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Synthesis, dopaminergic profile, and molecular dynamics calculations of *N*-aralkyl substituted 2-aminoindans

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Abstract—Brain dopaminergic system has a crucial role in the etiology of several neuropsychiatric disorders, including Parkinson's disease, depression, and schizophrenia. Several dopaminergic drugs are used to treat these pathologies, but many problems are attributed to these therapies. Within this context, the search for new more efficient dopaminergic agents with less adverse effects represents a vast research field. The aim of the present study was to synthesize *N*-[2-(4,5-dihydroxyphenyl)-methyl-ethyl]-4,5-dihydroxy-2-aminoindan hydrobromide (**3**), planned to be a dopamine ligand, and to evaluate its dopaminergic action profile. This compound was assayed as a diastereoisomeric mixture in two experimental models: stereotyped behavior (gnaw) and renal urinary response, after central administration. The pharmacological results showed that compound **3** significantly blocked the apomorphine-induced stereotypy and dopamine-induced diuresis and natriuresis in rats. Thus, compound **3** demonstrated an inhibitory effect on dopaminergic-induced behavior and renal action. *N*-[2-(-Methyl-ethyl)]-4,5-dihydroxy-2-aminoindan hydrobromide (**4**) was previously reported as an inotropic agent, and in the present work it was also re-evaluated as a diastereoisomeric mixture for its possible central action on the behavior parameters such as stereotypy and dopamine-induced diuresis and natriuresis in rats. Our results indicate that compound **4** produces an agonistic response, possibly through dopaminergic mechanisms. To better understand the experimental results we performed molecular dynamics simulations of two complexes: compound **3**/D_2DAR (dopamine receptor) and compound **4**/D_2DAR. The differential binding mode obtained for these complexes could explain the antagonist and agonist activity obtained for compounds **3** and **4**, respectively.

1. Introduction

Dopamine belongs to the monoaminergic group of neurotransmitters and constitutes about 80% of the content of these substances in the brain. The dopamine system is involved in the regulation of brain regions that subserve motor, cognitive, and motivational behaviors, and disruptions of dopamine function have been implicated in neurological and psychiatric disorders, including Parkinson's disease, depression and, mainly, schizophrenia.[1,2] In addition, brain dopamine (DA) is now recognized as an important modulator of systemic blood pressure through the regulation of fluid and sodium metabolism and vasopressin release.[3,4]

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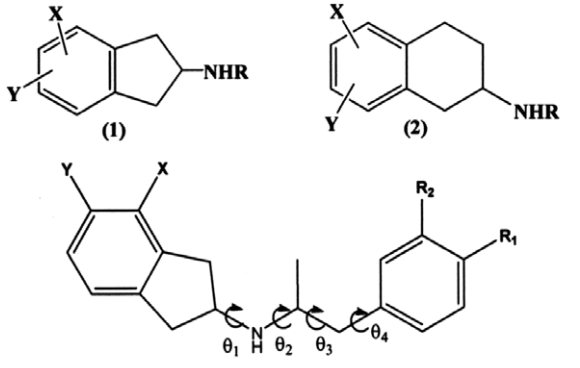
Schizophrenia is a chronic psychiatric illness that affects approximately 1% of the world population. Usually, a schizophrenic patient presents two main kinds of symptoms: positive (delusions, hallucinations) and negative (avolition, anhedonia, attentional impairment) symptoms. Most of the drugs used for treating this disorder are D_2-like receptor antagonists and are called typical antipsychotic. These drugs are effective only for treating the positive symptoms and present an important profile of adverse effects, mainly the extrapyramidal disturbances such as tardive dyskinesia.[1,2] For these reasons the search for new more efficient dopaminergic agents with lower adverse effects is still an extremely active research field.

During the last decades, a large amount of *N*-substituted 2-aminoindan (**1**) and 2-aminotetraline (**2**) analogs with peripheral and central nervous system action have been reported.[5–8] According to the well-established structure–biological relationship for the dopaminergic activity[9–12] as well as from our conformational studies,[13] we predict that compounds *N*-[2-(4,5-dihydroxyphenyl)-methyl-ethyl]-4,5-dihydroxy-2-aminoindan hydrobromide (**3**) and *N*-[2-(-methyl-ethyl)]-4,5-dihydroxy-2-aminoindan hydrobromide (**4**) could be ligands with dopaminergic activity. In particular since in their structures they have the fragment of the pharmacophore needed for the interaction with the dopaminergic receptor (*m*-hydroxyphenylethylamino fragment). Compound **3** represents a rigid analog of the dopamine α rotamer, while compound **4** has the same rotamer lacking the hydroxylic substituent on the aromatic ring in the aralkyl group (Fig. 1). Compound **4** was previously reported as an inotropic agent. In the present study we were promoted to synthesize compound **3** as a diastereoisomeric mixture and evaluated the pharmacological profiles of both, compounds **3** and **4**. Thus, taking in consideration their possible action on the dopaminergic system, we determined the behavioral parameters such as the stereotypy and dopamine-induced diuresis and natriuresis after central administration (ICV) in rats. In addition, for the better understanding of the mechanism of action of compounds **3** and **4** at molecular level, we simulated the molecular interactions of both compounds with the D_2 dopamine receptor (D_2DAR). These simulations were carried out using molecular dynamics (MD) calculations. Binding pocket of the D_2DAR was defined according to Teeter et al.,[14] while docking analysis was done using GROMACS software package.[15,16]



Compound	X	Y	R_1	R_2
3	OH	OH	OH	OH
4	OH	OH	H	H

Figure 1. Structures of compounds (**1**–**4**). Showing the four torsion angles θ_1–θ_4.

2. Results and discussion

2.1. Biological activity

Stereotypy is a major component of several psychiatric disorders, including childhood autism[17] or schizophrenia.[18] It is well-established that stereotypy (including sniffing or gnawing) is a dopamine-dependent behavior[19] and the neural substrate of apomorphine-induced stereotyped behavior in animals has been shown to include the central dopaminergic projections to the caudate-putamen region.[20] Apomorphine is known to be a mixed D_1/D_2 dopamine receptor agonist.[19,21] The activation of the D_1/D_2 dopamine receptors on striatum is expressed as the response of an excessive and repetitive behavior (stereotypy). Furthermore, it has been demonstrated that in adult and neonatal rats, lesioned with 6-hydroxydopamine (6OH-DA), the stereotypy behavior is the product of the activation of both receptor subtypes. Recently, receptor D_1 has been postulated as responsible for the initiation of the stereotypy behavior, while the participation of receptor D_2 involved the maintenance of such action, but not the initiation process. That is why the combined action of the D_1/D_2 antagonists blocks the stereotypy in a more effective way than the selective block through a selective antagonist of D_1.[22]

The results of the experiments reported in the present study demonstrated that compound **3** (0.6 μg/μl and 6 μg/μl), by itself, did not induce the stereotyped behavior (gnaw). On the other hand, the central administration of compound **3** significantly reduced (6 μg/μl) or inhibited (0.6 μg/μl) the stereotypy-induced by apomorphine (Fig. 3), indicating that in the central nervous system compound **3** acts as a dopaminergic antagonist. However, the selectivity as antagonist of the dopaminergic receptors involved in the stereotypy can be reached with the use of low doses.

The renal response to central administration of compound **3** was also evaluated[23–26] and is shown in Figure 4. Intracerebroventricular administration of dopamine increased urinary volume at 6 h and sodium excretion at 3 and 6 h periods of collection. Central pretreatment with compound **3** did not alter basal urinary volume or the sodium excretion, while completely blocked the diuresis and natriuresis-induced by DA (Fig. 4). Several neurotransmitters centrally applied are able to modulate

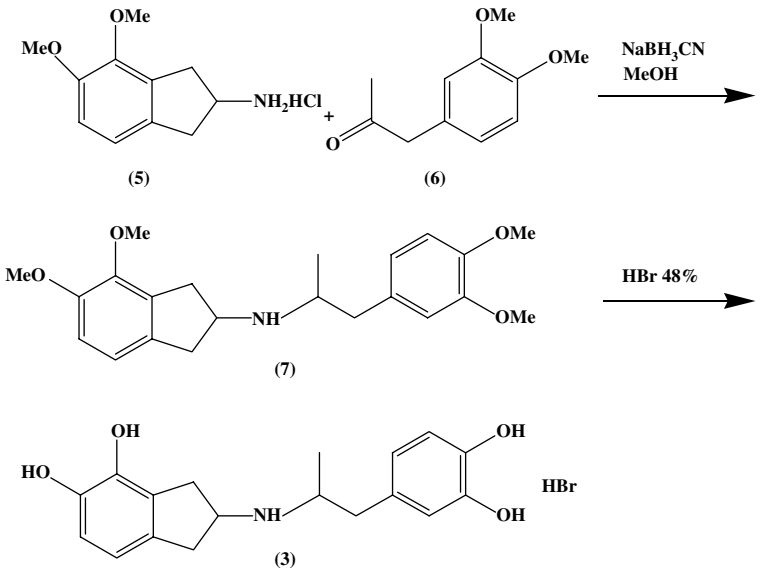


Figure 2. Synthetic route of *N*-[2-(3,4-dihydroxyphenyl)-methyl-ethyl]-4,5-dihydroxy-2-aminoindan bromohydride **4**.

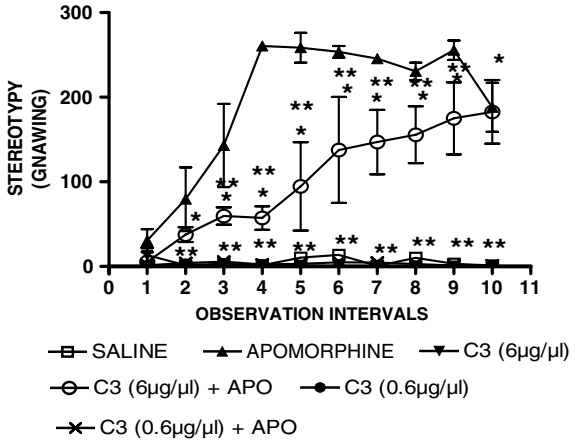


Figure 3. Effect of intracerebroventricular administration of compound **3** (0.6–6 µg/µl) on the stereotyped behavior (gnawing) in rats. On the ordinate are the numbers of gnawing for interval. On the abscise, the intervals of measurements of the behavior, observed during 4.5 min. The controls were injected with NaCl 0.9% and apomorphine (1 mg/kg, sc). The results are expressed as means ± SEM of four independent experiments. $^{*}p < 0.0001$ compared with the vehicle and $^{**}p < 0.0001$ compared with apomorphine. One- or two-way ANOVA was used, followed by the Duncan test. The results of compound **3** and compound **3** + apomorphine are overlapped with the saline control.

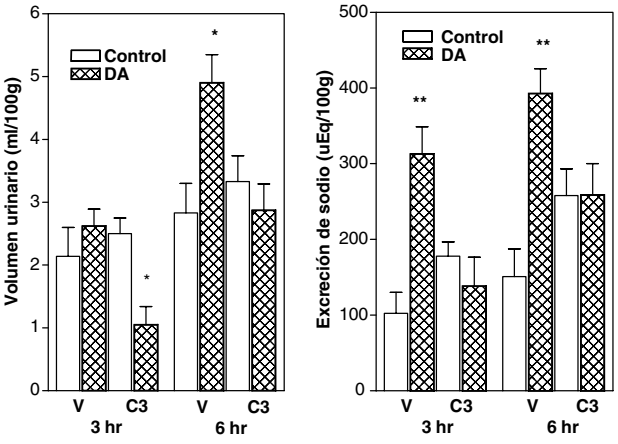


Figure 4. Effect of compound **3** on the DA-induced diuresis and natriuresis. Urinary response to ICV-DA in rats pre-treated with compound **3**. Groups of rats received an intracerebroventricular injection of compound **3** (C3) (50 µg/5 µl) ($N = 6–8$) or vehicle (V) (5 µl) ($N = 6–8$) 15 min before ICV-DA (100 µg/5 µl) or saline (5 µl) followed by water po (20 ml/kg). Significant *F*; ratios ($^{*}p < 0.05$ and $^{**}p < 0.01$) from two-way ANOVA were as follows: volume effect-DA at 6 h and C8 at 3 h; and Na^{+} effect, dopamine at 3 and 6 h.

the excretion of water and electrolytes.[23–26] DA has been postulated to function as a neurotransmitter, a neurohormone, or both and it has been involved in the regulation of fluid and electrolyte balance.[23,24] In effect, DA ICV-administered increases urinary volume and sodium excretion (Fig. 4), suggesting a role for the dopaminergic neurons besides the regulation of blood pressure and thirst, possibly through the control of vasopressin release.[4]

As shown in Figure 5, evaluation of compound **4** in the stereotypy assays demonstrated a significant increment in the licking, sniffing, and grooming behavior when compared to the saline control. These behaviors were blocked by haloperidol, a known antagonist of the dopaminergic receptors, suggesting an effect through dopaminergic mechanisms. It is important to mention that compound **4**-induced licking behavior was significantly higher than that of apomorphine. In regard to the renal effect observed in Figure 6, we demonstrate that intracerebroventricular administration of compound **4** significantly increased urinary volume and sodium excretion at the 6 h periods of collection ($p < 0.05$), while compound **3** was ineffective.

Our results of the inhibitory action of compound **3** on the stereotypy behavior (gnaw) induced by apomorphine and the diuresis and natriuresis-induced by

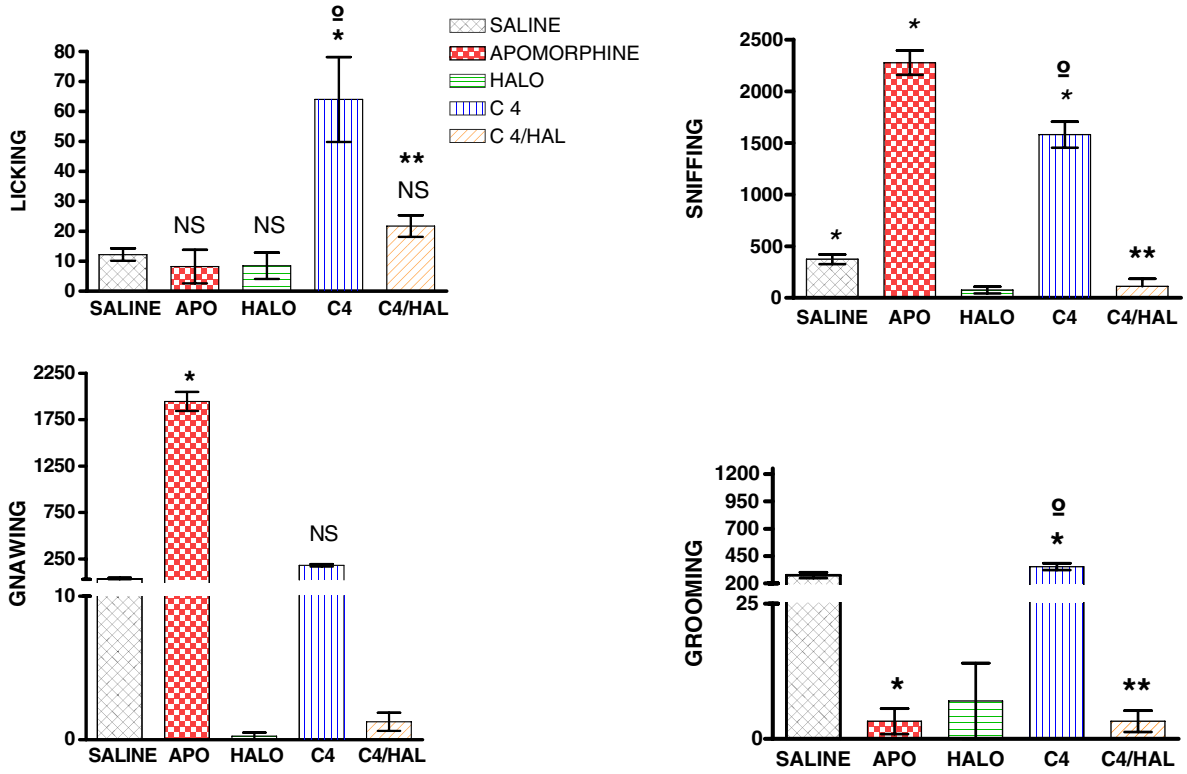


Figure 5. Effect of compound **4** (50 μg/5 μl) on the stereotyped behavior in rats blocked with haloperidol. On the ordinate, the sum of the behavior measurements. On the abscise, the tested compounds. The observations were carried out during 1 h. The results are expressed as means ± SEM of four independent experiments. The data were analyzed using one-way analysis of variance (ANOVA) and the test of Newman–Keul's. $^{*}p < 0.001$ compared to saline; $^{**}p < 0.001$ compared to compound **4**, $^{\circ}p < 0.05$ significant compared to apomorphine.

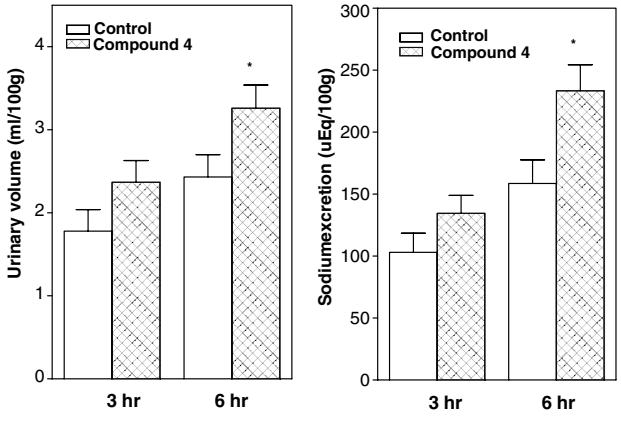


Figure 6. Effect of a single intracerebroventricular injection of compound **4** (50 μg/10 μl) on urine volume and sodium excretion in conscious hydrated rats. Significant differences (two-way ANOVA) are indicated. $^{*}p < 0.05$, compared with control-saline. $N = 6$–8 animals per group.

dopamine are in agreement with the predicted conformational theoretical study and strongly suggest that compound **3** represents a potential dopaminergic antagonist. The fact that compound **4** showed an opposite response to that shown by compound **3** with respect to the stereotypy behavior induced by apomorphine and the diuresis and natriuresis-induced by dopamine suggests that the incorporation of hydroxyl groups on the aromatic ring of the aralkyl fragment is responsible for the opposite response on the dopaminergic system. This would be in agreement with the reported results related to the antagonist responses when a hydroxyl group is present on the aromatic ring of the aralkyl fragment.[13] There are several explanations for the different activities obtained for compounds **3** and **4**. Probably the reason for the differential pharmacological response shown by the two compounds, **3** and **4**, could be due to the disposition of the hydroxyl groups on the aromatic ring of compound **4**, which could attribute a change in the conformational disposition of the latter in respect to the former, a fact that would not allow the adequate orientation and interaction with the dopamine receptor to promote the biological action of these compounds.[13]

To better understand the above experimental results, we conducted a computer-assisted molecular dynamics simulation of the molecular interactions of both compounds (**3** and **4**) with the D_2 dopamine receptor.

2.2. Molecular dynamics simulations

It should be noted that compounds **3** and **4** possess two chiral centers and therefore they are diastereomeric with the possibility of up to 4 isomers. However, we did not perform a diastereomeric resolution for the biological assays; thus, only one isomer of each compound was evaluated in the MD simulations. To choose one of the isomeric forms of **3** and **4**, we consider on one hand previously reported results[27] and on the other hand calculations

specially performed here determining the energetically preferred form of these compounds. Our previous results suggest that RR or RS forms would be the preferred isomers for these compounds.[27] We first optimize both isomers RR and RS of compounds **3** and **4** using DFT calculations. Our results indicate that RS isomeric forms are energetically preferred with respect to the RR isomers by 2.26 and 2.36 kcal/mol, for compounds **3** and **4**, respectively. Thus, on the basis of the DFT results we decide to use RS isomers of **3** and **4** for the MD simulations. In addition, to better characterize these isomeric forms we calculated their respective specific rotations $[\alpha]_D$.

Optical rotation calculations have been previously reported for different molecules.[28,29,32,35] Polavarapu[28] and Polavarapu and Chakraborty[29] reported calculations of $[\alpha]_D$ for several compounds using the Amos methodology[30] in the CADPAC program.[31] Polavarapu and Zhao[32] reported calculations of $[\alpha]_D$ using the Helgaker et al. methodology[33] using the DALTON program.[34] However, the accuracies of optical rotations calculated using the methodologies of Amos and Helgaker et al. are limited by their use of the HF/SCF methodology, in which electron correlation is neglected. More recently Stephens et al.[35] reported that DFT (B3LYP) provides substantially more accurate rotations than HF/SCF methodologies and therefore constitutes the current method of choice for stereochemical applications. They also showed that for these calculations, $[\alpha]_D$ values are strongly basis set dependent. In this sense large basis sets containing diffuse functions give very consistent results. On the basis of those results, we calculate the $[\alpha]_D$ using both RHF/6-31G(d) and B3LYP/6-311++G(2d,2p) calculations. Our calculations predict $[\alpha]_D$ values of 106.32 and 103.5 for compound **3** using RHF and DFT calculations, respectively. Whereas for compound **4**, these calculations predict $[\alpha]_D$ values of 31.37 and 28.96, respectively. However caution is required for these results. It should be noted that the above techniques apply to the calculations of $[\alpha]_D$ values of rigid molecules. In the case of conformationally flexible molecules (like compounds **3** and **4**), calculated $[\alpha]_D$ values are less accurate for two reasons. First, the $[\alpha]_D$ values of different conformations often differ in sign, reducing the magnitude of predicted $[\alpha]_D$ values. Second, errors due to uncertainties in conformational populations are introduced. In this case the $[\alpha]_D$ were calculated for the significantly populated conformations obtained from DFT calculations for compounds **3** and **4**.

The equilibrium state of the complexes was observed from onset of simulation until 10 ns. Temperature stabilized at 310 ± 4 K for both complexes. Whereas the Potential Energy stabilized at −462,000 ± 2000 kJ/mol and −467,500 ± 2000 kJ/mol for compound **3**/D_2DAR complex and compound **4**/D_2DAR complex, respectively. These parameters stabilized in a short time period (around 0.5 ns) suggesting that the system is well equilibrated (see Figs. S1, S2, S3, and S4 in supporting information).

Considering the 10 ns of MD simulation and from the time profiles it was concluded that some properties of the ligand–receptor complexes reached stable average values around 0.5 ns, whereas others takes longer time periods. For such reason to ensure full equilibration, only the last 9.5 ns were taken into account for the analysis. After discarding the first 0.5 ns of the trajectory, we follow the changes of spatial ordering of the ligand–receptor complexes (Fig. 7).

A highly conserved aspartic acid (Asp 86) in TMH3 is important for the binding of both agonists and antagonists to the D_2 receptor,[36–38] and its terminal carboxyl group may function as an anchoring point for ligands with a protonated amino group.[39] This structural requirement was also observed for the D_1 receptors.[40] In the present study, both ligands compounds **3** and **4** were docked into the receptor with the protonated amino group near Asp 86. After 10 ns of MD simulations, the ligands had moved slightly but in a different form compared with the initial position. Anyhow, the strong interaction with Asp 86 was maintained for both complexes (see Fig. 8), supporting the suggestion that Asp 86 functions as an anchoring point for ligands with a protonated amino group. From Figure 8a and b it is clear that a strong salt bridge forms for both compounds between the protonated amino groups and the carboxyl group of Asp 86.

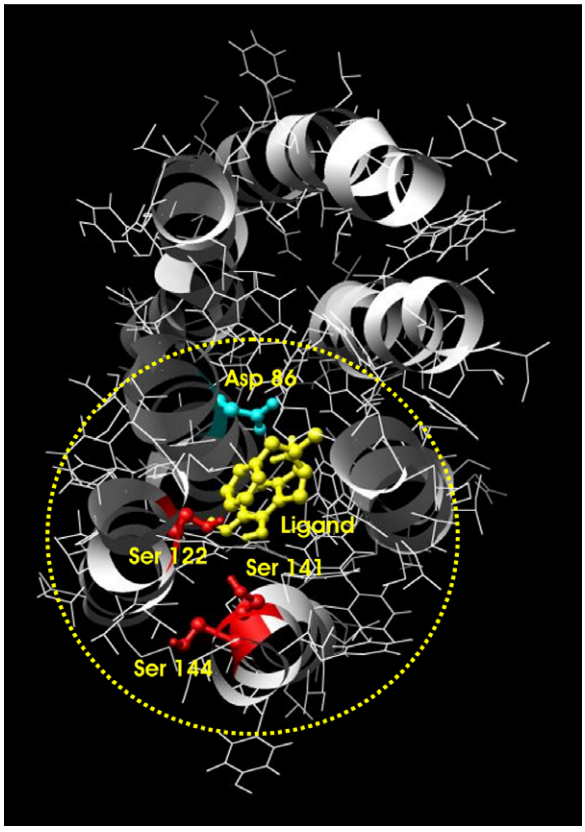


Figure 7. Spatial view of the dopamine D_2 receptor model from reference 15 using the chimera program as graphic interface. In this figure the binding pocket is denoted with a circle (dote line) including compound **3** (in yellow), Asp 86 (in light blue), Ser 122, Ser 141, and Ser 144 (in red).

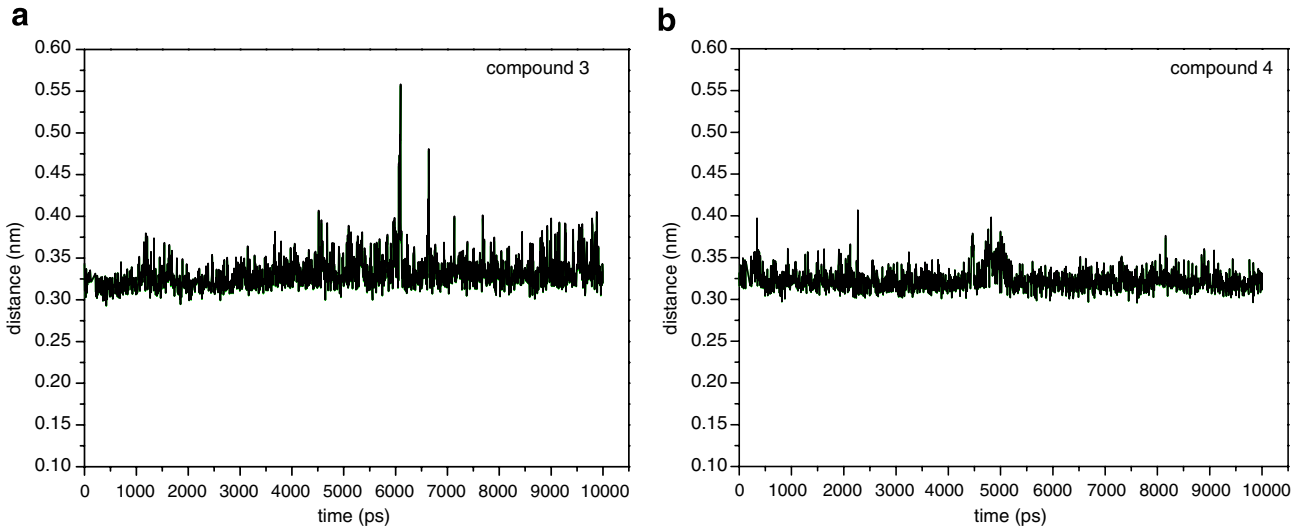


Figure 8. The length of salt bridge between the Asp 86 and the protonated amine group of compounds **3** (a) and **4** (b). A geometric average of interatomic distances between the three atoms of COO^- group with the nitrogen atom of amine was considered in this figure.

Pharmacological data with dopaminergic ligands[36,41] indicate that the meta-hydroxyl group of dopaminergic agonists is primarily important in stabilizing agonist 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 TMH5 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 TMH5 (Ser 141, Ser 144) and in TMH4 (Ser 122 and Ser 118) is important for agonist binding and receptor activation.[38,39,42,43] 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 the compound **4**/D_2DAR complex (Fig. 9). In this complex the strongest contributor to the network was Ser 141 (atomic distance 1.8 Å) which is consistent with the experimental observation that a Ser 141 Ala mutated receptor completely lost dopamine-induced activation.[14] It should be noted that the meta-hydroxyl group (X in Fig. 1) of compound **4** displayed another significant hydrogen bond interaction with Ser 118, however this interaction is weaker (atomic distance 2.0 Å) with respect to the hydrogen bond with Ser 141. In contrast to the above results, compound **3**/D_2DAR complex does not show hydrogen bond between meta-hydroxyl group and Ser 141 (Fig. 10). Only the starting conformation of compound **3** displayed this interaction due to the fact that we start the simulation using a spatial ordering possessing this interaction. However, during the rest of the 9.5 ns of simulation this interaction just disappears. In fact, none of the hydroxyl groups of compound **3** displayed any significant hydrogen bond with Ser 141. This is a striking difference with respect to the results obtained for compound **4**/D_2DAR complex. In the average complex after 10 ns MD, only the hydroxyl R_1 of compound **3** formed a strong hydrogen bond with Ser 122 (atomic distance of 2.0 Å) (Fig. 10).

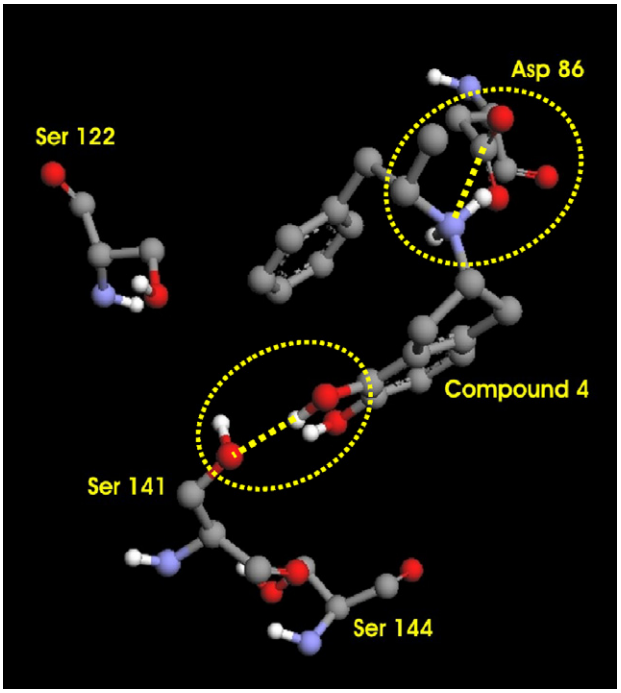


Figure 9. Interactions of compound **4** (ligand) with the D_2 dopamine receptor. Spatial view of two interactions: salt bridge (Asp 86 with protonated amino group) to the right and hydrogen bond between the meta-hydroxyl group (X in Fig. 1) with Ser 141. The rest of the aminoacids were deleted to better appreciate the molecular interactions and the spatial ordering of compound **4**.

The conformational behavior of the four torsional angles (θ_1–θ_4) of compounds **3** and **4** during the simulation is shown in Figures 11 and 12. From these figures it is clear that the conformational behavior of torsional angles θ_1, θ_2, and θ_3 are very similar for both compounds (Figs. 11 and 12a–c). Each of these three torsional angles displayed a highly preferred conformation; *gauche* + - form for θ_1 and θ_3 and *anti* form for θ_2. However it is interesting to note that the conformational behavior

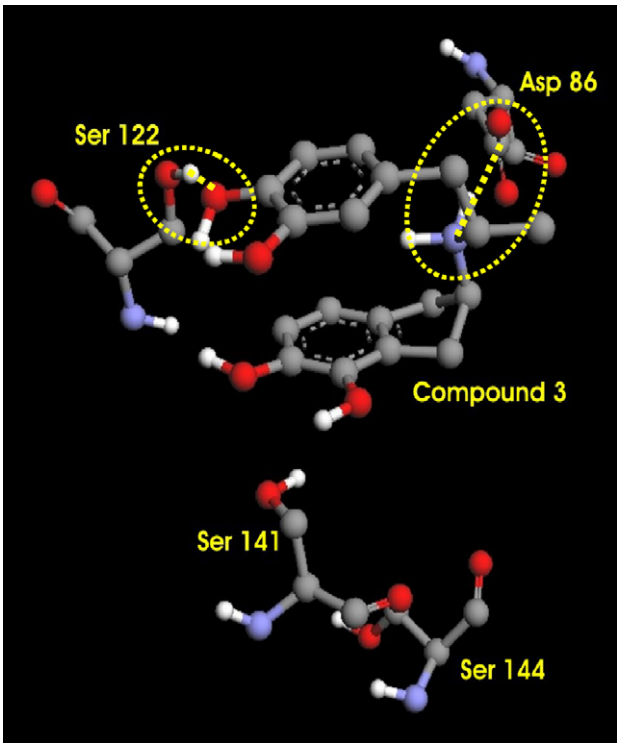


Figure 10. Interactions of compound **3** (ligand) with the D_2 dopamine receptor. Spatial view of two interactions: salt bridge (Asp 86 with protonated amino group) to the right and hydrogen bond between the R_1-hydroxyl group with Ser 122. The rest of the aminoacids were deleted to better appreciate the molecular interactions and the spatial ordering of compound **3**.

obtained for torsional angle θ_4 of compound **4** (Fig. 12d) is different from that of compound **3** (Fig. 11d). While θ_4 of compound **3** displays only one conformation (a perpendicular form with $\theta_4 \cong 90°$); θ_4 of compound **4** shows a conformational change from perpendicular ($\theta_4 \cong 90°$) to anti-perpendicular ($\theta_4 \cong -90°$) (see Fig. 12d). This conformational change occurs at ≅7 ns of simulation. This result is in a complete agreement with the different positional RMSD (root mean square deviation) profiles obtained for compounds **3** and **4**, respectively (Fig. 13). It appears that the strong hydrogen bond between the OH group at R_1 of compound **3** and Ser 122 maintains this ring in the same conformation during all the simulation. In contrast, the phenyl ring of compound **4** is allowed to perform an almost free rotation during the simulation, and therefore a conformational change takes place in this portion of the molecule.

Comparing the results obtained for both complexes (compound **3**/D_2DAR and compound **4**/D_2DAR), interesting conclusions might be obtained. Consistent with previous experimental[35,27] and theoretical[37,29] results, our simulations indicate the importance of the negatively charged aspartate 86 for the binding of compounds **3** (antagonist) and **4** (agonist). The main difference in binding mode between compounds **3** and **4** was that the agonist bound the receptor with the meta-hydroxyl in the direction of Ser 141 (in TMH5), while the antagonist has the hydroxyl group R_1 in the direction of Ser 122. The different conformation adopted by compounds **3** and **4** in their respective complexes might be well appreciated comparing Figures 9 and 10. Thus, it is reasonable to think that these different interactions with serine cluster would be the principal factor responsible for the different conformational behavior obtained for these complexes. Also it is reasonable to think that this different binding mode could be responsible for the antagonist and agonist effects displayed by compounds **3** and **4**, respectively.

In conclusion, compound **3** seems to have a profile of action coherent to dopaminergic function. On the basis of the results obtained on the apomorphine-induced stereotypy and centrally mediated renal actions, we can conclude that compound **3** has demonstrated to be a potential antagonist of the centrally mediated actions induced by dopamine. The different binding mode obtained from MD simulations for compound **3**/D_2DAR and compound **4**/D_2DAR complexes could explain, at least in part, the antagonist and agonist effect obtained for compounds **3** and **4**, respectively.

3. Experimental

3.1. Chemistry

Compound **3** was prepared through a reductive amination, combining a mixture of the derivative (**5**) and the ketone (**6**) as shown in Figure 2. Thus, the 4,5-dimethoxy-2-aminoindan hydrochloride (**5**) was obtained according to the procedure described in Refs. 6, 7, and 44 Subsequently, derivative (**7**) was hydrolyzed to produce compound **3** in good yield.[9]

Uncorrected melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. NMR spectra were recorded using a Jeol Eclipse 270 MHz spectrometer using $CDCl_3$ or DMSO-d_6 and are reported in ppm downfield from $CHCl_3$ or DMSO residual. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. Mass spectra were recorded on a Hewlett Packard 5995 mass spectrometer. The purity of all compounds was accessed by thin layer chromatography using different polarity solvents. Elemental analyses were performed on a Perkin-Elmer 2400CHN analyser, results were within ±0.4% of predicted values. All solvents were distilled and dried in the usual manner.

3.1.1. *N*-[2-(4,5-Dimethoxyphenyl)-methyl-ethyl]-4,5-dimethoxy-2-aminoindan hydrochloride (7). 4,5-Dimethoxy-2-aminoindan hydrochloride (**5**) (2.0 g, 10 mmol), the corresponding 4,5-dimethoxy-phenyl acetone (**6**) (1.94 g, 10 mmol), and sodium cyanoborohydride (0.94 g, 15 mmol) in CH_3OH 80 ml were stirred under a nitrogen atmosphere for 48 h. The resulting mixture was treated with an excess of 2 N HCl. The solvent was removed under reduced pressure, the remaining solid was dissolved in H_2O, and the aqueous solution was extracted with ether. The aqueous layer was made basic using 20% NaOH solution and extracted with $CHCl_3$.

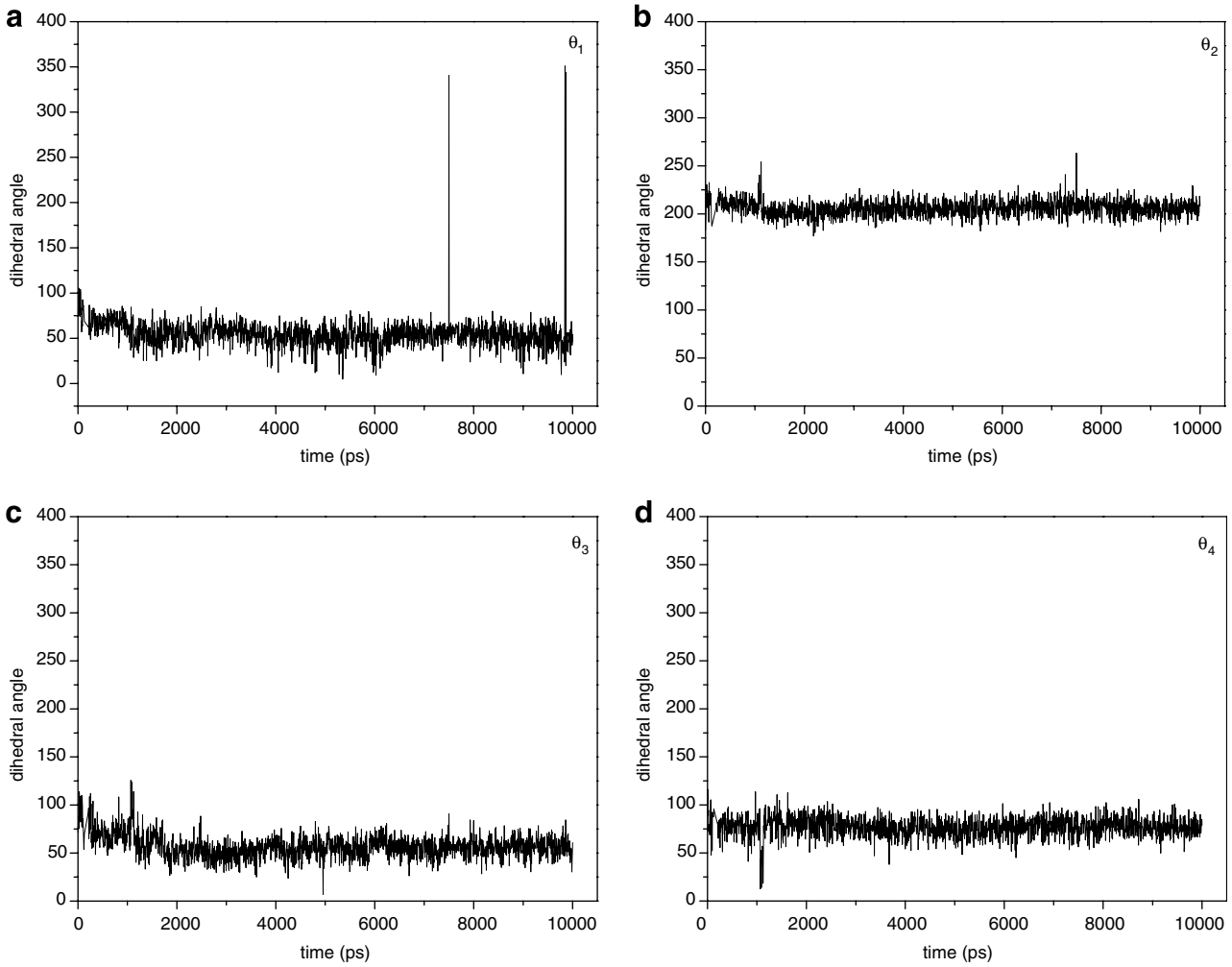


Figure 11. Evolution of the angles θ_1 (a), θ_2 (b), θ_3 (c), and θ_4 (d) of compound **3** with time.

The organic layer was dried over anhydrous magnesium sulfate; a steam of dry HCl was passed through the solution. Resulting crystals were collected by filtration. Recrystallization was carried out from isopropanol–ether (70%); mp 167–168 °C. IR (KBr) cm^{-1}: 2950 (CH_3); 2900 (CH_2); 2.800–2750, 1625, 1612, 1525 ($R_2NH_2^+Cl^-$); 1488, 1463 (C=C); 1275, 1263, 1075, 1028 (C–O–C); 800 (ArH). ^{1}H NMR ($CDCl_3$): δ 1.06 (d, 3H, J = 10.8 Hz, CH_3); 1.72 (bb, R_2NH); 2.36–2.48 (m, 2H, CH_2Ar); 2.55 (dd, 1H, J = 5.4 Hz, H_{ax} (C_1-Indan)); 2.65 (dd, 1H, J = 5.4 Hz, H_{ax} (C_3-Indan)); 3.06 (dd, 1H, J = 5.4 Hz, H_{eq} (C_1-Indan)); 3.16 (dd, 1H, J = 5.4 Hz, H_{eq} (C_3-Indan)); 2.93–3.26 (m, 1H, –CH(CH_3)CH_2Ar); 3.86 (m, 13H (–OCH_3)$_4$, CH(C_2-Indan)); 6.64 (dd, 1H, J = 8.1 and 2.97 Hz, ArH (C_6-aralkyl)); 6.67 (d, 1H, J = 8.1 Hz, ArH (C_5-Aralkyl)); 6.70 (d, 1H, J = 8.1 Hz, ArH, (C_6-Indan)); 6.75 (d, 1H, J = 2.97 Hz, ArH (C_2-Aralkyl)); 6.81 (d, 1H, J = 8.1 Hz, ArH (C_7-Indan)). ^{13}C NMR: 15 (CH_3); 35 (CH_2Ar); 37 (C_1-C_3 Indan); 41 (CH(CH_3)CH_2Ar); 51 (C_2-Indan); 56, 57, 60 (OCH_3)$_4$; 112, 113, 114, 121, 122, 130, 133, 134, 145, 148, 149, 152 (C, ArH). DEPT: 15(CH_3); inverted signals: 35 (CH_2Ar); 37 (C_1–C_3-Indan); 41 (CH(CH_3)CH_2Ar); 51 (C_2-Indan); 56, 57, 60 (OCH_3)$_4$; 112 (C_6-Aralkyl); 113 (C_5-Aralkyl); 114 (C_6-Indan); 121 (C_2-Aralkyl); 122 (C_7-Indan). MS *m/e*: the molecular ion did not show up, 220 (100%); 177 (50%); 146 (10%); 91 (6%), 77 (4%). $C_{22}H_{30}ClNO_4$: 407.94. Anal. Calcd C, 64.77; H, 7.41; N, 3.43. Found: C, 65.03; H, 7.17; N, 3.26.

3.1.2. *N*-[2-(4,5-Dihydroxyphenyl)-methyl-ethyl]-4,5-dihydroxy-2-aminoindan hydrobromide (3). The hydrochloride amine (**7**) (1.0 mmol) in 5 ml of 48% HBr was heated at reflux under a nitrogen atmosphere for 5 h. The solvent was removed under reduced pressure and the solid residue was recrystallized from isopropanol–ether (70%), mp 149–150 °C. IR (KBr) cm^{-1}: 3625–3050 (OH); 2975 (CH_3); 1625, 1512 ($R_2NH_2^+$); 1506 (C=C); 1300–1225 (C–O–H). ^{1}H NMR (DMSO-d_6): δ 1.07 (d, 3H, J = 6.43 Hz, CH_3); 2.5–3.47 (m, 7H, CH_2Ar, CH_2(C_1-Indan); CH_2(C_3-Indan); CH(CH_3)CH_2Ar); 3.95 (m, 1H, CH(C_2-Indan)); 6.51 (d, 1H, J = 7.91 Hz, ArH (C_6-Aralkyl)); 6.53 (d, 1H, J = 7.91 Hz, ArH (C_5-Aralkyl)); 6.61 (d, 1H, J = 7.91 Hz, ArH (C_6-Indan)); 6.64 (s, 1H, ArH, (C_2-Aralkyl)); 6.69 (d, 1H, J = 7.91 Hz, (C_7-Indan)); 8.04–8.60 (bb, $(OH)_4$); 8.90 (bb, $NH_2^+Br^-$). ^{13}C NMR: δ 15.70 (CH_3); 18.66 (CH_2Ar); 34.83 (C_1,C_3-Indan); 37.29 (CH(CH_3)CH_2Ar); 51.12 (C_2-Indan); 115.30 (C_6-Aralkyl); 116.28 (C_5-Aralkyl);

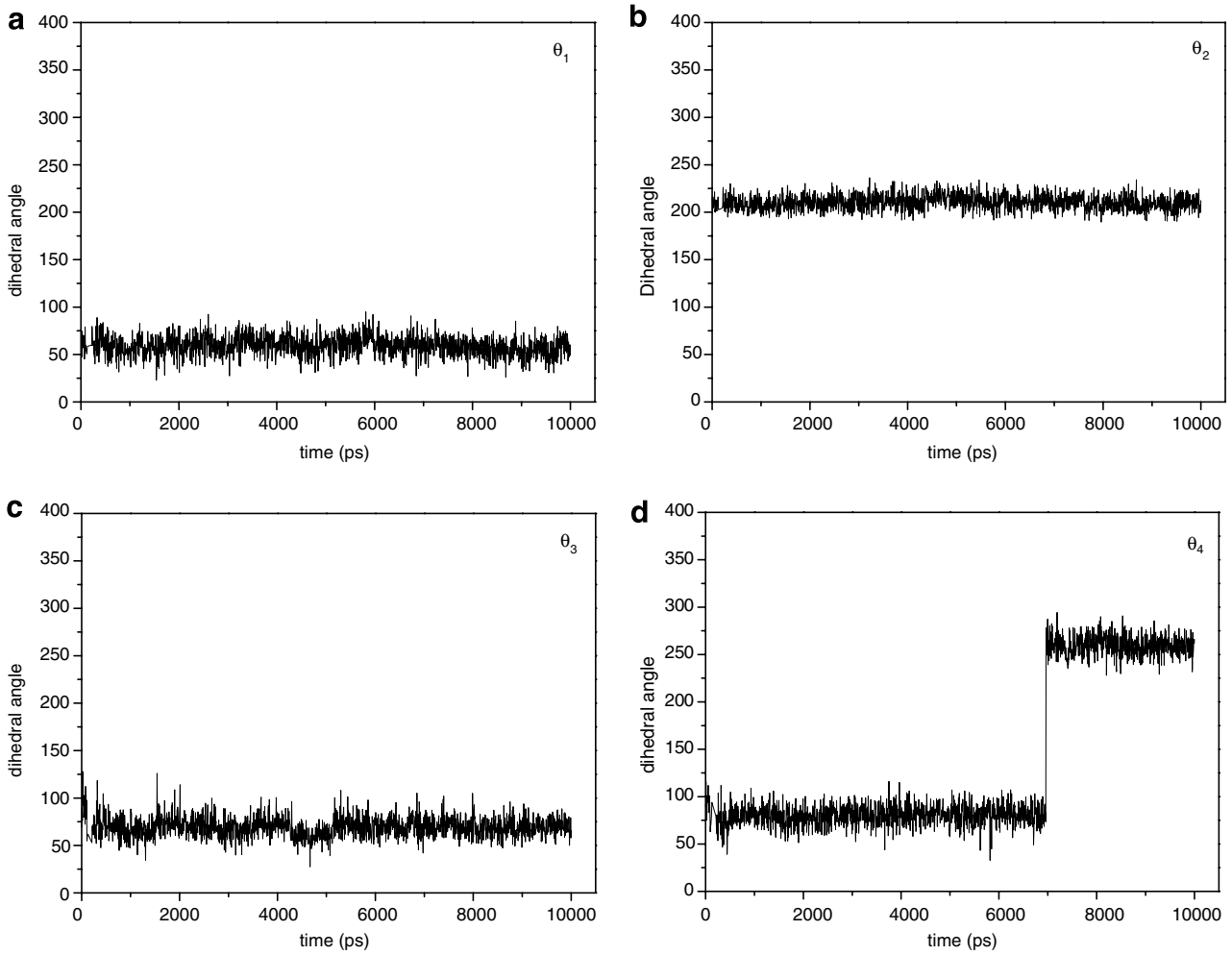


Figure 12. Evolution of the angles θ_1 (a), θ_2 (b), θ_3 (c), and θ_4 (d) of compound **4** with time.

117.21 (C_6-Indan); 121 (C_2-Aralkyl); 126.8 (C_7-Indan); 127, 128, 131, 142, 143, 145, 146 (qc). $C_{18}H_{22}BrNO_4$: 396.28. Anal. Calcd C, 54.56; H, 5.59; N, 3.54. Found: C, 54.93; H, 5.27; N, 3.59.

3.2. Biological activity evaluation

3.2.1. Behavioral study. Adult Sprague–Dawley rats (Centro Tecnológico, IVIC, Caracas), weighting 200–250 g, were housed under controlled conditions of temperature and light with free access to laboratory chow and water. An indwelling cannula was stereotaxically implanted, under pentobarbital anesthesia (40 mg/kg, ip), in the right lateral ventricle of each rat according to the following coordinates: 0.40 mm bregma, 1.1 mm lateral, 2.2 mm ventral. After three days of recovery from the surgical procedure, compounds **3** (0.6, 6.0 μg/5 μl) and **4** (50 μg/5 μl) were slowly infused into the lateral ventricle at a rate of 0.5 μl/min. Control rats were infused with vehicle, 0.9% NaCl. After 5 min of ICV infusion, animal behavior was monitored for 45 min. A group of rats pre-treated with compound **3** or vehicle were injected with apomorphine HCl (APO) (Sandoz S.A., Basel, Switzerland) (1 mg/kg, ip) one minute before the initiation of the behavioral observation. Animals were observed in transparent Plexiglas chambers ($32 \times 28 \times 28$ cm). Computer-assisted recording of the stereotyped (repetitive and purposeless) gnawing was carried out at 4.5 min interval for 45 min. Analysis of variance followed by Duncan's multiple range test was performed to determine a significant difference between means of each treatment group. Four rats were use for each measurement.

3.2.2. Renal action evaluation. Adult male Sprague–Dawley rats (220–280 g) were housed under controlled conditions of temperature and photoperiod (lights on from 06:00 to 18:00 h) and were provided with free access to laboratory chow and water. A cannula was implanted in the left lateral cerebroventricle, 1 mm caudal to the coronal suture and 1.5 mm lateral to the midsagittal suture, with the aid of a stereotaxic instrument and under pentobarbital anesthesia (40 mg/kg, ip). The cannula was secured to the skull with acrylic cement. A minimum of three days was allowed for recovery. Single ICV injections were made with a Hamilton syringe fitted with a stop to prevent needle penetration past the cannula tip.

The animals were randomly distributed into the following groups: V: control vehicle, receiving NaCl 0.9% (5 or 10 μl); DA: dopamine (100 μg/5 μl, ICV);

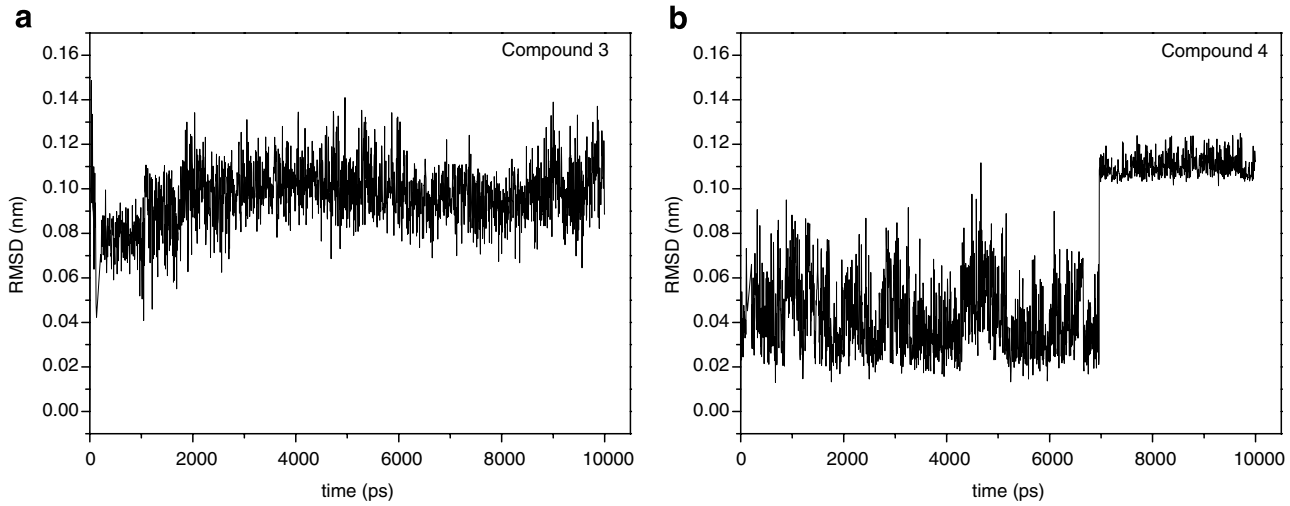


Figure 13. The root mean square deviation (rmsd) of both complexes during the 10 ns of MD simulation. (a) Compound **3**/D_2 DAR complex and (b) compound **4**/D_2DAR complex; in this case a conformational change might be observed around 7 ns of simulation.

C3: compound **3** (50 μg/5 μl, ICV); DA + C3 (15 min before DA), and C4: compound **4** (50 μg/10 μl, ICV). $N = 6–8$ animals per group. All the rats were weighed, orally loaded with 20 ml/kg of water, and than placed in individual metabolic cages. Urine was collected at 3 and 6 h; the bladder was emptied at 6 h by gentle suprapubic massage. Food and water were not available during the experiment. Ventricular cannula placement was confirmed postmortem by examining the distribution of an ICV injection of 5 μl of fast green dye, given before animal sacrifice. Data were used only if the dye was distributed in the lateral, third, and forth ventricles. Urine samples were assayed for sodium and potassium content by flame photometry. All data are presented as means ± SEM. Statistical differences between groups were analyzed using two-way analysis of variance (ANOVA) and by the Newman–Keul's Student range statistics.

The procedures used in these experiments were reviewed and approved by the Animal Care and Use Committee of The Central University of Venezuela, School of Pharmacy, Caracas, and Instituto de Investigaciones Clínicas. Dr. Américo Negrette, Facultad de Medicina. Universidad del Zulia.

3.3. Molecular dynamics simulations

A 3D model of the human dopamine D_2 receptor was used for the Molecular Dynamics (MD) simulations, based on the X-ray structure of bacteriorhodopsin[14] (PDB Acquisition Code: 1I15). The ligands' topologies were built using the Dundee PRODRG[45] server. For this purpose we used the previously optimized geometry at RHF/6-31G(d) level of theory of the global minimum of each ligand. In the present study, we have used an approach where manual docking was guided by information from site-directed mutagenesis and short docking simulations with both the receptor and the ligand free to move. Structurally similar parts of the ligands were oriented in similar positions in the receptor model which was described by Mansour et al.[36] Thus, these receptor–ligand complexes were prepared in order to obtain the input files for MD runs. Several docking positions were considered and the strongest receptor interactions were examined in detail.

The MD simulations and analysis are performed using the GROMACS 3.2.1 simulation package[15,16] and the GROMACS[46–50] united-atoms force field (FF) and the rigid SPC water model[51,52] in a cubic box with periodic boundary conditions. The receptor–ligand complexes were embedded in a box containing the SPC water model that extended to at least 1 nm between the receptor and the edge of the box resulting in a box of 7.17 nm of side lengths. The total number of water molecules was 11,030 for both simulations. Then three Na^+ ions were added to the systems by replacing water in random positions, thus making the whole system neutral. The time step for the simulations was 0.001 ps. for a complete simulation time of 10 ns. For long-ranged interactions the article-mesh Ewald (PME)[53–55] method was used with a 1 nm cut-off and a Fourier spacing of 0.12 nm. The MD protocol consisted of several preparatory steps; energy minimization using the conjugate gradient model,[56,57] density stabilization (NPT conditions), and finally production of the MD simulation trajectory. All production simulations are performed under NVT conditions at 310 K, using Berendsen's coupling algorithm[58] for keeping the temperature constant. The compressibility was 4.8×10^{-5} bar^{-1}. All coordinates are saved every 5 ps. The SETTLE[49] algorithm is used to keep water molecules rigid. Also the LINCS[59] algorithm was used to constrain all C_α atom position for the receptor in order to avoid desfolding problems.

3.4. Quantum mechanicals calculations

All calculations reported here were carried out using the Gaussian 03 program.[60] Geometrical optimizations for the low-energy forms of RR and RS isomeric forms of **3** and **4** were performed using B3LYP/6-31G(d)[61] calculations.

Optical rotation $[\alpha]_D$ for compounds **3** and **4** was calculated using the OptRot option[35] from Gaussian 03 program. This is a frequency-dependent calculation (CPHF = RdFreq) included in the route section. RHF/6-31G(d) and the B3LYP functional[62] with the 6-311++G(2d,2p)[63] base set have been used in these calculations. For the $[\alpha]_D$ calculations, the previously obtained B3LYP/6-31G(d) geometries were used.

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The author (Angel-Guío, JE) wishes to dedicate this manuscript to memory of his mother, Mrs. Ligia Guío de Angel (†).
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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.12.027.

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