INFLUENCE OF ARYLPIPERAZINES AROMATIC STRUCTURE OVER DIFFERENTIAL AFFINITY FOR 5-HT$_{1A}$ AND 5-HT$_{2A}$ RECEPTORS

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RESUMEN:

INFLUENCIA DE LA ESTRUCTURA AROMÁTICA DE LAS ARILPIPERAZINAS EN LA AFINIDAD DIFERENCIAL CON LOS RECEPTORES 5-HT$_{1A}$ Y 5-HT$_{2A}$

Las piperazinas son una familia de compuestos químicos muy amplia y con una gran capacidad para interactuar con diversos receptores serotonérgicos (5-HT). Debido a estas propiedades, estos compuestos tienen un importante potencial farmacológico, sin embargo muestran también algunos efectos tóxicos asociados. En la actualidad el subtipo 1A del receptor serotonérgico (5-HT$_{1A}$) ha resultado ser un importante blanco para el tratamiento eficaz de la depresión y ansiedad, mientras que el subtipo 2A del receptor serotonérgico (5-HT$_{2A}$) ha sido asociado con numerables efectos adversos.

En este estudio, se utilizan diversos métodos computacionales con el fin de efectuar una caracterización de los fragmentos estructurales y las propiedades químicas asociadas, responsables por la afinidad de las piperazinas para los receptores 5-HT$_{1A}$ y 5-HT$_{2A}$. En este trabajo, se discuten también, algunas propiedades de las estructuras aromáticas en las arilpiperazinas que son similares para los dos subtipos del receptor serotonérgico. Por otra parte se sugiere, que la substitución con calcógenos en la posición orto- y meta- así como el ligero incremento en el peso molecular son modificaciones que pueden aumentan la afinidad para el receptor 5-HT$_{1A}$; mientras que las arilpiperazinas con substitución por halógenos en las mismas posiciones además de un pequeño decremento en el peso molecular podrían incrementar la afinidad para el 5-HT$_{2A}$ receptor.

PALABRAS CLAVE: Piperazina; Receptor serotonérgico; Farmacóforos; Afinidad; Selectividad; Diseño de fármaco
SUMMARY:

The piperazines are a large family of compounds with an enormous potential for interacting with several serotonin (5-HT) receptors. Those compounds reveal prospect for use as drugs with diverse therapeutic applications, despite the fact that they also show some toxicological effects. Actually, the subtype 1A of 5-HT (5-HT_1A) receptor is responsible for efficient treatment of anxiety and depression, while subtype 2A of the 5-HT (5-HT_2A) receptor is accountable for several adverse effects. In this study, we applied several computational approaches to better describe the most important chemical and substructure properties that are responsible to influence the affinity of arylpiperazines to the 5-HT_1A or the 5-HT_2A receptors. In the present work we discuss some properties of the arylpiperazines aromatics structures that are similar for both serotonin receptors. However, consequently, we showed that the chalcogens substitution close to the benzene ortho- and meta-position as well as a slight increment in the molecular weight showed more affinity to the 5-HT_1A receptor. While arylpiperazines with halogens substitution at the same benzene position as well as a minor decrease in the molecular weight had more affinity for the 5-HT_2A receptors.

KEY WORDS: Piperazine; Serotonergic receptor; Pharmacophore; Affinity; Selectivity; Drug design.

INTRODUCTION

The interest in the role of serotonin (5-HT) and the mechanism of action of antipsychotic drugs (APDs) is the result to its direct and indirect effects on various 5-HT receptors, especially the 1A and 2A subtype serotonergic receptors (5-HT_1A and 5-HT_2A, respectively). Thus, both 5-HT_2A antagonism and 5-HT_1A agonism may be the most important of the 5-HT receptors for APD action. Postsynaptically, both 5-HT_1A and 5-HT_2A receptors are localised on the pyramidal neurones in the cortex, where the 5-HT_1A receptor inhibits neuronal output by activation of a hyperpolarising potassium current, and the 5-HT_2A facilitates output via activation of phospholipase C.

This opposition between the two 5-HT receptor subtypes suggests that agonists at 5-HT_1A receptors may modulate dopaminergic neurotransmission in the brain in a similar fashion to 5-HT_2A receptor antagonists. The 5-HT_1A receptor agonists can stimulate the release of dopamine (DA) in the prefrontal cortex as well as potentiate the effect of 2 subtype dopamine receptor (D_2) blockers on DA release. These studies suggest that 5-HT_1A receptor activation is critically involved in the regulation of DA release in these two brain regions, which are involved in key cognitive function.

5-HT_1A receptors are located both presynaptically and postsynaptically. The presynaptic receptors are also known as autoreceptors and are stimulated automatically upon release of serotonin. Activation of the 5-HT_1A autoreceptors inhibits the release of serotonin on a global level. Several studies suggest that atypical antipsychotics exert their effects on dopaminergic neurotransmission, at least in part, via activation of 5-HT_1A receptors, presumably due to concomitant potent 5-HT_2A and relatively weak D_2 receptor antagonism. Cai et al. suggested this may be a mechanism by which 5-HT_1A receptors modulate memory and anxiety.

The use of 5-HT_1A receptor agonists may substitute for 5-HT_2A antagonism and achieve many of the same benefits in combination with weak D_2 receptor blockade. All these studies focuses on the regulation of central 5-HT_1A receptor function as an ideal target to antidepressant drugs by 5-HT_1A receptor agonists underlies the therapeutic efficacy of these drugs.

The 5-HT_1A receptor is present in high density in serotonergic cell body areas, in particular the dorsal and median raphe nuclei, as well as in cortical and limbic areas (e.g. frontal cortex, entorhinal cortex, hippocampus, amygdala, septum). It’s also present in the hypothalamus where play important roles in the regulation of neuroendocrine function and responses to stress.

Anxiolytic or antidepressant efficacy may be due in part to compensatory changes distal to the 5-HT_1A receptor receptor, such as regulation of G protein expression or reduced capacity of the receptor to activate G protein due to regulatory processes (e.g. phosphorylation) at the level of the G protein.

The increase in serotonin neurotransmission, due to somatodendritic autoreceptor desensitization, to normo-sensitive 5-HT_1A receptors in certain brain regions (e.g. hippocampus or cortex) and to sub-sensitive 5-HT_1A receptors in other brain regions (e.g. amygdala or hypothalamus) underlies the therapeutic efficacy of these drugs (Figure 1).
Figure 1. Anxiolytic and Antidepressant Drug Effects on Serotonergic Neurotransmission (Adaptation from Hensler et al.7).
The 5-HT1A receptor is located on serotonergic cell bodies and dendrites, functioning as the somatodendritic autoreceptor.
Several compounds are agonists at the 5-HT1A receptor, comprising anxiolytic and antidepressant activity.

By blocking the serotonin transporter (SERT) or inhibiting monoamine oxidase (MAO), antidepressant drugs increase the synaptic concentration of the serotonin neurotransmitter (5-HT).

The piperazines (Figure 2) are an important family of compounds with vast pharmacological properties from their interactions with several 5-HT receptors, in particular, the 5-HT1A. However, one assumes that this family has the same mechanism of action and toxicity as amphetamines and ecstasy11-16. Actually, the most adverse effects are supposed to be due to the agonist interaction of the piperazines with the 5-HT2A17. Indeed, Capela et al.,18-19 demonstrated, in vitro, that the overstimulation of 5-HT2A receptor is responsible for the cortical neuron’s death.

Figure 2. Piperazine functional group.

Furthermore, the adverse effects on 5-HT2A receptors associated with piperazine consumption are usually schizophrenic symptoms and cerebral cortex disorders18,20, in part, due to the 5-HT2A receptors agonist interaction. On the other hand, the 5-HT1A receptors are responsible for a central modulation of affective disorders, such as anxiety and depression, revealing an enormous potential for antipsychotic drugs21, as previously referred.

Our goal consisted on identifying the chemical and molecular properties in the piperazine family that determine the selectivity and the affinity for 5-HT1A and 5-HT2A receptors. So, it is possible to improve drug design for compounds with more affinity and selectivity for the 5-HT1A receptor. For this purpose, mathematics and statistics methods were used for our analysis from the arylpiperazines in relation to the two receptor targets.

In the last decade, several Quantitative-Structure Activity Relationships (QSAR) studies were made of arylpiperazines their
Molecular and chemical properties relevant to the 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors, as well, to pharmacophores\textsuperscript{22-25}. It was shown that electro-topologic structure and substructure distances were the principal factors involved in the structure-activity correlation, although they also were related to the compounds’ lipophilicity\textsuperscript{25}.

The structural diversity of 5-HT\textsubscript{2A} ligands represents a challenge for pharmacophore definition, although some proposed models exist, as we already referred. Tammy et al.\textsuperscript{26} believed that the presence of a basic nitrogen group as a central point of the ligand-receptor interaction maybe questionable. Indeed, their research illustrated, at least in the piperidine family, that the basic nitrogen group substitution didn’t affect ligand’s affinity in relation to 5-HT\textsubscript{2A} receptors and reduced, actually, the possibility of interaction with other receptors. So, our research tried a new biochemical interaction approach that makes possible the design of new drugs and computational simulations.

MATERIALS AND METHODS

Data Set

The pharmacophore characterizations were developed from a group of molecular descriptors (1D, 2D and 3D) obtained from eDragon through a study set of a hundred and twenty four arylpiperazine derivates (Tables 1-7 in the annexes).

Computational methods

Structures of all arylpiperazine derivates were drawn on the ChemDrawn\textsuperscript{27} software package, pre-optimized by molecular mechanics using the MM2 force field. The final structure was obtained by subsequent optimization with AM1 semi empirical Hamiltonian, implemented in the MOPAC 6.0 program\textsuperscript{28}.

Molecular descriptors (n=1666) were calculated for each molecular structure using eDragon software\textsuperscript{29-32} while the appropriate descriptor selection was made by means of the genetic algorithm (GA) program designed in Matlab v7.0 for this purpose. After a previous analysis of the initial descriptor set considering: a variability of more than 90\% by descriptor and the elimination of the descriptors that present more than 80\% of correlation between them, we obtained 572 and 573 final descriptors from eDragon for the 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors, respectively.

For GA analyses\textsuperscript{33,34}, the procedure was composed of 600 chromosomes chosen by a probabilistic form and the crossover was performed uniformly and with a probability of 50\%. This procedure was fixed at 1 000 effective iterations. Next, the mutation procedure was carried out by changing the genes (variables) of the chromosomes with a fixed probability (50\%). The crossover/mutation steps were repeated several times until they had reached a fixed stop criterion. In our model, the stop criterion was fixed at 100 000 iterations. The new chromosomes obtained after crossover/mutation procedure were evaluated with the objective function of leave-one-out internal cross-validation (Q\textsubscript{2,LOO}) and was only included in the population if the Q\textsubscript{2,LOO} value was higher than any of the chromosomes already considered in the initial population.

To avoid procedure complications by the structural diversity present in the piperazine's data set, we performed the subdivision of the initial number of piperazines into five subgroups by their structural similarity through cluster analysis from Moloc software\textsuperscript{35,36}. For each cluster, a model was obtained by the combination of the GA and other parameters for model validation, such as the bootstrap internal cross-validation (Q\textsubscript{2,BOO}) and the leave-multiple-out internal cross-validation (Q\textsubscript{2,LMO}). The Q\textsubscript{2,LMO} was calculated considering only a group of molecules (around 33\%) for the model construction. While the Q\textsubscript{2,BOO}, a more accepted internal cross-validation, was calculated by taking samples randomly and repeatedly of size N (where N is the molecules number) for the construction of the model and predicting the remaining molecules. This procedure was repeated several times (we used 5000 repetitions) and the Q\textsubscript{2,BOO} represented the mean predictability coefficient. For the multiple linear regression (MLR) evaluation we used the Statistical Package\textsuperscript{37,38}. Therefore, we elaborated the pharmacophore model of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors, after flexible alignment. The pharmacophore groups identification as well as the flexible alignment were performed with Moloc software\textsuperscript{36}.

RESULTS AND DISCUSSION

The vast number of molecular descriptors obtained in eDragon software were simplified by eliminating the low variation and high correlated descriptors in the data set and, therefore, eliminating a large part of background noise from the essential information in our descriptors. The next step consisted of cluster analysis and, consequently, the formation of subgroups from initial piperazines set by the application of the respective method in Moloc software\textsuperscript{35} (Figure 3). That previous procedure allowed us to get five groups of piperazines with high structural similarity and made possible a better extrapolation of the mutual information shared by the flexible alignment in each group.
Figure 3 presents the initial study set (129 piperazines) division into several clusters by structural similarity shared between them. The number of clusters is proportional to structural similarity shared by the molecules in each subgroup. The selected clusters are pointed out in the figure by the numbers (1-5), which we believe to be a better equilibrium between the chemical characterization and the application domain.

![Dendogram obtained by the Moloc cluster analysis of the initial molecular set. The selected subgroups (1 until 5) are marked in red.](image)

It is interesting to note that the five clusters cloud did not point up the same molecules for each receptor (as can be observed in Table 1), because not every molecule from the study set showed simultaneously a known pKi for 5-HT1A and 5-HT2A receptors. For each selected cluster a predictive model was obtained with the combination of the GA and the validation method previously referred. The obtained models and the selected descriptors are presented in the Tables 2 and 3.

| Table 1. The number of compounds obtained in each cluster for 5-HT1A and 5-HT2A receptors from cluster analysis in Matlab software. |
|---|---|---|---|---|---|---|
|                  | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 | Total  |
| N° of 5-HT1A compounds | 32        | 22        | 32        | 17        | 11        | 114   |
| N° of 5-HT2A compounds | 16        | 23        | 24        | 16        | 11        | 90    |
It's important to remember that the interpretation of molecular properties are extremely difficult, in particular, some classes of eDragon descriptors. Therefore, we used a correlation matrix analysis between the selected descriptors of each cluster and the easily interpretable descriptor family, such as constitutional descriptors, functional groups, atom-centred fragments and few other descriptors, to a better understanding of the structural similarity criteria applied in pharmacophore models by Moloc.
The GA selected descriptors are strongly related to flexibility (number of rotatable bonds), molecular weight, aromatic substructure and atom type involved in the aromatic substitution; and all they represent the principal factors involved in 5-HT receptor selectivity and affinity. Besides the principal piperazine group, both receptors had affinity to aromatic groups with strong electrotopological or electronegative substituents and the presence of RCONHR bonds in the molecule, which are similar to the peptide bonds observed in several intrinsic proteins in humans. The two subtypes of serotonin receptors shared an empathy with heteroatoms' alpha carbons, such as oxygen and nitrogen elements, and aliphatic amines. On the opposition, we observed the lack of empathy with the bromine element and some functional groups, more exactly -N(CO)₂ and -Cₓ₃ (where X is a certain chalcogen or halogen element).

On the one hand, the 5-HT₁₅ receptor showed more specificity for arylpiperazines with chalcogens, such as oxygen and sulphur elements, in particular. On the other hand, the 5-HT₂₅ receptors had more affinity for arylpiperazines with halogens, such as fluorine and chlorine elements. Besides all that, both receptors revealed a crescent affinity when those periodic elements were present in the arylpiperazine aromatic structures at ortho- and meta- positions, in particular. The effect of halogen or chalcogen elements on the arylpiperazine selectivity is probably related to the spatial arrangement of the group in the aromatic ring and its influence on the final arylpiperazine conformation, as observed by Gaillard et al.²⁴ and López-Rodríguez et al.²⁵.

Although molecular weight and saturation index were more or less the same in each cluster, we noted a very small tendency for the 5-HT₁₅ receptor when those factors were slide arises. While 5-HT₂₅ receptor had more affinity for arylpiperazines with less molecular weight and saturation index, the number of substituents in aromatic group of arylpiperazine, which allowed rotative bonds, was more daring for the 5-HT₂₅ receptor.

In pharmacophore construction, we selected one group of molecules that demonstrate an elevated affinity index for one receptor and, simultaneously, a minor affinity index for the other receptor. So those subgroups from the study set should represent the most important intrinsic physico-chemical properties for each serotonin receptor and, therefore, facilitate our interpretation. Next, each previous subgroup was submitted to flexible alignment by a superposition algorithm from Moloc software.³⁵ This made possible the maximum superposition of molecules and their saturated bonds, in particular. So, common molecular properties were valued in pharmacophore model construction (Figure 4).

Figure 4. Illustrations of 5-HT₁₅ and 5-HT₂₅ pharmacophores in the left and right pictures, respectively, obtained from Moloc software.

Legend - The pictures of the spheres representing specific three-dimensional conformational characteristics, more exactly: Dark blue sphere - Hydrogen atom donors (acid group). Light blue sphere - Aromaticity. Red sphere - Hydrogen atom acceptors (base group)

Figure 4 show a basic centre in both models, more exactly, the existence of a proton acceptor in the N₄ functional group from piperazine (red sphere), while the light blue sphere demonstrated the steric effect due to aromatic substructure predominance around the N₄ atom. However, the 5-HT₂₅ model exhibited a more steric shield without losing the N₄ atom's basic character. The presence of electronegativity group density, such as the carbonyl group (C=O), shared proportionately a certain basic character in the molecule and it was more visible in 5-HT₁₅ model. Furthermore, acid groups (dark blue sphere) revealed a higher influence in the 5-HT₂₅ receptor interaction, instead of the 5-HT₁₅ receptor, due to their positions in relation to hydrophobic centres of arylpiperazines. These results were in accordance with Chidester et al.³⁵. In spite of all this, the pharmacophore constituted a standard model from the common properties given by certain number of molecules and, so, was dependent on local molecular properties from some privileged structures. The N₄ atom's basic character, the carbonyl group and the aromatic rings' pi (π) density were some examples of such privileged structures present in our study set.
As we can note, both pharmacophore models share some common characteristics, such as aromatic rings and one basic group, mainly a basic nitrogen group. Actually, those structures had the predisposition to form a triangular and linear rearrangement\(^\text{20,26}\). We observed a major complexity in the 5-HT\(_{2A}\) pharmacophore due to the existence of carbonyl group between a N4 atom (see Figure 5) and an aromatic ring in some arylpiperazines (molecules 78, 92, 128 and 129 in the annexes). These circumstances tend to modify the pharmacophore’s model itself but preserving its high affinity for the receptor.

![Figure 5. Representation of N4 atom present in the arylpiperazine functional group.](image)

In the performed analysis we studied the properties of the arylpiperazines family principally those aspects related to the aromatic neighbouring. Therefore, the analysis of ligand-receptor interaction needs a further study, such as molecular docking, for a better understanding of how the analysed molecular properties are adjusted with the local environment of the actives sites in the 5-HT receptors.

**CONCLUSIONS**

We can conclude that arylpiperazines family share similar aromatic structure properties of interaction with 5-HT\(_{1A}\) and 5-HT\(_{2A}\) receptors, such as i) strong electrotopologic or electronegative substituents, ii) substructures with hydrogen donor and acceptor groups between aromatic group and N4, iii) N and O elements as substituents in benzene (Bz-N1-N4) with the capacity of establish hydrogen bonds, iv) and antipathy with Br element and specific groups (N(CO)\(_2\) and CX\(_3\), X = halogen or chalcogen).

Furthermore, there also exits diverse substructures that allowed altering the intrinsic arylpiperazine affinity to select a specific subtype of 5-HT receptor. The 5-HT\(_{1A}\) pharmacophore model studied demonstrate that i) a basic group high accessibility, ii) an electronegative substructure near the ortho position aromatic ring and iii) and opposite lipophilic and electrostatic effects in the nitrogen substituent were extremely important for the affinity of arylpiperazines family. In particular, the presence of aromatic substructures at ortho- and meta- positions, inhibition of their rotative bonds and the attenuated increase of the saturation index and molecular weight are chemical properties that allowed the increase of arylpiperazines’ affinity for the 5-HT\(_{1A}\) receptor to the detriment of the 5-HT\(_{2A}\) receptor.

Finally, arylpiperazines without H-donor groups or halogen atoms and with electronic density or chalcogens (e.g. O and S) close to the benzene ortho- and meta- substitution position were associated with high increment of 5-HT\(_{1A}\) receptor affinity. Our study allows confirming previously experimental data and, more importantly, to understand the electrotopologic and three-dimensional arylpiperzine's substructures importance in the selectivity of subtype 5-HT receptors.

However, ligand-receptor interaction properties need further investigation thought molecular docking or other computational approach.

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Comment of the reviewer Prof. Pilar Muñiz Rodríguez PhD. Titular del Área de Bioquímica y Biología Molecular de la Facultad de Ciencias de la Universidad de Burgos. España.

The piperazines are a family of chemical compounds with different pharmacological properties including those arising from the
result of interaction with serotonin receptors. The authors, by computational methods, establish a relationship between the structure of the interaction with different piperazines with two types of 5-HT receptor antagonists.

The influence of the substituents of benzene ring as the molecular weight of arilpiperazines was discussed and a model for understand the substructures importance in the selectivity of subtype 5-HT was established. Further in vivo studies are needed to confirm the data that the authors observed using computational model.


The authors have developed an extraordinary effort using a highquality set of tools. Bibliography is generous and it is very well updated.

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ANNEXES
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INFLUENCE OF ARYLPiperazines AROMATIC STRUCTURE OVER DIFFERENTIAL AFFINITY FOR 5-HT1A AND 5-HT2A RECEPTORS