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ORIGINAL PAPER

Using a pharmacophore representation concept to elucidate molecular similarity of dopamine antagonists

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Abstract The pharmacophoric concept plays an important role in ligand-based drug design methods to describe the similarity and diversity of molecules, and could also be exploited as a molecular representation scheme. A three-point pharmacophore method was used as a molecular representation perception. This procedure was implemented for dopamine antagonists of the D_2 receptor subtype. The molecular structures of the antagonists included in this analysis were categorized into two structurally distinct classes. Using structural superposition with internal energy minimization, two pharmacophore models were deduced. Based on these two models other D_2 antagonists that fulfil them were derived and studied. This procedure aided the identification of the common 3D patterns present in diverse molecules that act at the same biological target and the extraction of a common molecular framework for the two structural classes. The pharmacophoric information was found to be suitable for guiding superposition of structurally diverse molecules, using a more biologically meaningful selection of the targeting points.

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Keywords Dopamine antagonists · Molecular informatics · Molecular similarity · Pharmacophore generation · Structural superposition

Introduction

A major area of interest in computational drug research is the design of small organic molecules with high affinity of binding towards a given protein receptor.

In the absence of a receptor's 3D structure, drug discovery and design efforts often rely on ligand-based techniques, such as pharmacophore development and QSAR (quantitative structure—activity relationships) studies [1].

The ligand-based or the active analogue approach depends on models inferred from different known ligands at best together with their measured biological activities towards the receptor. The key idea is 'molecular mimicry', which states that structurally similar compounds are more likely to exhibit similar properties [1, 2].

The concept of chemical structure similarity is widely used but poorly defined, with many divergent approaches having been proposed. Similarity and diversity of molecules depend on their 3D chemical structure and their biological activity [2–4]. Molecular diversity and similarity analysis can be accomplished through pharmacophore generation.

Pharmacophore modelling assumes that a set of chemical features in a molecule is recognized at a receptor site and is responsible for the molecule's biological activity. Key elements in developing a pharmacophore model are the determination of

functional groups essential for binding, their correspondence from one ligand to another, and their common spatial arrangement [1].

This paper describes a procedure for the utilization of the pharmacophore concept as a molecular representation scheme. According to this approach, molecules may be classified according to their biological effects or physicochemical properties and their 3D structure. This methodology could enhance the features considered to be important for the specific biological activity. Additionally, it could be exploited to deduce molecular similarity between structurally diverse molecules based on their biological activity, facilitating also structural superposition.

We examine the features of this procedure in the context of analysing dopamine (DA) antagonists for the D_2 receptor subtype. DA neurotransmitter plays a critical role in cellular signalling processes responsible for information transfer in neurons functioning in the nervous system. Dopamine receptors (DRs) belong to the superfamily of GPCRs. There are five reported sequences for the human DRs. Based on their pharmacological behaviour, they are subdivided into the D_1-like (D_1 and D_5) and the D_2-like (D_2, D_3 and D_4) subfamilies [5, 6]. DRs are ideal targets for treating schizophrenia and Parkinson's disease [6, 7]. Since it has been generally accepted that a DA D_2 antagonistic component is required for antipsychotic activity, most pharmacological approaches, which are currently under investigation rely on the development of compounds which interfere to some extent with DA D_2-like receptors [7, 8].

Methods

Structures of dopamine antagonists and binding affinity data

The Cambridge Structural Database (CSD) [9] was searched for 3D crystal structures of DA antagonists acting as antipsychotics or neuroleptics. Sets of well-known DA D_2 receptor antagonists of various chemical classes were selected.

Additional information was looked up at the SuperDrug conformational database [10], a free resource of computed 3D-structures for WHO-classified drugs that provides data for drug name, synonyms, formula, CAS-number, ATC (Anatomical Therapeutic Chemical)-code, 2D-similarity screening (Tanimoto coefficients) and an automatic 3D-superposition algorithm.

Binding affinity is quantitatively represented by the inhibition constant (termed K_i). It is empirically measured as the concentration of drug required to block half the total receptor population. High-affinity drugs have low K_i values. Experimental K_i values available from the GPCRDB information system, the PDSP K_i Database and other published data were used as an indication of the binding potency of the examined D_2 antagonists [11–13].

Based on the structural variety among DA antagonists and the available binding affinity data, 11 molecules were initially chosen: *haloperidol*, *raclopride*, *risperidone*, *zetidoline*, *sulpiride*, *spiperone*, *clozapine*, *loxapine*, *octoclothepin*, *clothiapine* and *chlorpromazine*. Among them, *haloperidol* is one of the most frequently used antipsychotics, *chlorpromazine* was the first antipsychotic drug used during the 1950s and 1960s, while *risperidone* and *clozapine* belong to the new generation of antipsychotics often classified as atypical.

The molecular structures of the antagonists included in this analysis were categorized into two classes: (a) class I antagonists that have two aromatic or ring moieties connected by a flexible linker with an amine group and (b) class II bulky tetracyclic or tricyclic (in the case of chlorpromazine) antagonists in a non-planar (v-like) conformation of the tricyclic system [14]. The molecules of class I: *haloperidol*, *raclopride*, *risperidone*, *zetidoline*, *sulpiride* and *spiperone* are shown as 2D structure diagrams in Fig. 1 and as 3D representations in Fig. 2. Respectively, 2D and 3D representations of *clozapine*, *loxapine*, *clothiapine*, *octoclothepin* and *chlorpromazine*, constituting class II, are depicted in Figs. 3 and 4.

Determination of bioactive conformation

The conformation observed in the crystal structure of an unbound molecule is not necessarily the biologically active form. Molecules with several rotatable bonds may adopt several different conformations, some of them being favourable because of low internal energies, others being less favourable because of van der Waals or electrostatic repulsion between non-bonded atoms or groups. The biological activity is supposed to depend on one conformation hidden among all low-energy conformations of the molecule [15].

The determination of the bioactive conformation is a very significant step in most strategies used in computer-aided drug design. It is often a non-trivial task, since most drug-candidate molecules have numerous low-energy conformations [16, 17]. Several methods are available for generating conformational ensembles. However, the main issue is how to choose which method is likely to give the best results [17]. Size and

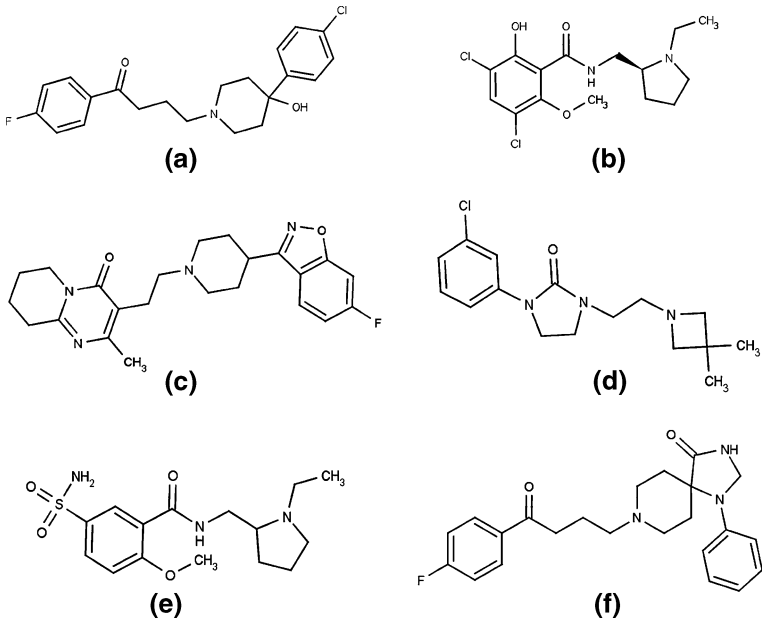

Fig. 1 Two-dimensional structure diagrams of class I molecules: (**a**) *haloperidol*, (**b**) *raclopride*, (**c**) *risperidone*, (**d**) *zetidoline*, (**e**) *sulpiride* and (**f**) *spiperone*

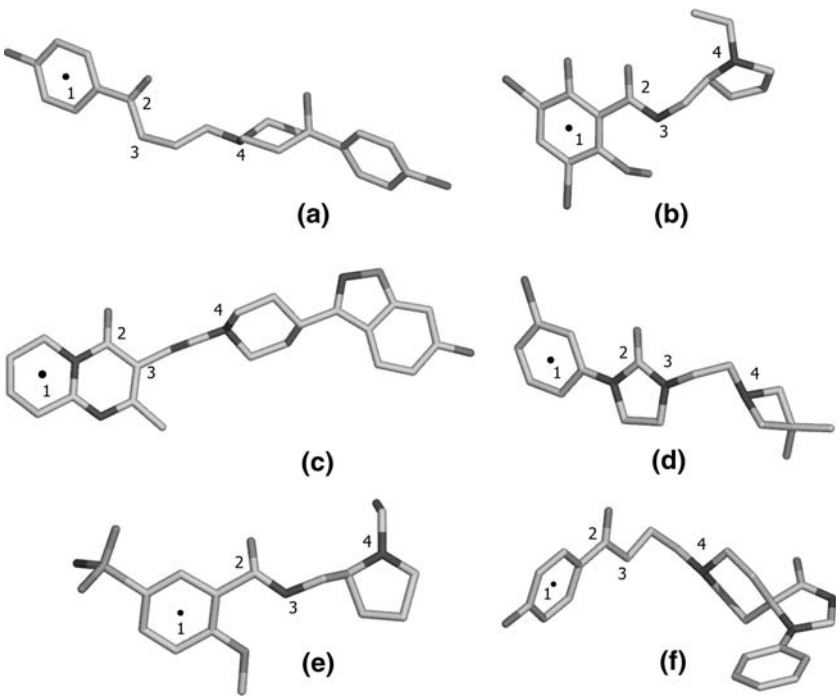

Fig. 2 Three-dimensional structural representation of class I molecules: (**a**) *haloperidol*, (**b**) *raclopride*, (**c**) *risperidone*, (**d**) *zetidoline*, (**e**) *sulpiride*, and (**f**) *spiperone*. The targeting points (the centroid of an aromatic ring and three atoms) are indicated in each structure. These points are numbered in order to show the assignment (or matching) of the atoms of one molecule to the atoms of the other molecules

flexibility of molecules have major impact on the quality of generated conformational ensembles, as they in part reflect the depth of conformational space [18]. It has been deduced that geometrically similar structures should be collected in order to increase the probability of finding the bioactive conformation among the generated ensembles [17]. In many real-life applications, determination of bioactive conformation would be impossible to perform if a reference ligand of limited conformational flexibility is not available, or if a set of distinct ligands showing conformational rigidity in complementary parts of their molecular skeletons is not known [19].

Pharmacophore generation

Pharmacophore modelling assumes that a set of chemical features in a molecule is recognized at a receptor site and is responsible for the molecule's biological activity (bioactivity). Pharmacophore

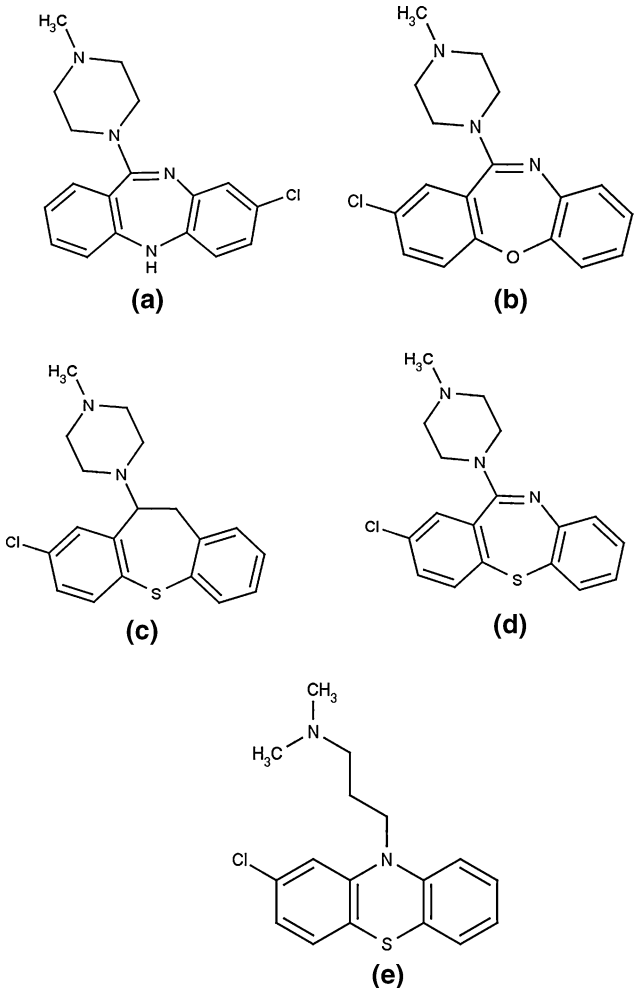

Fig. 3 Two-dimensional structure diagrams of class II molecules: (**a**) *clozapine*, (**b**) *loxapine*, (**c**) *octoclothepin*, (**d**) *clothiapine* and (**e**) *chlorpromazine*

generation is the procedure for the extraction of the most important of these common chemical features from a series of molecules with a similar mechanism of action. The pharmacophore concept is based specifically on interactions that are observed in drug receptor complexes: hydrogen bonding, charge transfer, electrostatic and hydrophobic interactions [20].

The basic steps of this procedure include: *the definition of the pharmacophoric groups* accomplished by the identification of pharmacophorically equivalent elements and *the determination of the bioactive conformation* (pharmacologically active) of the molecules using structural superposition in conjunction with internal energy minimization [1]. The step of chemical feature definition and identification affects the universality and selectivity of the resulting model. Although selectivity is a major issue in pharamcophore generation and validation, only universal pharmacophores represent a mode of action instead of a group of already known ligands [20]. General chemical feature types such as lipophilic, aromatic lipophilic points and hydrogen bond donors and acceptors are examined, in accordance with other published approaches [20].

Molecular modelling studies

Molecular modelling studies were performed using molecular mechanics, implemented by the Energy program [21]. Conformational geometries and energies were calculated using an extension of the Scott & Scheraga empirical force field. The energy terms included bond stretching/bending, van der Waals interactions, torsional and electrostatic potentials. All energy minimizations were carried out using a quasi-Newton method [21] and different starting points to cover the conformational space.

Molecular mechanics calculates the internal energy of a molecule. The lowest energy conformation of a molecule is favoured. Knowledge of the molecule's conformation is important, because the structure of a molecule has a great effect on its binding activity. Strictly speaking, internal energy values can only be directly compared for conformational isomers or geometric isomers that have the same number and types of bonds.

Structural superposition with internal energy minimization (targeting)

With the Energy program it is possible to superimpose in space a pair of molecules, one of which is treated as flexible and is placed onto the other molecule, which is treated as a rigid structure (reference molecule). This procedure allows a simultaneous and independent minimization of the internal energy of the molecules in their respective conformational space, while keeping target groups close in space (*targeting*).

A qualitative notion of a good structural superposition for the pairwise targeting procedure was used. A superposition was good if (a) the internal energy of each molecule was low with respect to their initial (crystal structure) energy, (b) their aromatic groups overlap and (c) their H-bond donors and acceptors overlap.

In order to deduce a common structural framework for the examined antagonists, we must select the same reference molecule structure in each pairwise superposition. The reference molecule should be in its bioactive conformation for pharmacophore generation.

In this study, we chose to implement superposition based on the selection of four non-collinear atoms in each molecule.

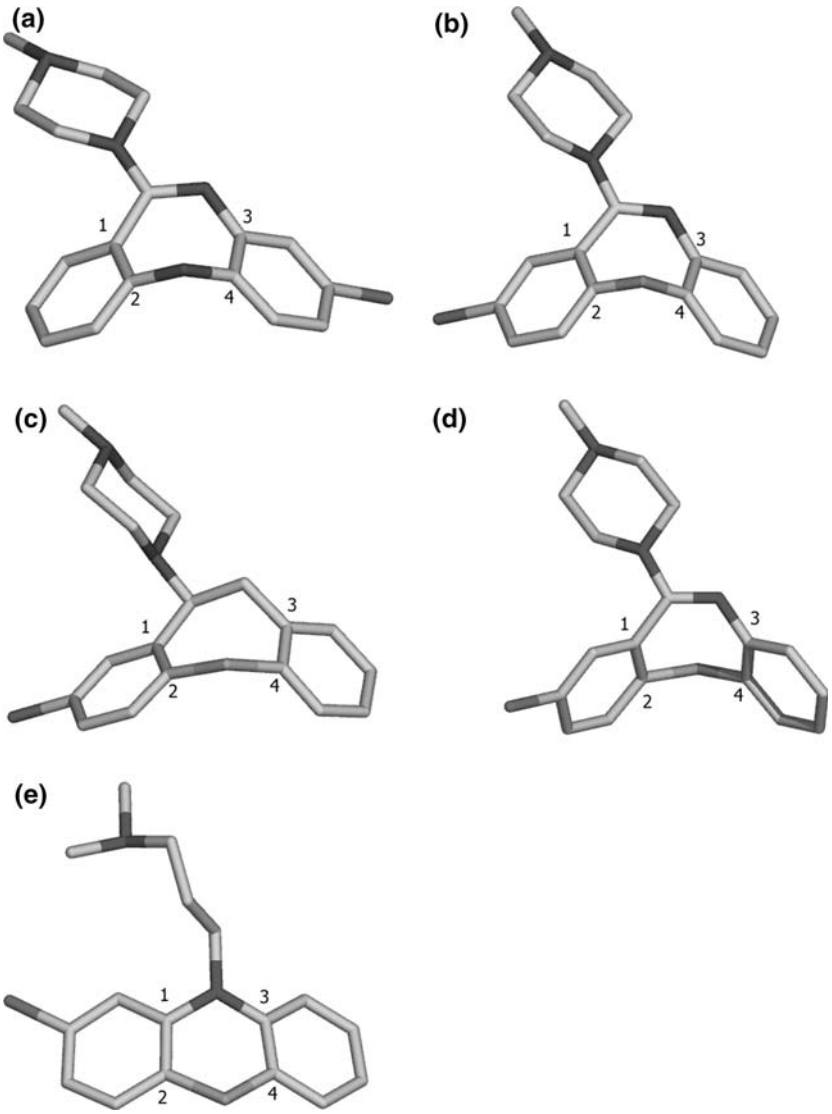

Fig. 4 Three-dimensional structural representation of class II molecules: (**a**) *clozapine*, (**b**) *loxapine*, (**c**) *octoclothepin*, (**d**) *clothiapine* and (**e**) *chlorpromazine*. The targeting points are indicated in each structure

Modelling of structure class I

For this structural class, *haloperidol* was selected as the reference molecule. One conformational assumption was made that concerns the coplanar arrangement of the aromatic ring and the carbonyl group of the molecule. This is supported by the crystal structures of the members of class I present in CSD [22, 23].

The targeting points were selected based on the major 'active elements' of the molecule: *aromatic ring*, *carbonyl* and *amine group*, derived from structure—activity studies [24].

The next step was the determination of *haloperidol*'s putative bioactive conformation. This was deduced with the following process: targeting was implemented using the crystal structure of *haloperidol* both as *flexible* and *rigid* structure, along with the conformational assumption and four targeting atom pairs. Thus the molecule was let to decrease its internal energy 'retaining' however valuable structural information from the crystal structure conformation. Subsequently, all the other molecules of *class I* were fitted on this template.

Modelling of structure class II

The members of the second class have more rigid and similar structures. This simplified the selection of the *reference* molecule and the choice of the fitting points. The antipsychotic drug *loxapine* was used as reference. Its bioactive conformation was deduced with the procedure described above. Subsequently, all the other molecules of *class II* were fitted on this template.

Conformational flexibility

Due to the putative character of the bioactive conformation on which the pharmacophore derivation is based, one must take into account a certain conformational flexibility in order to search for or design other molecules that fulfil the particular model. This can be accomplished with the introduction of a range of values for the geometric elements of the model [25, 26]. For this purpose, one could exploit the values from both the crystal structures and the putative bioactive conformations generated by the targeting procedure.

Results

Pharmacophore model of class I

Using the proposed bioactive structure of *haloperidol* and four non-collinear atoms as targeting points, we calculated the putative bioactive structures for the other five members and deduced a common pharmacophore. The targeting points are marked on the molecular structures in Fig. 2.

Total internal energy values of the antagonists in their crystal structure and in their putative bioactive conformation are shown in Table 1. Also tabulated are binding affinity data (K_i values).

Figure 5 depicts the common structural arrangement of the proposed bioactive conformation of the examined antagonists as derived from the targeting procedure. The common structural framework facilitates the identification of the fundamental set of active elements and their relative 3D orientations.

A simple way to define a pharmacophore model is in terms of three active group positions and three distances. In particular, we chose the centroid of the aromatic ring (dummy atom) as point A, the carbonyl oxygen as point B and the amine nitrogen as point C. The three distances used were (AB), (BC) and (AC). Table 2 contains the values of the geometric elements of the proposed model for the members of class I in their crystal and targeted conformation.

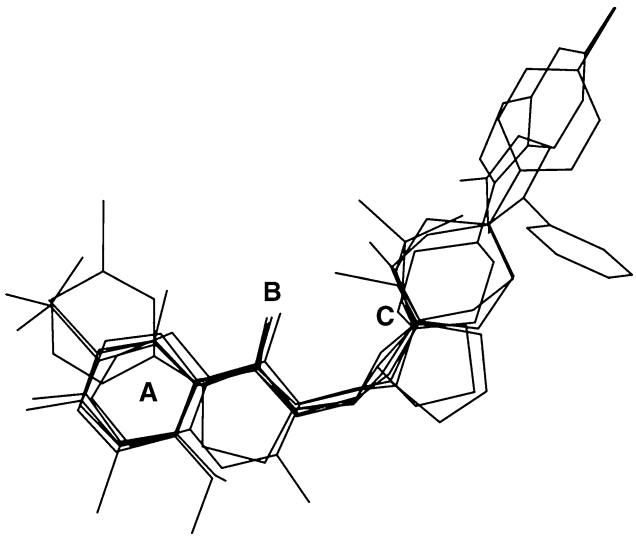

Fig. 5 Structural superposition of class I molecules in their putative bioactive conformation. Depicted are the centroid of the aromatic ring (**A**), the carbonyl oxygen (**B**) and the amine nitrogen (**C**)

These values provide an inference of which of the geometric features of the model exhibit greater variation between the crystallographic structure and the proposed bioactive conformation, as well as an estimation of the conformation flexibility of structure class I. Moreover, due to the existence of the flexible (three-membered alkyl) chain, one should exploit the information gained by the exploration of energetically favourable spatial arrangements for the reference molecule (conformational analysis of *haloperidol*) regarding especially the range of values for (AC).

Table 1 Internal energy values of the crystal structures and the targeted conformations for class I molecules, along with binding affinity

Molecule	$E_{initial}$ (kcal/mol)	$E_{targeted}$ (kcal/mol)	K_i
Haloperidol	–0.447	–1.905	+++
Raclopride	–0.753	–1.546	+++
Risperidone	–1.026	–3.031	+++
Zetidoline	–0.145	–1.435	++
Sulpiride	–0.448	–1.36	++
Spiperone	–0.263	–2.523	+++

+++ indicates inhibition constant (K_i) <10 nM; ++ indicates $10 < K_i < 100$ nM; + indicates $100 < K_i < 1{,}000$ nM. They give half-maximal inhibition of $[^3H]$*Spiperone* (D_2)

Checking pharmacophore model I

Using SuperDrug, we searched for other molecules based on their 2D similarity (Tanimoto coefficients) [27] with *haloperidol* and their ATC classification (into one of the 12 subcategories of the category ANTIPSYCHOTICS). We chose to study molecules with available experimental 3D structure from CSD.

The range of values for the three distances of model I, along with the respective values for three representative examples of molecules gathered following the above-described procedure, are shown in Table 2. The 2D structures of these molecules are shown in Fig. 6a–c.

Table 2 The values of the geometric elements of the model of class I in their crystal structure and targeted conformation

Also tabulated are the average, standard deviation and range of values for the three distances as well as the distance values for three representative examples of molecules derived based on model I

	(AB) (Å)		(BC) (Å)		(AC) (Å)	
	Crystal	Targeted	Crystal	Targeted	Crystal	Targeted
Haloperidol	3.62	3.62	4.32	3.86	7.34	6.95
Raclopride	3.65	3.65	4.19	3.84	7.32	6.90
Risperidone	3.56	3.56	4.63	3.23	7.29	6.40
Zetidoline	4.02	4.02	4.69	3.52	7.55	7.43
Sulpiride	3.57	3.57	4.65	3.68	7.32	6.89
Spiperone	3.62	3.62	4.80	3.58	6.53	6.94
Average	3.67	3.67	4.55	3.62	7.23	6.92
Standard deviation	0.17	0.17	0.24	0.23	0.35	0.33
Range (min–max)	3.5–4.0		3.2–4.8		6.4–7.6	
Benperidol	3.58	3.58	5.05	3.59	7.63	6.73
Bromperidol	3.63	3.63	4.32	3.22	7.37	6.45
Moperone	3.61	3.61	5.11	4.36	7.57	7.23

Pharmacophore model of class II

The same procedure was followed for the second class of antagonists using as template for structural superposition the putative bioactive conformation of *loxapine*, to derive a common pharmacophore for class II compounds.

Table 3 summarizes total internal energy values of the antagonists in their crystal structure and in their putative bioactive conformation. Also tabulated are typical binding affinity data (K_i values).

Figure 7 shows the common structural arrangement of the proposed bioactive conformation of the examined antagonists of class II, derived from the implementation of the targeting procedure.

The pharmacophoric model should include common elements to describe structures that possess the tricyclic system with different central ring and with or without a piperazine ring.

Thus in accordance with the model of class I, this pharmacophore was defined in terms of the centroids of the two terminal rings of the tricyclic system as points A, B and the distal amine nitrogen as point C, along with the corresponding distances (AB), (BC) and (AC).

Table 4 reports the values of the geometric elements of the pharmacophore model for the members of class II in their crystal structure and targeted conformation.

Checking pharmacophore model II

The same methodology was implemented for the second model. The corresponding range of values and three typical examples are tabulated in Table 4. The 2D structures of these molecules are shown in Fig. 6d–f.

Extension to other antipsychotic compounds

An interesting subcategory of antipsychotics is diphenylbutylpiperidine derivatives (such as *penfluridol*, *pimozide*, *fluspirilene*).

The study of *penfluridol* (Fig. 6g) depicts that this molecule shares common chemical features with both classes. Similarly to class I molecules, *penfluridol* has a flexible three-membered alkyl chain between the aromatic rings and the tertiary amine, but it has a second aromatic moiety instead of a ketone carbonyl. Additionally, in *penfluridol*, as in class II, there is a system of two aromatic rings in a non-planar (v-like) conformation. The common characteristic of carbonyl oxygen and an aromatic ring is their electron density localization. This indicates the pharmacophoric equivalence of the two groups [28].

The structure of *penfluridol* can be exploited to derive a common structural framework between the two classes, indicating the primary binding groups whose topographical arrangement is fundamental to the activity of these drug classes.

Another interesting aspect is the utilization of the pharmacophoric elements of the two models in order to superimpose and compare two molecules without obvious structural similarity (e.g. *loxapine* and *haloperidol*), taking into account their biological activity.

A general common molecular framework for feature comparison

In order to achieve a common structural framework for the two classes of DA D_2 antagonists, based on the assumption of a common binding site, we selected *loxapine* as the reference molecule due to its more rigid structure against the members of class I.

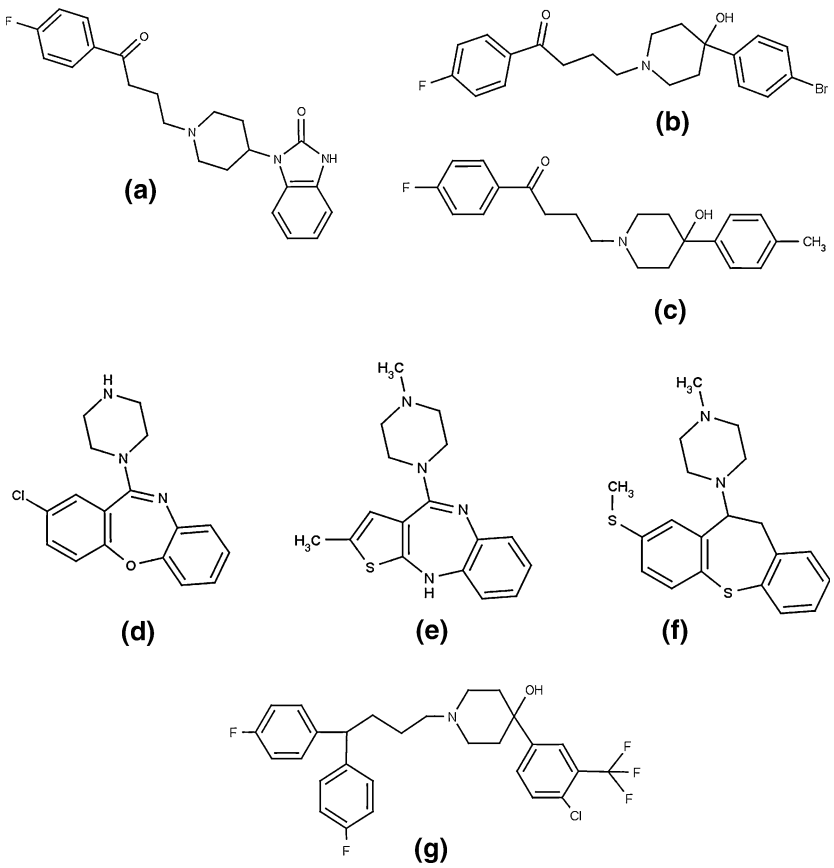

Fig. 6 Two-dimensional structure diagrams for representative examples of molecules derived based on the two pharmacophore models: (**a**) *benperidol*, (**b**) *bromperidol*, (**c**) *moperone*, (**d**) *amoxapine*, (**e**) *olanzapine*, (**f**) *metitepine* and (**g**) *penfluridol*

First we applied targeting of *penfluridol* upon the putative bioactive conformation of *loxapine*, without using any conformational assumption. This way we exploited the greater similarity of *penfluridol* vs *loxapine* against the corresponding resemblance of *haloperidol* vs loxapine. Indeed, the 2D-similarity screening (Tanimoto coefficients) in the SuperDrug database for these two pairs was 43.57 and 34.58, respectively, while the 2D score of *penfluridol* vs *haloperidol* was 62.99. Then, using the targeted structure of *penfluridol* as reference structure, we superimposed *haloperidol* and the other members of class I.

Table 3 Internal energy values of the crystal structures and the targeted conformations for class II molecules, along with binding affinity

Molecule	$E_{initial}$ (kcal/mol)	$E_{targeted}$ (kcal/mol)	K_i
Clozapine	–0.345	–1.759	+
Loxapine	–0.142	–1.586	++
Octoclothepin	–0.769	–0.806	++
Clothiapine	0.007	-1.587	+++
Chlorpromazine	–0.576	–1.367	+++

+++ indicates inhibition constant (K_i) <10 nM; ++ indicates $10 < K_i < 100$ nM; + indicates $100 < K_i < 1{,}000$ nM. They give half-maximal inhibition of *[*^{3}H*]Spiperone* (D_2)

Figure 8 shows the superposition of the structurally diverse antagonists: *loxapine*, *haloperidol* and *penfluridol*, in a common structural framework.

This pharmacophoric procedure resulted in a more biologically meaningful superposition of *loxapine* and *haloperidol* with respect to available automatic 3D-superposition approaches (e.g. the 3D-superposition procedure available at the SuperDrug web server) [10]. Indeed, fast superposition algorithms tend to have certain limitations when applied to molecules with considerably different overall geometry [29].

Additionally, this molecular framework depicts the common features and their specific topographical arrangement as primary requirements for the biological activity of these molecules (as DA antagonists). These include an aromatic ring, a ketone carbonyl and an amine moiety or two aromatic rings and an amine moiety.

Discussion

In the absence of a 3D structure for a particular receptor protein of therapeutic interest, drug discovery and design efforts are often based on a model inferred

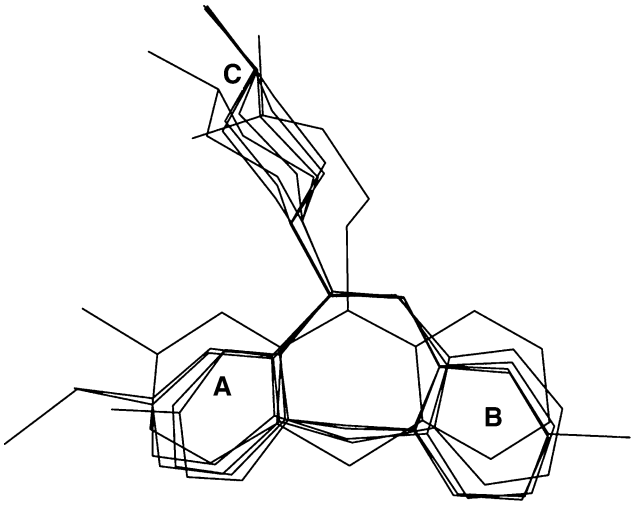

Fig. 7 Structural superposition of class II molecules in their putative bioactive conformation. Depicted are the centroids of the two terminal rings of the tricyclic system (**A**, **B**) and the distal amine nitrogen (**C**)

from the different ligands that bind to it. Ligand-based drug design approach depends on a principle, which states that structurally similar compounds are more likely to exhibit similar properties ('molecular mimicry') [1–4].

One question is how to define a similarity between two structures. The terms *molecular similarity* and *molecular diversity* have various definitions in function of chosen criteria. Molecular similarity can be described using pharmacophore features. A pharmacophore is commonly defined as an arrangement of molecular features or fragments forming a necessary but not sufficient condition for biological activity [1].

Pharmacophore generation can be implemented using the targeting procedure, which is the structural superposition of a series of molecules with a similar mechanism of action, in conjunction with internal energy minimization and the subsequent identification of the common chemical features.

In this paper, a three-point pharmacophore method was used as a molecular representation perception. It can aid the identification of the common 3D patterns present in diverse molecules that act at the same biological target. This procedure was implemented for DA antagonists of the D_2 receptor subtype. Since there is not a detailed crystal structure of a DR, nor a closely related G-protein coupled receptor (with significant homology to DR) is available, ligand-based modelling strategies can be exploited.

The molecular structures of the antagonists included in this analysis were categorized into two classes: class I and class II antagonists.

Class I antagonists have two aromatic or ring moieties connected by a flexible linker with an amine group. The targeting procedure allowed the molecules to lower their internal energy and obtain a common structural orientation. The pharmacophore model was defined in terms of the centroid of an aromatic ring as point A, the carbonyl oxygen as point B and the amine nitrogen as point C, along with the corresponding distances (AB), (BC) and (AC). Based on the distance values of the crystal structures and the putative bioactive conformations of the class I molecules, we deduced a range of values for the geometric elements of the model (Table 2). In Table 2, are also depicted three typical examples of molecules with 2D similarity with *haloperidol* and ATC classification as antipsychotics. Their distance values were in compliance with the range of the corresponding distance features of the model. Indeed, small deviations from the proposed range in the case of the crystal conformation could be eliminated looking at their targeted conformation, due to the structural flexibility of the molecules.

Table 4 The values of the geometric elements of the model of class II in their crystal structure and targeted conformation

Also tabulated are the average, standard deviation and range of values for the three distances as well as the distance values for three representative examples of molecules derived based on model II

	(AB) (Å)		(BC) (Å)		(AC) (Å)	
	Crystal	Targeted	Crystal	Targeted	Crystal	Targeted
Clozapine	4.61	4.61	7.72	7.80	5.97	5.80
Loxapine	4.51	4.52	7.73	7.70	6.19	6.24
Octoclothepin	5.02	5.02	7.71	7.71	6.05	6.10
Clothiapine	4.69	5.02	7.73	7.71	6.09	6.10
Chlorpromazine	4.80	4.80	6.81	7.00	5.12	5.10
Average	4.73	4.79	7.54	7.58	5.88	5.87
Standard deviation	0.19	0.23	0.41	0.33	0.43	0.46
Range (min–max)	4.5–5.0		6.8–7.8		5.1–6.2	
Amoxapine	4.60	4.60	7.69	7.83	6.15	5.75
Olanzapine	4.64	4.64	7.78	7.77	5.88	5.9
Metitepine	5.11	5.11	7.68	7.67	6.17	6.22

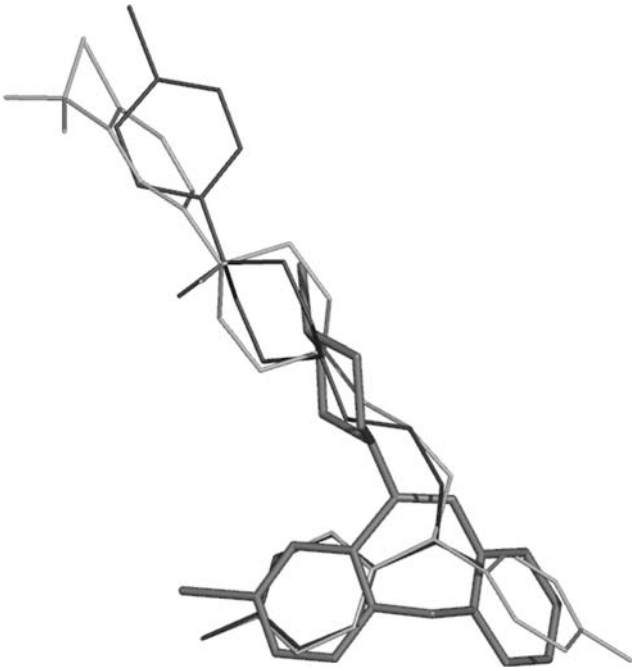

Fig. 8 Superposition of the structurally diverse D_2 antagonists *loxapine* (*thick grey line*), *haloperidol* (*black line*) and *penfluridol* (*light grey line*)

Class II is composed of bulky tetracyclic or tricyclic antagonists in a non-planar (v-like) conformation of the tricyclic system. The molecules in their targeted conformation acquired a common structural orientation of lower energy. The pharmacophore of this class was defined in terms of the centroids of the two terminal rings of the tricyclic system as points A, B and the distal amine nitrogen as point C, along with the corresponding distances (AB), (BC) and (AC). In order to check the validity of the derived model, we searched for other D_2 antagonists that fulfil the pharmacophore. Their geometric elements were in accordance with the proposed range of values in the pharmacophore model (Table 4).

An interesting group of antagonists was diphenylbutylpiperidine derivatives (e.g. *penfluridol*). Especially the study of *penfluridol* depicted that this molecule shared common chemical features with both classes, indicating the pharmacophoric equivalence of the carbonyl oxygen and an aromatic ring.

Therefore, we combined the two pharmacophores in a general common molecular framework that could offer insight into the significance of chemical features for binding to D_2 receptors. These components included an aromatic ring, a ketone carbonyl and an amine moiety or two aromatic rings and an amine moiety [30, 31].

Another interesting aspect was that the pharmacophoric concept might be exploited in order to derive a more biologically meaningful superposition of structurally diverse molecules that bind in the same binding site. This was demonstrated for the case of *loxapine* and *haloperidol* that belong to the two different structural groups, studied in this paper. Based on the common pharmacophoric components of the two groups that each molecule belongs to and the existence of a molecule that shares common chemical features with both of them (in this case *penfluridol*), one could infer a more reasonable selection of fitting points between the two structures, compared to automatic superposition procedures.

Therefore this procedure might be used to infer molecular similarity between structurally diverse molecules, using the biological information implied in the pharmacophore concept. The underlying assumption exploited is that a specific biological interaction is obtained either via a set of geometric features common to the data set of ligands, or alternatively, they may be chemical attributes, translated into geometrical features (e.g. hydrogen bonds, coordinates of hydrophobic atoms, points representing charged groups, etc.).

The pharmacophoric information appeared also to be suitable for guiding structural superposition. This could be accomplished by assigning different degrees of importance to the various components of the molecular representations, which results in a more biologically meaningful selection of the targeting points.

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