhaps play a role in reinforcement and reward. A subpopulation of these receptors appears to be autoreceptors, which modulate dopamine synthesis, release and neuronal activity. These observations have led to the hypothesis that D_3 receptor may be an appropriate target in the treat-

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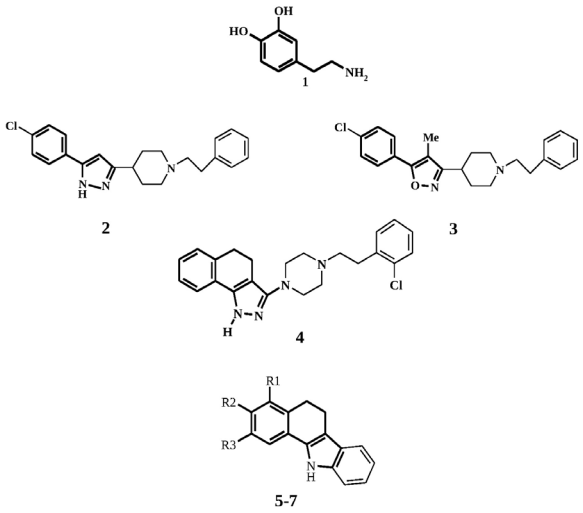


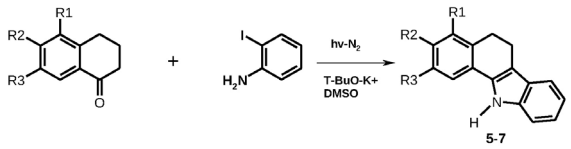
Compounds	R-1	R-2	R-3
5	-OMe	-H	-H
6	-H	-OMe	-H
7	-H	-H	-OMe

Figure 1. Comparison between compounds **2-4** (D_4 receptor antagonists) and compounds **5-7**.

ment of neuropsychiatric disorders, such as schizophrenia and drug addiction [3]. The role of D_3 sites in disease, however, remains to be established.

D_4 receptors are located over the dopaminergic system in areas that control emotions and knowledge. Much emphasis has been placed on investigating the role of D_4 receptor in disorders involving the dopaminergic mesocorticolimbic pathway, such as schizophrenia. This is in part due to studies which have revealed a predominant mesocorticolimbic distribution of this receptor with relatively high levels in the thalamus, hypothalamus, hippocampus, amygdala, nucleus accumbens, globus pallidus, and much of the cerebral cortex. These receptors are also expressed in the basal ganglia in low levels, where a selective D_4 receptor ligand has been suggested to be less prone to induce extrapyramidal secondary effects [4].

It has been also found that serotonin plays an important role in the regulation of dopaminergic neurotransmission, since it inhibits the release of dopamine at the level of the basal ganglia by axo-axonal connections through $5HT_{2A}$ receptors. The antagonism of $5HT_{2A}$ receptor increase dopaminergic neurotransmission in the striatum and prefrontal cortex. An increase in the release of dopamine also occurs in these brain regions as 5HT1A receptors become activated by increased serotonergic tone, which in



Compounds	R-1	R-2	R-3
5	-OMe	-H	-H
6	-H	-OMe	-H
7	-H	-H	-OMe

Figure 2. Synthetic route of methoxylated indols **5-7**.

turn results in a considerable diminution of unwanted side effects, such as, negative symptoms (denials and memory impairment), involuntary extrapyramidal movements and hyperprolactinemia [1-4].

Based on the search for new ligands with antipsychotic properties we refer to the compounds **2** and **3**. There is evidence showing that compounds **2** and **3** are highly selective toward dopamine D_4 receptor as antagonists, while they exhibit selectivity to other protein G coupled receptors in the CNS; they also showed affinity for voltage sensitive channels such as sodium, calcium and potassium. Meanwhile, compound **4** has shown higher selectivity and affinity for dopamine D4 receptor and ionic channels [5].

The comparison between compounds **2-4** (D4 receptor antagonists) and compounds **5-7** (Fig. 1) indicates that the basic nuclei are strongly related since they have the same comparative relation with the benzene ring attached to the five-member heteroaromatic ring, which could be in a free rotation form (compounds **2** and **3**) as well as in a rigid form (compound **4**). Compounds **5-7** differentiate from compounds **2-4** only by the fact that they have an aromatic ring and they do not have the substituted 4-piperidin ring or the substituted 1-piperazinic ring. Compounds **5-7** were obtained by Barolo *et al.* [6], starting with previously synthesized 1-tetralones from 1-iodoanilines as shown on Figure 2. The formation of these products by the SRN1 mechanism is depicted in Figure 3.

When o-iodoaniline receives one electron from the nucleophile, its radical anion is formed. This radical anion affords a radical by fragmentation of the C-I bond (eq 1), giving a new radical anion by reaction with the nucleophile (eq 2). This radical anion affords the substitution product by electron transfer to the substrate (eq 3) which by dehydration yields product (eq 4).

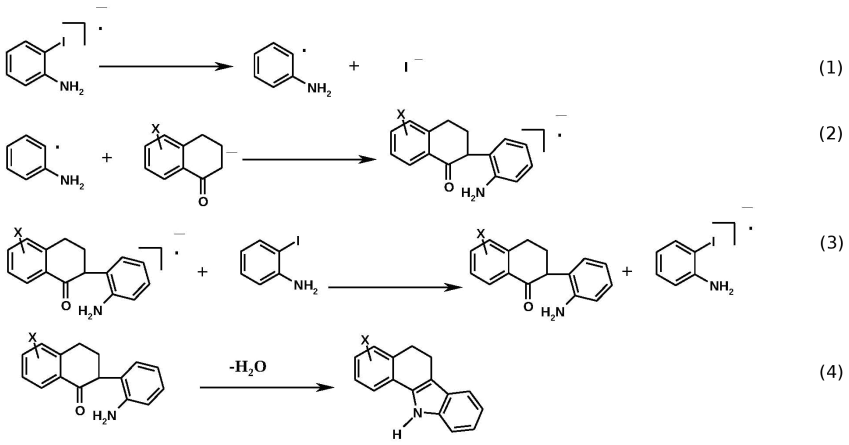


Figure 3. Reaction of o-iodoaniline with 4-tetralones by the $S_{RN}1$ mechanism.

In the light of evidence, compounds **5-7** were resynthesized and pharmacologically evaluated to determine their possible antagonistic action on central dopaminergic receptors by studying behavioural parameters such as stereotypy. In addition, a molecular modelling study was performed on compounds **5-7**. First a comparative conformational and electronic study on these structures was performed by using quantum mechanic calculations. The aim of this study was to compare the stereoelectronic aspects displayed by compounds **4-7**. In a second step MD simulations were carried out on compounds **5-7** interacting with the dopamine D_2 receptor.

MATERIALS AND METHODS

Organic syntheses

Uncorrected melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. Nuclear magnetic resonance (NMR) were recorded using a Brucker Advance NMR spectrometer at 300 MHz (^{1}H) and 100 MHz (^{13}C) and reported in ppm downfield (δ) from TMS as internal standard. The purity of all compounds was accessed by thin layer chromatography using mixtures of different polarity solvents. All solvents were distilled and dried by the usual mode.

Synthesis of methoxylated indoles (5-7)

In a flat bottomed three neck flask equipped with a magnetic stirrer and an inlet of nitrogen gas, 10 mL of dry and degasified DMSO with potassium tert-butoxide (0.227 g; 2.02 mmol) and the corresponding tetralones (0.352 g; 2.0 mmol) (a-c) were mixed, then o-iodoaniline (0.109 g; 0.50 mmol) was added 15 min later and the mixture irradiated by 180 min. The reaction was stopped with an excess of ammonium nitrate and 60 mL of water. The mixture was extracted with 20 mL of CH_2Cl_2, three times, and the organic extract washed with water and dried with sodium sulfate. The final product was purified by column chromatography using petroleum ether and acetone as eluants.

1-methoxy-5,11-dihydro-6H-benzo-(α)-carbazol (**5**). White crystals, showed a melting point of 141-142 °C. ^{1}H NMR ($CDCl_3$) δ: 8.13 (s. 1H), 7.55 (d, 1H. J=7.0 Hz), 7.37-7.33 (m, 1H), 7.25-7.07 (m, 3H), 6.96 (d, 1H, J= 7.7 Hz), 6.79 (d, 1H, J= 8.0 Hz), 3.86 (s, 3H), 3.12-2.90 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 157.05, 137.00, 132.95, 129.91, 127.48, 127.02, 124.22, 122.30, 119.83, 118.80, 112.77, 112.58, 111.04, 109.53, 55.57, 21.37, 19.05.

2-methoxy-5,11-dihydro-6H-benzo-(α)-carbazol (**6**). White crystals, showed a melting point of 168-169 °C. ^{1}H NMR ($CDCl_3$) δ: 8.05 (br. S, 1H), 7.53-7.59 (cplx. m, 1H), 7.35-730 (cplx. m, 1H), 7.22-7.06 (cplx. m, 3H), 6.84 (d, 1H, J= 2.6 Hz), 6.74 (dd, 1H, J= 8.4, 2.6 Hz), 3.8 (s, 3H), 3.07-2.89 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 158.67, 138.53, 136.78, 127.62, 122.09, 121.77, 120.88, 119.80, 118.37, 114.92, 111.34, 110.93, 110.80, 55.30, 29.99, 19.67.

3-methoxy-5,11-dihydro-6H-benzo-(α)-carbazol (**7**). White crystals, showed a melting point of 126-127 °C. ^{1}H NMR ($CDCl_3$) δ: 8.12 (s. 1H), 7.54 (d, 1H. J= 7.5 Hz), 7.33 (d, 1H J= 7.0 Hz), 7.20-7.07 (m, 3H), 6.86 (d, 1H, J= 2.6 Hz), 6.69 (dd, 1H, J= 8.0, 2.6Hz), 3.82 (s, 3H), 2.94 (s, 2H). ^{13}C NMR ($CDCl_3$) δ: 158.50, 137.00, 132.98, 129.80, 129.13, 128.72, 127.43, 122.39, 119.83, 118.80, 113.25, 111.18, 111.09, 106.49, 55.38, 28.62, 19.91.

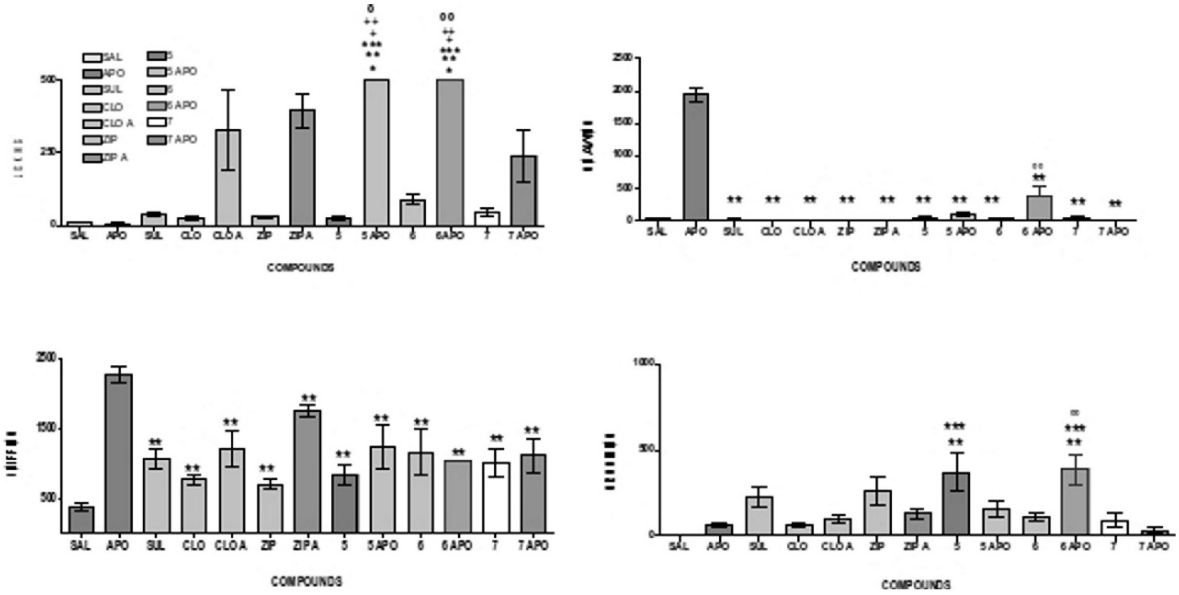


Figure 4. Effect of compound **5**, **6** and **7** on rat's behavior and on apomorphine-induced stereotypy. On the ordinate, the sum of the behavior measured during a period of one hour. On the abscissas, the drug treatment groups: SAL = saline, APO = apomorphine (1 mg/kg, i.p.), CLO = clozapine (1 mg/kg, i.p.), SUL = sulpiride (1 mg/kg, i.p.), ZIP = ziprasidone (1 mg/kg i.p.), 5 = compound **5** injected ICV at a dose of 10 µg/5 µl, 6 = compound **6** injected ICV at a dose of 10 µg/5 µl, 7 = compound **7** injected ICV at a dose of 10 µg/5 µL. The results were expressed as mean ± SEM of four independent measurements. * Significantly different from saline group, ** Significantly different from apomorphine group, *** Significantly different from clozapine group, + Significantly different from sulpiride group, ++ Significantly different from ziprasidone, ° significantly different from 5 10 ug *vs.* 5 10ug /APO group, °° significantly different from 6 10 µg *vs.* 6 10 µg /APO group (one-way ANOVA followed by Newman-Keuls test).

Pharmacological activity evaluation

Behavioural study

Adult Sprague-Dawley rats (Bioterio de la Facultad de Veterinaria de LUZ, Maracaibo, Venezuela), weighting 200-250 g, were housed under controlled conditions of temperature and light with free access to laboratory chow and water. Before the administration of the drug, a stainless steel cannula was implanted into the right lateral ventricle of each rat, under anaesthesia with xylazine (Setton® al 2 %) (1mg/kg., i.p) and ketamine (1 mg/kg., i.p), according to the following coordinates: antero-posterior -0,40 mm from Bregma, 1.2 mm lateral and 3 mm ventral, using a stereotaxic frame for rats. Cannulas less than 4 mm length were hand-made from 20G syringe needles; they were sealed with silicone and then fixed to rat´s skull with acrylic cement [7,8]. The ICV injection was carried out by using a 10 µL Hamilton syringe provided with a luer taper to precise application of the compounds. After three days of recovery from the surgical procedure, rats were treated with the compounds **5**, **6** and **7** at the doses of 10 µg/5 µL (Fig. 4), sulpiride (Tocris Bioscience) 1 mg/kg, i.p., clozapina (Tocris Bioscience) 1 mg/kg, i.p., ziprasidona (Geodon®) 1 mg/kg, i.p. Control rats were infused with vehicle, 0.9 % NaCl. After ten minutes of ICV administrations, animal behaviour was monitored for 60 min. A group of rats pre-treated with the compounds **5**, **6** and **7**, clozapine **8**, ziprasidone **9** or vehicle were injected with apomorphine HCl (APO) (Sandoz S.A., Basel, Switzerland) (1 mg/kg, i.p.), 10 min before the initiation of the behavioural observation. Animals were observed in a transparent Plexiglas chambers (32 x 28 x28 cm). Computer-assisted recording of the stereotyped (repetitive and purposeless) gnawing (the cage or body); sniffing; licking (the cage) and grooming was carried out at 6 minute interval for 60 min. One way analysis of variance (ANOVA) followed by Newman-Keuls test was performed to determine a significant difference between means of each treatment group. Four rats were used for each measurement.

Molecular modelling. Theoretical Calculation

All reported calculations were done using the GAUSSIAN 03 program [9]. Preliminary conformational study was done employing the GAS-

COS algorithm [14] combined with semiempirical (AM1) calculations. Once the different conformations were obtained, the preference forms were taken and they were optimized by RHF/6-31 G (d) calculations.

Molecular dynamics simulations

It must be pointed out that the principal goal of the molecular dynamics simulations performed here is not to obtain a new D_2 DR by homology. Our aim in this study is less ambitious; we wish to obtain a reasonable indication of the relationship between the structures of compounds **5**-**7** and their potential affinities for the binding pocket of D_2 DR. Thus, for such purpose we considered more appropriate to use a previously reported and extensively tested model for the D_2 DR [10]. The MD simulations and analysis are performed using the GROMACS 3.2.1 simulation package [11] and the GROMACS [12] united-atoms force field (FF) and the rigid SPC water model [13]. The ligands' topologies and charges were built using the Dundee PRODRG server [14]. In the present study, we have used an approach where manual docking was guided by information from site-directed mutagenesis. Thus, all the simulations performed here were carried out following the same procedure previously reported [10] (for more detail see these references).

The model used here is lacking the intra and extra-cellular loops which play a role in the stabilization of the whole system while immersed in the cellular membrane. In our simulations, the whole membrane has been excluded, and replaced by mere bulk water making a relatively simple but yet useful model of this system. We "fix" this problem by constraining the Cα of the trans-membrane helixes during the simulations time.

RESULTS AND DISCUSSION

Chemistry

Compounds **5**-**7** were synthesized from previously prepared 1-tetralones by Rossi *et al.* [6] Thus, although these compounds have been previously reported, the most relevant aspects of such synthesis are summarized here in Figures 2 and 3. Elemental analyses as well as other experimental data are described in details in the experimental section.

Biological assays

Compounds **5**-**7** were pharmacologically evaluated in order to establish their possible antagonistic action on the central dopaminergic receptors by measuring behavioural parameters such as stereotypy in rats after intracerebroventricular administration of these compounds at doses of 10 μg/5 μL. A computational quantum study was performed taking into account the structural similarity of compounds **5**-**7** with compounds **2**-**4** and their high affinity as antagonists for the D4 receptor [5]. The study was based on comparative conformational and electronic calculations of structures which have a stereoelectronic similarity between the basic nuclei of compounds **4** and **5**-**7**.

Thus, compounds **5**-**7** were pharmacologically evaluated to determine their possible antagonistic action on central dopaminergic receptors by studying behavioural parameters such as stereotypy in rats and its effect on the apomorphine-induced stereotyped behaviour. Stereotypy is a major component of several psychiatric disorders, including childhood autism [15] and schizophrenia [16]. It is well established that stereotypy (including sniffing or gnawing) is a dopamine-dependent behaviour [17], and the neural substrate of apomorphine-induced stereotyped behaviour in animals have been shown to include the central dopaminergic projections to the caudate-putamen region [18]. Apomorphine is known to be a mixed D_1/D_2 dopamine receptors agonist [17]. The activation of the D_1/D_2 dopamine receptors on striatum is expressed as the response of an excessive and repetitive behaviour (stereotypy).

Our results (Fig. 4) demonstrates that compounds **5**-**7**, at a dose of 10 μg/5 μL, did not induce stereotyped behaviour in the animals; yet, at this same dose, they were able to diminish apomorphine-induced gnawing while they also reduced, although partially, apomorphine-induced sniffing. Compounds **5**-**7** alone did not induce licking in the rats; yet, co-administered with apomorphine increased this behaviour in manner similar to that observed with clozapine and ziprasidone.

These results are in concordance with previous studies that report that clozapine **8** (an atypical antipsychotic) is able to inhibit apomorphine-induced stereotypy without completely affecting sniffing behaviour and locomotion of the rodents (Fig. 5). Clozapine **8** augments serotonergic tone (and as a result, indirectly activates $5HT_{1A}$ receptors) and reduces dopaminergic transmission produced by the concomitant blockade of $5HT_{2A}$ receptors (mainly) and D_2 receptors. These mechanisms would all together

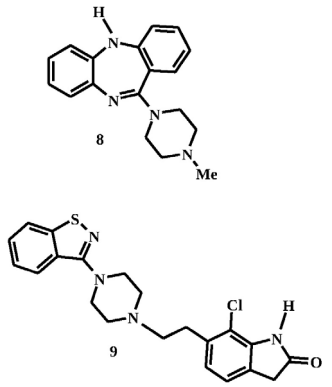


Figure 5. Atypical antipsychotic clozapine **8** and ziprasidone **9**.

moderate the reduction of the dopaminergic function, which explains clozapine's efficacy on positive symptoms with less production of extrapyramidal involuntary movements. At the same time, it also produces a serotonergic blockade, which would explain its efficacy on negative symptoms and mood disturbances [19].

Other studies have demonstrated that ziprasidone **9** behaves in a manner similar to clozapine **8**, being able to reduce apomorphine-induced sniffing and locomotion in rats without producing a complete suppression of those behaviours [20]. It is well known that ziprasidone **9** is an antipsychotic that basically acts by blocking of D_2 y $5HT_{2A}$ receptors, although its relative affinity is considerably higher for $5HT_{2A}$ receptors (8-fold greater than for D_2). In addition, this drug is able to block 5HT1D, $5HT_{2C}$, α1-adrenergic and H1 histamine receptors. Ziprasidone **9** also acts as a moderate inhibitor of serotonin and norepinephrine uptake and as a $5HT_{1A}$ receptor agonist.

On the other hand, the blockade of $5HT_{2A}$ is clinically related to the control of negative symptoms and neutralization of the extrapyramidal effects associated with the dopaminergic blockade. Yet, due to its agonist activity on $5HT_{1A}$ receptors, ziprasidone **9** has two pharmacological characteristics that distinguish it from other antipsychotics: an improvement of movement control with a minor incidence of extrapyramidal effects and a prevention of the onset of resistance to insulin. Its potent antagonism on $5HT_{2C}$ receptors seems to contribute to its antipsychotic effect.

As a whole, we observe that there is a concordance between the behavioural profiles induced by our compounds **5**-**7** (Fig. 4) and those reported for clozapine 8 and ziprasidone 9 19,20, and thus, it is plausible to suggest that compounds **5**-**7** could be acting as potential atypical antipsychotic agents.

These results allow us to hypothesize that the incorporation of a new aromatic ring in compounds **5**-**7** and the suppression of the piperazinic ring substituent or the substituted 1-piperazinic ring, such as in compounds **2**-**4**, offer the development of new nuclei with dopaminergic activity.

To evaluate this possibility, we conducted theoretical calculations based on the conformational study of compounds **4**-**7**. As previously indicated, compounds **5**-**7** were synthesized and tested as dopaminergic antagonists based on the structural similarity between those compounds and the basic nuclei known for D_4 dopamine receptor antagonists. The question that arises is if there really exists a stereoelectronic similarity between compound **4** and the synthesized and tested molecules. If the answer is affirmative, it is possible to think that these compounds may act by a molecular mechanism similar to compound **4**. Although the simple observation and comparison of those basic nuclei would show that there is an apparent similarity (Fig. 1), it is clear that a more direct form of evaluating this potential similarity is to calculate the conformational and electronic properties of these compounds and to compare them.

Molecular modelling

The molecular modeling study was carried out in two steps. In the first one a comparative conformational study was carried out on compounds **4**-**7** using *ab initio* [RHF/6-31G (d)] calculations. In a second step we performed molecular dynamics (MD) calculations trying to simulate the molecular interactions between compounds **5**-**7** reported here with the dopamine D_2 receptor.

Conformational study

The conformational study of compounds **5**-**7** is relatively simple since these molecules are quite rigid and the only conformational change that might occur is that given by the "up" or "down" inter-conversion of ring B (Fig. 6a). Quantum mechanic calculations at the HF/6-31G (d) level of theory showed that the two conformers are iso-energetic. The theoretical study of this conformational change at a HF/6-31G level of calculation (d) showed that the

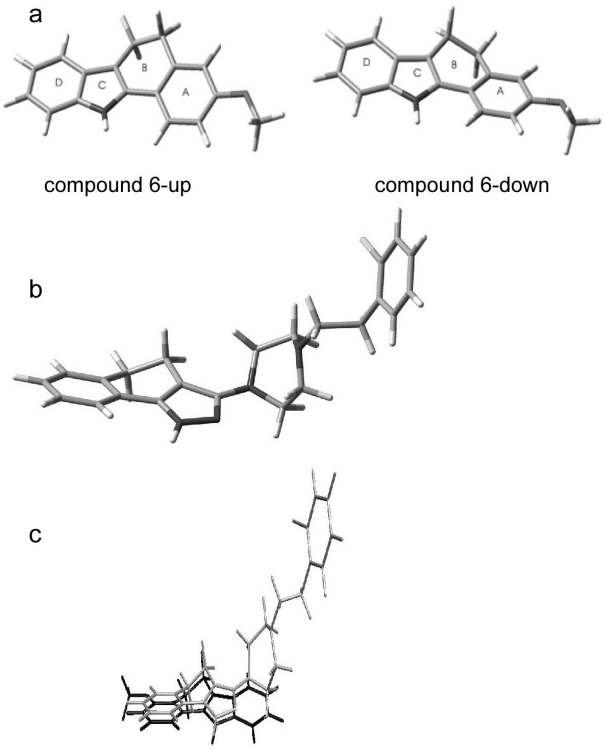


Figure 6. a) spatial view of the "up" and "down" conformations of compound **6**; **b**) spatial view obtained for the low-energy conformation of compound **4**; **c**) stereoview of overlapping of the low-energy conformations obtained for compounds **4** (ligth gray) and **5** (black).

"up" or "down" conformation of ring B has the same value of energy, and it corresponds to the energetic preferred conformers of these molecules.

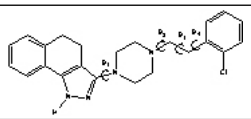
On the other hand, compound **4** (Table 1) have four free rotations (torsion angles θ1-θ4), giving place to 81 theoretical possible conformations following the rules of Conformational Multidimensional Analysis (CMDA). Therefore, for this molecule an exploratory conformational study was made using the GASCOS 21 algorithm combined with semi-empirical AM1 calculations. This conformational study showed that compound **4** displays a marked molecular flexibility giving extended, semi-extended and semi-folded conformations. The total number of obtained conformations was 6 (Table 1), being a semi-extended conformation the one of minimum energy (Fig. 4b).

Once the preferred energetic conformations of these compounds were obtained, it was interesting to compare them to each other to see their similarities and differences. Figure 4c shows a spatial visualization of the superposition of the preferred conformations of compounds **4** and **5**. As can be observed, there exists a very good superposition between the basic nuclei of these compounds. Nevertheless, it is evident that there is not a complete conformational superposition between compound **4** (indicated in gray) and molecule **5** (drawn in black, Fig. 5). This is because compound **4** has a ring connected to the basic nuclei by a flexible chain that compounds **5**-**7** do not have.

In order to confirm the above results in the second step of our study we performed MD simulations on compounds **5**-**7** interacting with the model of the dopamine D_2 receptor.

Molecular dynamic simulations

Comparing the results obtained for the different complexes, interesting general conclusions might be obtained. Consistent with previous experimental and theoretical results, our simulations indicate the importance of the negatively charged aspartate 86 for the binding of these ligands. A highly conserved aspartic acid (Asp 86) in trans-membrane helix 3 (TM3) is important for the binding of both agonists and antagonists to the D_2 receptor [22,23], and its terminal carboxyl group may function as an anchoring point



Conformation	θ_1	θ_2	θ_3	θ_4	ΔE(Kcal/mol)
1	-171.8	75.06	179.47	-84.51	0
2	45.04	-88.51	177.33	-83.22	0.51
3	160.37	-66.76	-86.95	-91.35	0.73
4	163.56	-179.35	-143.56	-83.81	1.53
5	31.27	63.71	-139.47	-102.91	2.42
6	109.15	179.54	-152.32	-84.62	3.20

Table 1. Conformations obtained for compound **4**, showing the torsional angles and energy gap.

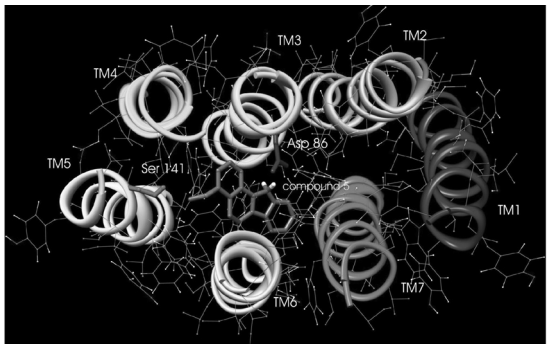


Figure 7. Spatial view of the dopamine D2 receptor. In this figure the binding pocket is denoted including compound 5, Asp 86 and Ser 141.

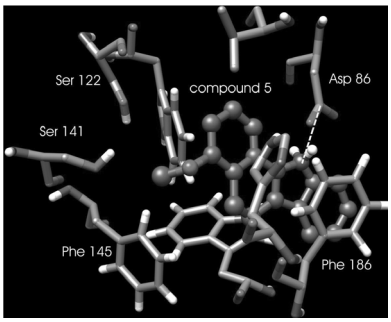


Figure 8. Interactions of compound **5** (ligand) with the D2 dopamine receptor. Spatial view of the stabilizing interactions: salt bridge (Asp 86 with protonated amino group) and hydrophobic interactions with the aromatic residue Phe 186. The rest of the amino acids were deleted to better appreciate the molecular interactions and the spatial ordering of compound **5**.

for ligands with a protonated amino group. In the present study, all the compounds simulated were docked into the receptor with the protonated amino group near to Asp 86. After 3 ns of MD simulations, the ligands had moved only slightly from the initial position. The strong interaction with Asp 86 was maintained for all the complexes, supporting the suggestion that Asp 86 functions as an anchoring point for ligands with a protonated amino group.

It was suggested that the serine cluster and dopamine form a hydrogen bonding network. Such a hydrogen-bonding network was reproduced by the MD simulation of these complexes (Fig. 7). In these complexes the strongest contributor to the network was Ser 141 which is consistent with the experimental observation that a Ser 141 Ala mutated receptor completely lost dopamine induced activation [23]. However two significant differences were observed comparing the results obtained for compounds **5**-**7** with those previously reported for compounds acting as agonists at the dopamine D_2 receptor. The first one is related with the type of stabilizing hydrogen bond obtained for these compounds and the other important difference corresponds to the different spatial ordering adopted by the hydrophobic moiety of these compounds (ring D). It should be noted that compounds **5**-**7** possess metoxy groups at the ring A and therefore the only possibility they have to give hydrogen bonds with serine 141 is acting as proton acceptor. It has been previously reported that this interaction is significantly weaker in comparison with those interactions where the hydroxyl group of the ligand (in general agonist) is the proton donor counterpart. Pharmacological data with dopaminergic ligands [23], indicate that the hydroxyl groups of dopaminergic ligands are primarily important in stabilizing the binding, suggesting that the serine residues (141 and 144) of the D_2 receptor may not be equally important for binding affinity. Individual mutation of serines 141 and 144 in TM5 to alanine produced asymmetrical effects on dopamine receptor binding. These results indicated that Ser 141 might be differentially important for dopamine binding. In addition site-directed mutagenesis studies have indicated that a cluster of serine residues in TM5 (Ser 141, Ser 144) and in TM4 (Ser 122 and Ser 118) is important for agonist binding and receptor activation [22,24]. With respect to the hydrophobic portion of compounds **5**-**7**, these moieties are located in the same region observed for other D_2 ligands like for instance tetrahydroisoquinolines (THIQs) [25-27]. However, it is interesting to note that compouds **5**-**7** are rigid molecules possessing a constrained conformational flexibility and therefore the available spatial ordering for these hydrophobic moieties is restricted giving a very poor molecular interactions with the aromatic residues located in the binding pocket: Trp 182, Phe 82 and Phe 186. In fact rings D displayed only an adequate distance to interact with Phe 186 (Fig. 8). These theoretical results are in agreement with the experimental data suggesting that compounds **5**-**7** could act as antagonists at the human D_2 dopamine receptor.

CONCLUSIONS

In conclusion, compounds **5**-**7** seem to have a profile of action with a dopaminergic function. On the basis of the results obtained from the apomorphine-induced stereotypy and molecular modelling studies, it can be concluded that

compounds **5**-**7** are potential antagonists on the central actions mediated by dopamine. However, it must be pointed out that in vitro binding data which could support the conclusion that the compounds are binding to dopamine receptors has not been carried out. Therefore our results might be considered as preliminary ones until such results will be available.

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