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Molecular recognition and binding mechanism of *N*-alkyl-benzyltetrahydroisoquinolines to the D_1 dopamine receptor. A computational approach

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Abstract

In order to better understand, at sub-molecular level, the minimal structural requirements for the recognition process in the inhibitory activity, a series of *N*-alkyl-benzyltethrahydroisoquinolines (BTHIQs) were examined as Dopamine D_1 receptor antagonist variants. According to the cardinal role of the electrostatic factors during this interaction, ab initio and density functional theory (DFT) calculations were performed for a better understanding of the recognition process at the sub-molecular level. RHF/3-21G, RHF/6-31G(d) and B3LYP/6-31G++(d,p) in the gas phase, plus DFT calculations using the IPCM solvation model were carried out for all the complexes. We simulate the electronic interactions between BTHIQs with its biological receptor in terms of smaller molecules. $H_3C-COOH$ was used to mimic the side chain of Aspartic acid and CH_3OH mimicked the side chain of Serine; alternative moieties present on the BTHIQ derivatives were used as the different partenaires. Using the above mentioned computational model, we are able to interpret the basic behaviours and predict some additional features of BTHIQ–Dopamine D_1 receptor interaction.

Keywords: Ab initio and density functional theory calculations; Binding mechanism of *N*-alkyl-benzyltethrahydroisoquinolines; D_1-Dopamine receptor; Interactions including solvent effect

1. Introduction

The application of gene cloning techniques has allowed the identification of five dopamine receptor subtypes which can be classified into two classes: D_1-like dopamine receptors (D_1 and D_5) and D_2-like dopamine receptors (D_2, D_3 and D_4) [1,2]. The D_2-like dopamine receptors show high affinities for drugs (antagonist) used for the treatment of schizophrenia (antipsychotics) and those (agonist) used in the treatment of Parkinson's disease [1]. However, at present little is understood concerning the physiological significance of this receptor subtype multiplicity. Future research endeavours should lead to better understanding of these receptor subtypes and eventually to new drugs with more specific actions.

Previously we described the synthesis of (R)-norroefractine [3] a monophenolic unmethylated 1-benzyl-1,2,3,4-tetrahydroisoquinoline (BTHIQ). We

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have also accomplished the synthesis of racemic monophenolic *N*-alkyl-BTHIQs by a new method incorporating a 'one-pot' cyclization—reduction—alkylation sequence [4]. All those compounds were reported to bind to D_1 dopamine receptor (D_1-d-r) [3,4]. In addition an enantioselective synthesis of dopaminergic (R) and (S) benzyltetrahydroisoquinolines was previously reported [5]. However, in those papers the mechanism of action of BTHIQs at molecular level was not taken into account.

Although there are currently available interesting data about the general characteristics of the D_1-d-r, the molecular mechanism by which dopamine binding to the receptor induces G protein is still unknown. Thus, deduction of the characteristics of the D_1-d-r ligands of relatively small molecular dimensions (i.e. BTHIQs) should be a fruitful addition to the current knowledge of the D_1-d-r. Such a chemical approach to the molecular pharmacology of those compounds could give us a more detailed structural profile for the D_1-d-r which has not yet been entirely elucidated. While helpful empirical rules and correlations are emerging from in vitro activity studies, this is obviously a very complex phenomenon which will undoubtedly require the joint effort of many disciplines to be elucidated (including of course computational approach).

As it was said before, the mechanism by which dopamine bound to the receptor induces G protein activity is unknown but it most likely involves a cascade of intramolecular reactions. In particular, charged and conserved amino acid residues found in transmembrane domains should participate in dopamine recognition.

The molecular recognition and the binding mechanism of dopamine D_1 ligand into its biological receptors might be subdivided into two major steps (A,B) followed by a third phase (C) frequently referred to as an internal interaction:

A: Long range interaction (electrostatic preorientation).
B: Short-range interaction (electronic attachment followed by a 'fine-tune' via aromatic ring orientation) of the BTHIQs compounds.
C: Internal interaction (include fit, conformational readjustment of the protein molecules).

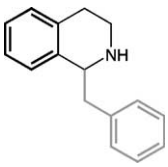
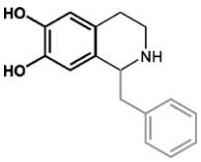
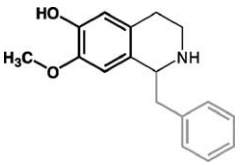
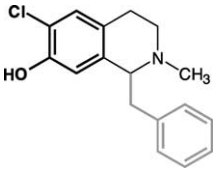
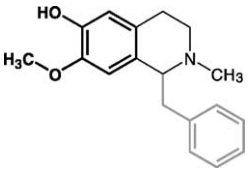
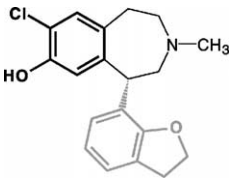
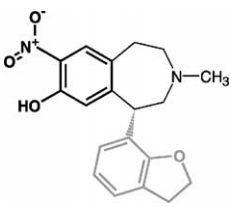
It is assumed that the driving force in the early preorientational phase (A) is electrostatic. This is due to the attraction between Asp 103 in transmembrane III and two Ser (199 and 202) in transmembrane domain *V* both of which could interact with the amine and phenolic hydroxyl groups of dopamine, respectively, [6]. This model was tested experimentally and proven valid although the two serine residues in transmembrane domain *V* differentially affect agonist binding [6].

In the present theoretical study, which is exploratory in its nature, we have focused our attention in the first A and B phases. The scope of the present paper does not include the final C binding phase.

Molecular recognition and the converse concept of specificity [7] are explained in mechanistic and reductionistic terms by a stereoelectronic complementarity between the binding molecule and the receptor [8]. In this context it is obvious that the knowledge of the stereoelectronic attributes and properties of BTHIQs compounds will contribute significantly to the elucidation of the mechanism of action at molecular level.

Molecular interactions between molecules are determined fundamentally by molecular size and shape and charge distribution, but when one inspects molecules in these terms one becomes aware that they rarely have a unique description and there may be several different forms or species in equilibrium. In this case an interesting question arises: if one changes drug structure to alter the equilibrium, can one relate the consequences to changes in biological activity? If one can, one may obtain some insight into the mechanism of drug action and provide a method for drug design. The complete molecular structure of the D_1-d-r is not known. How then can theoretical calculations contribute in this area? One way is to seek explanations of differences. We cannot study the absolute, but we can make comparisons, so we will study no single molecular species, but series of chemically closely related compounds and seek explanations of differences between members of an apparently homogeneous series (see Table 1), these may be correlated with differences in the biological activities. Observing the structures and biological activities of representative dopamine D_1 receptor antagonists it is possible to draw a general structural picture for these

Table 1
Structures and biological activities (IC_{50}) of representative BTHIQ compounds acting as dopamine D_1 receptor inhibitors

Structure	IC_{50} (μ/mol)
	248.80 [9]
	9.27 [9]
	23.22
	5.75×10^{-2} [10]
	1.84
	5.80×10^{-3} [11]
	1.80×10^{-4} [11]

compounds. Among the salient molecular features common are:

(a) At least one polar group at C_7; specifically a OH group on the benzene ring A at C_7 position is present in all potent compounds.
(b) A basic, usually tertiary nitrogen group which is part of a piperidine (or equivalent) ring.
(c) At least one aromatic ring, usually a benzyl group is attached to C_1.

The main molecular features apparently required for dopamine D_1-receptor inhibitory activity have led to a schematic representation of the pharmacophoric patron, as shown in Fig. 2.

In the present exploratory theoretical study, we simulate the electrostatic interactions between BTHIQ derivatives with its biological receptor in terms of smaller model molecules. With the knowledge of the cardinal role of the electrostatic factors in the molecular interactions, ab initio and density functional theory (DFT) calculations were performed for a better understanding of the recognition process at the sub-molecular level. Thus, we report here the results of a study of BTHIQs with emphasis on the parameters for the construction of a more complete dopamine D_1 receptor model: such a model would provide the basis for comparison of various antagonists producing the same response, and for an exploration of the effect that the molecular enviroment in the receptor could have on such a process.

2. Methods

All the calculations reported here were carried out using the GAUSSIAN 98 program [12].

For the molecular interaction (MI) simulations, all the complexes under investigation were initially optimised using RHF/6-31G(d,p) level of theory. Correlation effects were included using DFT with the Becke 3-Lee-Yang-Parr (B3LYP) [13] functional and the 6-31++G(d,p) basis set for all the complexes obtained at the lower level of computation. During the DFT calculations, the RHF/6-31G(d,p) geometries were kept fixed.

While gas phase predictions are appropriate for many purposes, they are inadequate for describing

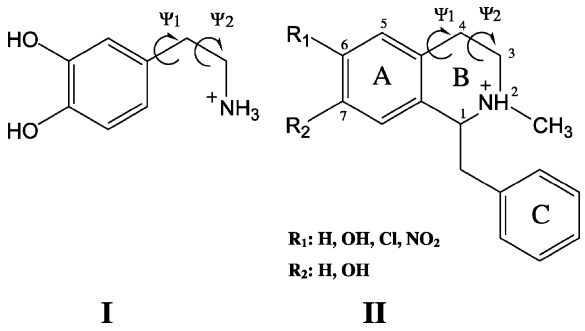


Fig. 1. General structural feature of dopamine and BTHIQs. Definition of the spatial orientation of atomic nuclei used for molecule including the numeric definition of relevant dihedral angles.

the characteristic of polar molecules in solution. Electrostatic effects are often much less important for species placed in a solvent with a high dielectric constant than they are in the gas phase. Recently we have demonstrated the importance of including solvation energies in studying the relative binding free energies of ligand–receptor interactions [14]. Therefore, we have performed DFT (B3LYP/6-31++G(d,p)) calculations using the IPCM solvation model with explicit inclusion of solvent environment to gain deeper insight on the effect of the different molecular interactions reported here. Isodensity polarized continuum model (IPCM) defines the cavity as an isodensity surface of the molecule [15]. It should be emphasised, however, that the evaluation of the solvent effect implies a comparison to the gas phase results. Thus both sets of results (without and with the inclusion of the solvent) are required.

The conformational study of dopamine and its D_1 receptor–antagonists was performed from ab initio and DFT calculations. Rotational energy profiles around torsional angles have been determined by using RHF/3-21G, RHF/6-31G(d) and B3LYP/6-31G(d) calculations. The energy has been calculated at 30° intervals of the dihedral angles.

The electronic study of the compounds was carried out by using molecular electrostatic potentials (MEPs). MEPs have been shown to provide reliable information, both on the interaction sites of molecules with point charges and on the comparative reactivities of these sites [16–19]. These MEPs were calculated by using RHF/6-31 + G(d) wave function from the SPARTAN program [20]. DFT/6-31G(d) optimised coordinates were imported into PC SPARTAN. To generate the wave functions, HF/6-31 + G(d) single point calculations were performed from PC SPARTAN.

3. Results and discussion

3.1. Stereo-electronic complementarity between dopamine and BTHIQs

The essential conformational problem in dopamine concerns the overall orientation of the side chain with respect to the conjugated ring and is described by the two principal torsion angles ψ_1 and ψ_2 (Fig. 1). The first of these torsion angles defines the position of the plane of the side chain with respect to the plane of the ring (coplanar or perpendicular) and the second, the orientation of the polar head with respect to the ring (*trans* or *gauche*). Dopamine may be considered as a 1,2-disubstituted ethane, therefore they will have *gauche* (*syn*-clinal) and *trans* (*anti*-periplanar) forms, but, where does one form change to the other and how far from an energy minimum? It is clear, therefore, that the information about local and global minima of dopamine is not enough. We need to have at least a good notion of the shape and also some indication about the dynamic behaviour of the internal degree of freedom of dopamine. Probably, the most comprehensive computational method to answer the above questions is to evaluate the potential energy surface (PES) of two independent variables (ψ_1 and ψ_2), associated with dopamine in Fig. 1. Fig. 3 shows the PES obtained for dopamine in the cationic form. In this surface the values obtained for

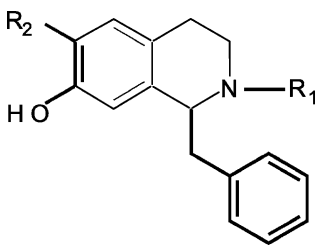


Fig. 2. Pharmacophoric patron of BTHIQ derivatives acting as dopamine-D_1-receptor inhibitors. Schematic representation; the salient structural features are denoted in bold.

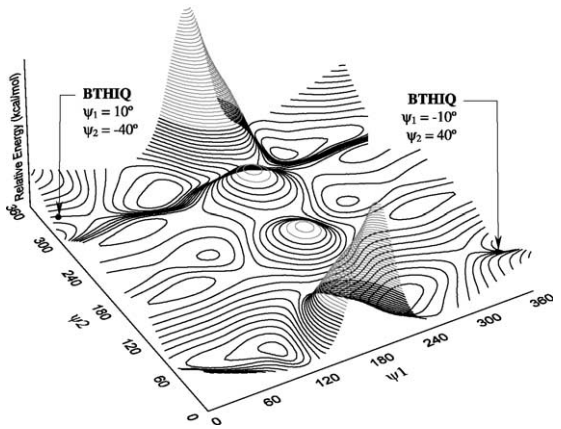


Fig. 3. Conformational PES obtained for dopamine in its cationic form from RHF/3-21G calculations. Full cycle of rotation (from 0 to 360°) is shown for variables ψ_1 and ψ_2.

the torsional angles ψ_1 and ψ_2 of compound II (Fig. 1), a representative BTHIQ molecule have been included for comparison. In Fig. 3 there are six clearly defined potential wells corresponding to regions close to the expected six (ψ_1, ψ_2) rotamers of dopamine. This surface clearly indicates that the g^+ and g^- minima of ψ_2 are noticeably shifting, in the opposite directions, with respect to the *anti* conformer.

Because the 3-21G basis set is a small one, we have attempted a partial verification of the above results by using a more extended basis set (RHF/6-31G(d)) and DFT calculations. For economic reasons we did not recalculate the whole conformational energy surface but only the essential parts of it. Thus, we have calculated the energies of the perpendicular and antiperpendicular conformers by rotating the ring (varying ψ_1 as the side chain is fixed with $\psi_2 = 180°$ (Fig. 4a). In turn, we have evaluated the g^+, *a* and g^- conformers by rotating the side chain (varying ψ_2) as the aromatic ring is fixed with $\psi_1 = 90°$ (Fig. 4b). The *gauche* effect (i.e. *gauche* is more stable than *anti*) might be appreciated in this curve.

The barrier of ψ_1 is about 4.3 and 5.0 kcal/mol at DFT and RHF/6-31G(d) levels, respectively (Fig. 4a); while the barrier of ψ_2 is about 8 and 7.6 kcal/mol at DFT and RHF/6-31G(d) levels, respectively (Fig. 4b). Thus, DFT calculations suggest that dopamine possesses a moderate but significant molecular flexibility. Our results are in good agreement with those previously reported for a protonated 2-phenyl-ethyl-amine [21]. However, for dopamine the g^+ and g^- conformers are not equivalent; this is a striking difference which might be attributed to the presence of the OH groups in the aromatic ring.

It is interesting to note that compound **II** displays the ψ_1 and ψ_2 torsional angles very close to those obtained for the folded conformations of dopamine. Accepting the validity of the DFT calculations and of the obtained results, dopamine seems to possess a significant molecular flexibility, therefore it is reasonable to think that BTHIQs could mimetize the spatial ordering of dopamine in its folded conformation or in a closely related form.

Once the preferred conformations of compounds **I** and **II** were obtained, in an attempt to find

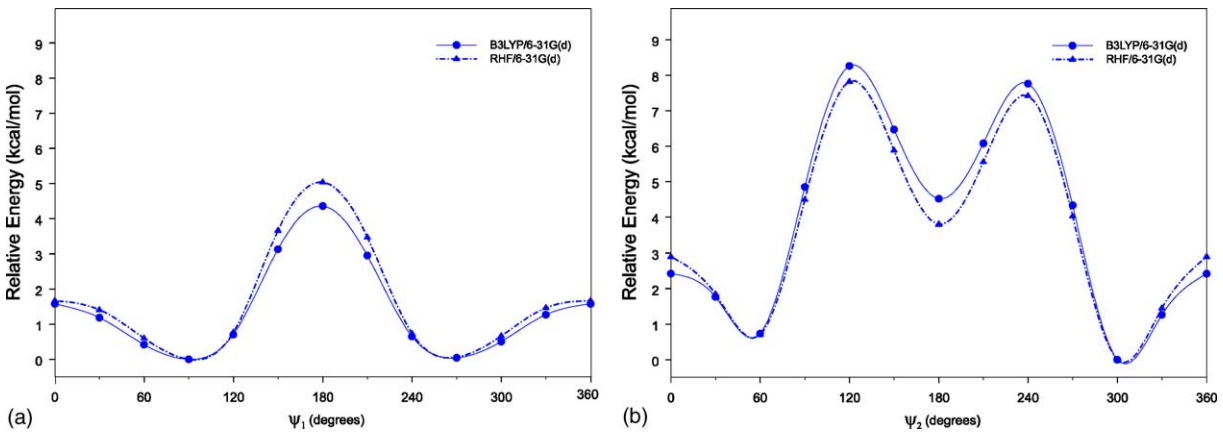


Fig. 4. Rotational energy barrier profiles computed at the RHF/6-31G(d) and DFT levels of theory obtained for dopamine (**I**).

the potentially reactive sites for dopamine (**I**) and compound (**II**), we have evaluated the electronic aspects of the molecules using MEPs. MEPs are of particular value because they permit visualisation and assessment of the capacity of a molecule to interact electrostatically with a binding site. MEPs can be interpreted in terms of a stereoelectronic pharmacophore condensing all available information on the electrostatic forces underlying affinity and specificity.

Fig. 5 shows the MEPs obtained for compounds **I** and **II**. This figure indicates that the MEP of dopamine in a folded conformation shows a remarkable similarity with that obtained for compound **II**. The close electronic similarity between dopamine and BTHIQ derivatives could account for their common affinity for the D_1-d-r. This assumption is supported by a qualitative comparison of the isopotential maps obtained through the ab initio computer modeling of dopamine and several BTHIQ structures (results not shown).

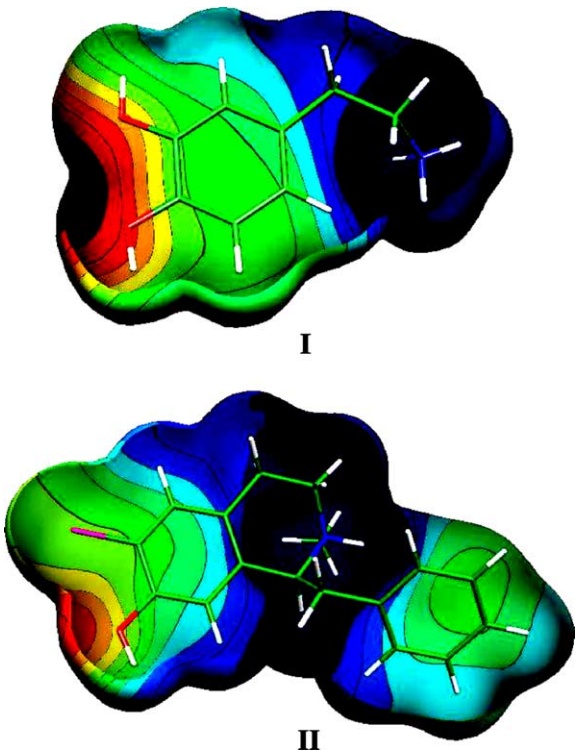


Fig. 5. MEP energy isosurfaces for compounds **I** and **II** obtained from RHF/6-31G(d,p) calculations.

The analyses of MEPs showed two characteristic regions, one with negative potential and another with positive potential. The negative region is generated by the presence of the OH group at C_7; while the positive region corresponds to the large positive potential around the nitrogen cationic head. In the case of BTHIQs, the electrostatic potential surrounding the aromatic ring C itself does not appear to suggest any characteristic electrostatic interaction (or at least any strong interaction). Therefore, the role of this fragment appears to be one of a 'topological marker' for the proper orientation of the entire molecule at the receptor site allowing optimum interaction of polar substituents with the receptor. Another possibility, however, is that the ring C itself contributes its own interaction with the receptor site through dispersion forces. This problem will be discussed in Section 3.2.3.

3.2. Binding mechanism of BTHIQs to the D_1 dopamine receptors

Reduced model. Several model compounds were used to mimic BTHIQs and related molecules, and to enable quantum mechanical (QM) molecular orbital (MO) calculations. The use of model compounds to calculate MEPs and simulate MI is necessary since BTHIQs and the putative active sites of the D_1-d-r are too large for accurate QM MO calculations and the number of ligand-models to be screened is large. Moreover a model compound representing the modified active BTHIQ moiety may be desirable in order to evaluate the ability of the ligands to interact with the dopamine D_1-receptor pocket. By using a model, one avoids dealing with complexities due to the rest of the BTHIQ molecule. Thus a better understanding of the inherent electronic properties of the active moieties of BTHIQs reflected in the MEPs and MI may be gained. When choosing a model compound, the ability to reproduce electronic properties of the entire molecule (i.e. BTHIQs) was considered. In this pilot study we mainly consider two variations on the BTHIQ structure: (i) our attention was focussed on changes in the phenolic OH at C_7 in BTHIQs with variation on substituents at the adjacent carbon (C_6) (Fig. 6A); (ii) variations which could significantly affect the nature of the potential around the basic nitrogen, for example

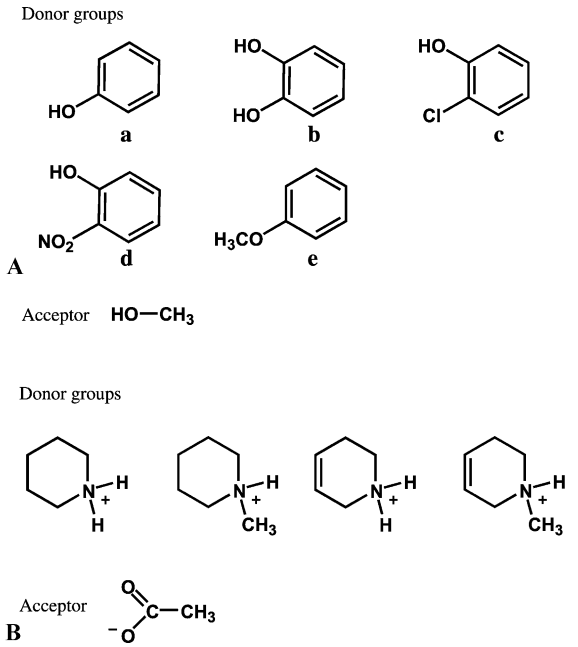


Fig. 6. Reduced models selected to mimic the reactive moieties of BTHIQs. CH_3OH and CH_3COOH were used to mimetize the side chain of Ser and Aspartic acid, respectively.

changes in the substituent on the nitrogen atom as well as in the flexibility of this ring (Fig. 6B).

Methylic alcohol (CH_3OH) was used to mimic the side chain of Serine. Similarly acetic acid (CH_3COOH) mimicked the side chain of Aspartic acid. Alternative moieties present on the BTHIQs (denoted in bold in Table 1 and shown isolated in Fig. 6) were used as the different partenaires (interacting counter parts).

3.2.1. Electrostatic attraction as the basis of preorientation

The preorientation is expected to occur before the ligand can actually bind to the D_1 receptor. The notion of preorientation is based on the idea that in molecular recognition atoms do not see atoms, but the electrostatic field of one of the molecules experiences the electrostatic fields of the other molecule.

Different model systems that mimic alternative moieties present on natural and synthetic BTHIQ molecules (displayed in Fig. 6) were investigated using the MEP approach in an effort to better understand the structural factors accounting for their biological activities. The electrostatic potential has long been applied as a guide to molecular reactive behaviour [22–24]; for instance, the most negative values of MEP were interpreted as identifying and ranking sites for electrophilic attack, while its overall pattern served as the basis for qualitative analyses of biological recognition interactions.

Fig. 7 shows the MEPs obtained for the reduced models a–d displayed in Fig. 6. It is interesting to note that the maximum values of MEP were obtained for the H atom of the phenolic OH indicating the acid character of this atom (the different values of $V(r)$ are denoted in this figure). The increased electron density at the H position and the result of the electrowithdrawing effect of neighboring chlorine and nitro groups might be also appreciated in this figure. Interestingly compounds possessing electrowithdrawing groups at the adjacent carbon (C_6) of the phenolic group displayed increased biological activity (see Table 1).

Fig. 8 shows the MEPs obtained for the reduced models displayed in Fig. 6B. These MEPs show the minimum values, $V(r)$ min, in the vicinity of the lone pair regions of N, which is in accordance with experimental findings, indicating that this heteroatom is H-bond acceptor. The value of the potential is slightly reduced by the presence of a CH_3 group. Moreover, although the presence of an unsaturated double bond introduce a conformational change, this situation does not introduce appreciable electronic changes.

3.2.2. Electronic attachment

In phase B there is a short range electrostatic interaction, which may result in Brönsted (H-bonded) complexes formation. These are achieved for the Asp 103 and Ser 199, or Ser 204, respectively. We simulate the electrostatic interactions between BTHIQs with the dopamine D_1 receptor in terms of small model molecules (Fig. 6).

The energies of interaction (EI) were calculated with the approximation neglecting the superimposition of error due to the difference between the total energies of the complex with the sum of the total energies of the components:

$$\mathrm{EI} = E_{\mathrm{Cx}} - (E_{\mathrm{BC}} - E_{\mathrm{AC}})$$

where EI is the energy interaction, E_{Cx} the complex energy, E_{BC} the energy of proton-donor component

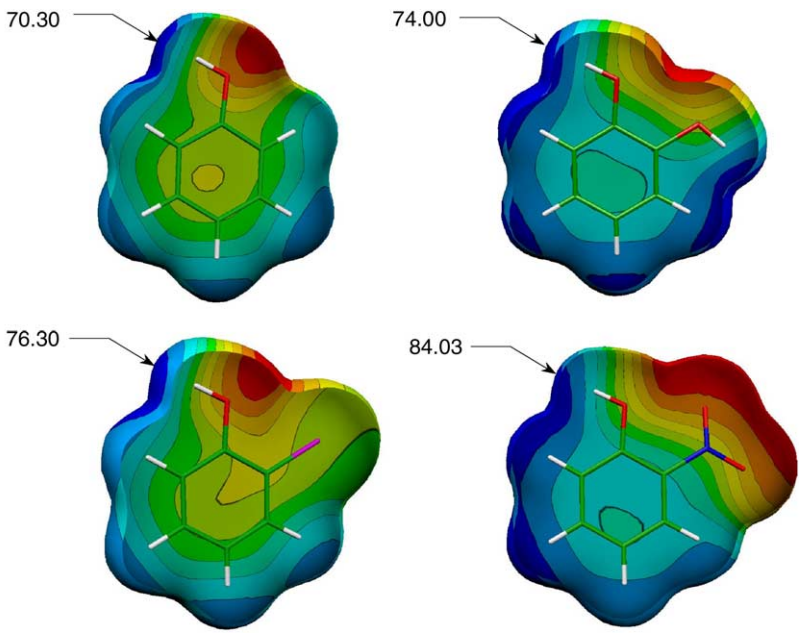


Fig. 7. MEP energy isosurfaces for the reduced models a–d obtained from RHF/6-31G(d,p) calculations.

(i.e. Brönsted acid), and E_{AC} the energy of proton acceptor component (i.e. Brönsted base).

The energies of all the complexes obtained and their components are listed in a summarized way in Table 2; while the interaction energies obtained for the different complexes are shown in Table 3.

Observing the results obtained for complexes **I**–**III** (Fig. 9a and Table 3) it is clear that the most favored interaction occurs when the CH_3–OH group is acting as acceptor while the phenol group is the donor counter parts (compare the energies obtained for complexes **I** and **III**. This result is in

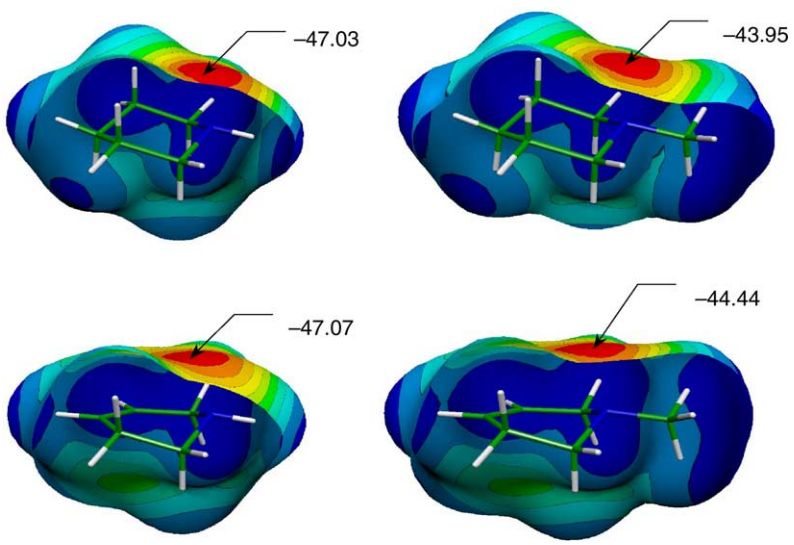


Fig. 8. MEP energy isosurfaces for the reduced models f–i obtained from RHF/6-31G(d,p) calculations.

Table 2
Energies (hartree) obtained at three levels of theory for the complexes and their components

Model compounds	RHF/6-31G**	B3LYP 6-31++G**	
		In vacuo	IPCM
Methanol	−115.046709586	−115.733802314	−115.741803387
a Phenol	−305.573755062	−307.491973865	−307.501889293
b Catechol	−380.426278834	−382.710935177	−382.731344906
c Chloro-phenol	−764.468134044	−767.082574279	−767.094633544
d Nitro-phenol	−509.032450713	−511.986385812	−512.005082855
e Methoxy-benzene	−344.596958913	−346.795250672	−346.800967537
Acetic acid	−227.822171555	−229.103261319	−229.114339899
f Piperidine	−250.207430902	−251.929175671	−251.933020445
g Methyl-piperidine	−289.238924648	−291.241193951	−291.243224610
h Tetrahydro-pyridine	−249.014553443	−250.69461484	−250.699661054
i Methyl-tetrahydro-pyridine	−288.046307074	−290.006808341	−290.010456523
Complexes			
I	−420.627769654	−423.232173937	−423.243579382
II	−459.650892148	−462.535433625	−462.544478111
III	−420.632071335	−423.237660588	−423.249594905
IV	−495.484534785	−498.456667964	−498.474832922
V	−879.527979234	−882.829761646	−882.839592958
VI	−624.094639926	−627.735623279	−627.751690807
VII	−478.045666056	−481.049638881	−481.061547964
VII	−517.076727520	−520.361214644	−520.368948179
IX	−476.852845990	−479.815234848	−479.828844265
X	−515.884181586	−519.126868410	−519.136488717

accordance with our chemical intuition; after all the proton in a phenol group is more acidic than the proton of an alcohol. The replacement of an hydroxyl by a metoxy group gives a decreased interaction (compare the results obtained for complexes **II** and **III**). These results could explain the lack of activity reported for several natural products possessing metoxy substituents at C_7 [25].

On the other hand the results obtained for complexes **IV**–**VI** (Fig. 9b) indicate that the interaction energies obtained for complexes **V** and **VI** are favored over the rest of the complexes. These results illustrate very well the electrowithdrawing effect of chlorine and nitro group at the neighboring C_6. These results are in agreement with experimental data reporting that compounds possessing chlorine and nitro groups at C_6 were the most active in their respective series [9–11].

Fig. 10 gives the geometries obtained for the **VII**–**X** complexes. Starting geometries converged to the same kind of hydrogen–bonding interactions for such complexes, independent of the level of theory used. It is interesting to note that these complexes are energetically favored with respect to the complexes **I**–**VI**. These results could indicate that the $>NH^+$–CH_3/Asp interactions would be the driving force for the BTHIQ/D_1-d-r complex.

Table 3
Interaction energies (kcal/mol) obtained for the different complexes

Complexes	RHF/6-31G**	B3LYP 6-31++G**	
		In vacuo	IPCM
I	−4.584	−4.015	0.071
II	−4.533	−4.004	−1.071
III	−7.283	−7.458	−3.704
IV	−7.245	−7.486	−1.057
V	−8.243	−8.399	−1.980
VI	−9.714	−9.686	−3.015
VII	−10.080	−10.794	−8.903
VII	−9.781	−10.517	−7.143
IX	−10.116	−10.893	−9.314
X	−9.854	−10.541	−7.337

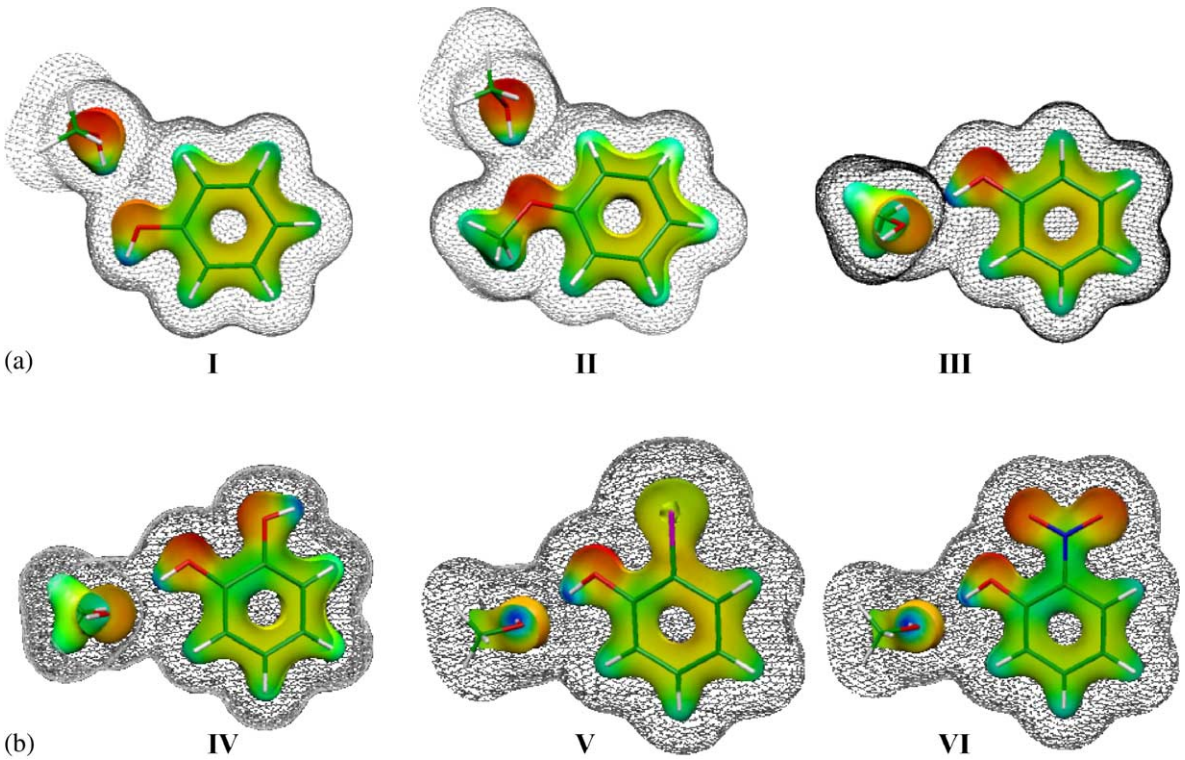


Fig. 9. (a) Spatial view of complexes **I**–**III** obtained from RHF/6-31G(d,p) calculations. The interactions are shown as insolvent surfaces mesh of electronic densities. (b) Spatial view of complexes **VI**–**VI** obtained from RHF/6-31G(d,p) calculations. The interactions are shown as involvent surfaces mesh of electronic densities.

The MI energy of complexes **VII**–**X** was found to change significantly in function of the spatial orientation of the lone pairs of nitrogen atom; being the complexes with the lone pair in axial position the preferred forms. In other words it appears that the complex geometry in this case is highly dependent on the molecular interaction.

3.2.3. The benzyl-group (CH_2–Ph) of BTHIQs (aromatic ring orientation)

To obtain a clear profile of the overall recognition process, it is necessary to underline the role of the benzyl-group (CH_2–Ph), which could play a determinant role in the BTHIQs structure to adopt the proper orientation allowing optimum interactions of ligand with the receptor.

The conformational intricacies of benzyl-group of BTHIQs is itself very complex and therefore it was evaluated in a separate paper. Thus, an exhaustive conformational study of this flexible moiety of BTHIQs is reported in the companion paper [26]. We take the main conclusions of such study in order to determine the structural role of the benzyl-group in the binding mechanism of BTHIQs.

RHF/6-31G(d,p) indicate [26] that the flexible side chain of BTHIQs display a moderate molecular flexibility; being the extended *trans* conformation the preferred form for this moiety. With respect to the conformational behavior of the ring B of BTHIQs, the energy minima having energies below 2 kcal/mol are separated by a transition barriers of approximately 10.25 kcal/mol and hence, the molecule cannot be expected to move easily from one conformation to the other by gaining this energy from other favorable interactions. In contrast phenyl derivatives display a major molecular flexibility. Thus, our results [26] indicate that the different substituents at C_1 in BTHIQ molecules introduce a significant steric hindrance

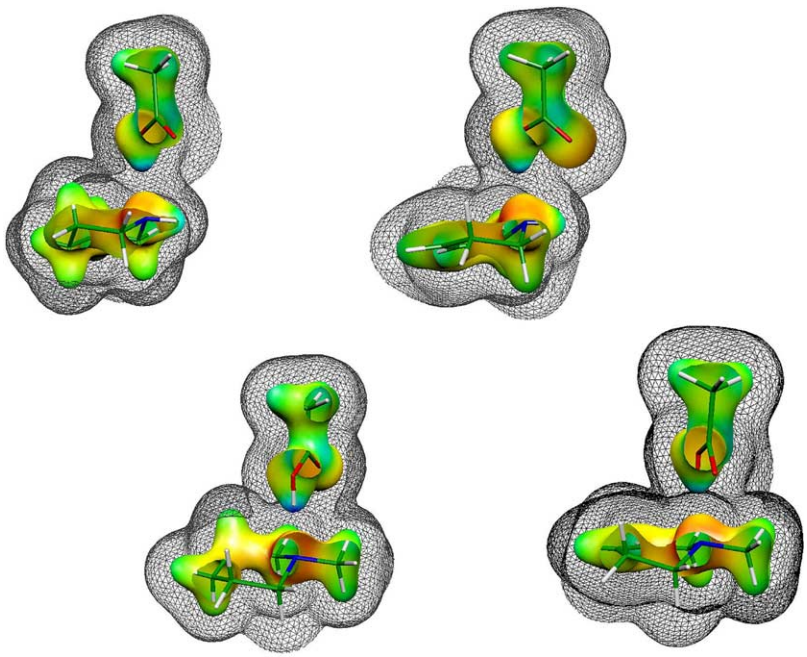


Fig. 10. Spatial view of complexes **VII**–**X** obtained from RHF/6-31G(d,p) calculations. The interactions are shown as involvent surfaces mesh of electronic densities.

which might, in turn, be responsible for a conformational restriction determining the spatial orientation of the lone pairs of N atom favoring or not the electronic attachment with the side chain of Asp residue. Although the above concepts were formulated independently, it seems that they are interdependent. However, it is clear that on the basis of our calculations it is not possible to discard the possibility of a third binding site which could take place through dispersion forces. Further theoretical and experimental works testing conformationally constrained analogues are necessary to confirm our results.

3.3. A molecular model for the binding mechanism of BTHIQs

Finally we wish to discuss some details about a putative overall recognition process between the BTHIQs molecules and the Dopamine D_1 receptor.

The main molecular features apparently required for the inhibitory activity and the observed effects of changes in structure on activity have led to a reduced representation of the receptor, as shown in Fig. 11, where the two major complementary features of the receptor site have been inferred. In order to obtain this complex we simulate both interactions (SITES I and II) simultaneously.

Before a ligand can bind, there must be a relatively rigid complementary crevice inside the receptor that complements the ligand in shape in order to be able to accommodate it. Since complementary is rarely

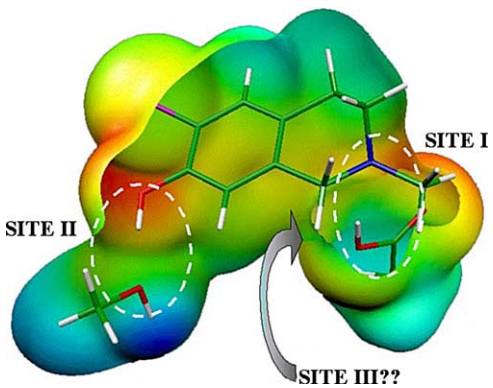


Fig. 11. Schematic representation of a reduced model for the dopamine-D_1-receptor, showing the putative binding sites.

perfect, more than one ligand can fit in the same crevice. Depending on the detailed mechanism of the binding process, the structural characteristics of the small ligand can assume varying degrees of importance. At least, two general cases can be distinguished: (a) one possibility is that binding take place by a stepwise process in which an initial interaction between a single segment of the ligand (in any conformation) and its subsite is followed by a rapid rearrangement of the conformation of the small molecule so as to permit the binding of the remaining segments to their subsites; (b) another possibility is that at any instant, only those molecules having the appropriate single 'biologically relevant' conformation are able to bind to the receptor; each portion of the ligand binds simultaneously to the appropriate subsite of the binding site.

In fact, there are various ways in which BTHIQs may be involved but it is not yet possible to do more than speculate. Accepting the validity of our theoretical calculations, it seems that an intermediate model sharing aspects of both extreme situations (models (a) and (b)) is in particular probable in the case of BTHIQs.

On the basis of our results and using the simple notion of receptor-site occupancy, one may seek chemical features common to dopamine and its competitive antagonist to suggest chemical binding sites. Thus, one may imagine that the amine group of BTHIQ engages the receptor at a specific site (SITE I, Fig. 11). We propose this interaction as the driving one because our results indicate that the molecular interaction between $>NH^{+}-CH_3$ and Asp 103 would become the major component of the recognition process. In addition the conformational restriction observed in the ring B of BTHIQs appears to play a determinant role on this interaction.

If isoquinoleinic nitrogen engages the receptor at the site which would otherwise accommodate the $-NH_3^{+}$ of dopamine, then one may envisage that the rest of the BTHIQ molecule contributes additional binding by interacting with at least one accessory region. The molecular structure of dopamine D_1 receptor antagonists appears to be quite critical for activity. The fact that activity is markedly affected by altering the substituents at C_6 and C_7 suggests a co-operative effect between active groups, and one may consider that the HO at C_7 makes a specific contribution to binding. It should be noted that the molecular interaction at the SITE II is not restricted to a determinate spatial orientation of the OH group; and therefore it is reasonable to think that a rapid arrangement of this portion could takes place. Thus a kind of stepwise binding involving first the (SITE I) followed by the rest of the molecule (SITE II) seems a reasonable possibility and there is some strongly suggestive evidence that this phenomenon takes place. There is not a single and particular substituent at C_1 but different hydrophobic groups which seem to be well tolerated to produce the biological response. It is clear, however, that not any conformation is operative. This is an additional support for an intermediate model. On the basis of our results it is not prudent to discard the possibility of a third binding site, for example a hydrophobic interaction due to the aromatic ring C, and this is still an open question. The proposed molecular model must therefore be refined and tested with the use of molecules known to act as agonists, partial agonists and antagonists on the dopamine D_1-receptor, in order to produce a realistic scale of energies that would be predictive for the activity of other untested compounds. The ability to explain new antagonists structurally related to the BTHIQs reported here on this basis is currently being explored.

4. Conclusions

To better understand how BTHIQs interact with dopamine D_1 receptors, the conformational, electrostatic and physicochemical properties of these agents have been studied using ab initio and DFT calculations.

A putative binding mechanism of BTHIQ and its mimetics to dopamine D_1 receptor has been prepared on the theoretical calculation grounds. The results of calculations presented above show that the molecular mechanism postulated for the recognition process of the dopamine D_1-receptor is energetically feasible. To date, the general picture is that both $>NH^{+}-CH_3$ and HO at C_7 groups (SITES I and II, respectively) play a key role in the molecular recognition process. Such process is facilitated by the presence of an electrowithdrawing group placed near to the HO at C_7. In addition an appropriate ring orientation of

the aromatic ring C of BTHIQs may be operative stabilizing this process.

We were able to explain, on the basis of a combined computational model, several structural characteristics of BTHIQ–D_1-d-r complexes. With this approach we could interpret the conformational and electrostatic factors of BTHIQs, in spite of the missing 3D structure of dopamine D_1 receptor. Our preliminary model might serve as a good basis for rational drug design of the dopamine D_1 binding inhibitors. In this sense, theoretical calculations appear to be of great use for the evaluation of molecular interaction in the molecular recognition process of BTHIQs and its congeners. However, it must be pointed out that high level theory calculations and the inclusion of solvent effects are crucial in order to obtain satisfactory accuracy in the electronic distributions of these compounds. This information is essential to the understanding of the structure-inhibitory activity of BTHIQs congeners from a medicinal chemistry point of view.

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