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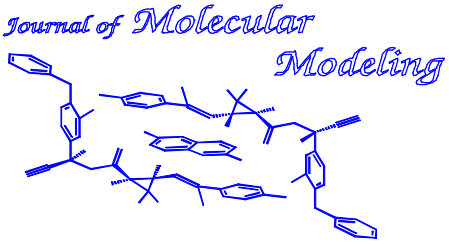

Ergoline Derivates as a Probe for Featuring the 5-HT$_{1A}$ Receptor Pharmacophore

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Abstract

A 5-HT$_{1A}$ pharmacophore has been obtained employing a set of rigid templates encompassing the 5-HT structure. The use of rigid templates allowed us to overcome the discrepancy found when flexible structures where the energy of the active conformers are sometimes higher than the global minimum energy are used. On the basis of the results herein reported the three-dimensional requirements necessary for the binding interaction have been defined within this set of molecules. In this study forbidden zones of the receptor have been characterised.
The pharmacophore model derived places some agonist/antagonist pharmacophore models appeared in the literature in question.

Keywords: 5-HT$_{1A}$, pharmacophore model, rigid template, ergoline derivative.

Introduction

Drugs acting as direct serotonin agonists or those that enhance central serotoninergic functions have been shown to affect memory, depression, anxiety, pain, emesis, and other important centrally mediated functions in humans. [1]

The discovery that the anxiolytic activity of Buspirone, whose mechanism of action was unknown for a long time and was thought to involve dopamine, is due to the 5-HT$_{1A}$ receptor activation, was a stimulus for a detailed investigation on the 5-HT$_{1A}$ receptor subfamily and its therapeutic implications. [2]

A large number of compounds has been assessed for their ability to bind selectively at the 5-HT$_{1A}$ receptor to identify therapeutic agents devoid of unwanted effects associated with multiple receptor interactions.

From this extensive investigation, some chemical classes such as indolealkylamines, ergolines and related substructures, aminotetralines, notably among them 8-OH-DPAT the prototypical 5-HT$_{1A}$ agonist, and some heterogeneous classes such as arylpiperazines, arylpiperidines and aryloxyalkylamines,

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have been shown to display remarkable affinity to the 5-HT_{1A} receptor. [3]

The ergoline derivatives have attracted great interest for their broad spectrum of pharmacological action that includes central, neurohumoral and peripheral effects.

Structural modification of the ergoline skeleton allows the modulation of these activities by selectively enhancing some and suppressing other, leading to a number of clinically useful agents such as the memory enhancer Nicergoline or the selective serotonin antagonists Metergoline and Methylsergide employed for the treatment of migraine. [4]

Among the multiple receptor interactions, the serotoninergic component is one of the most common, being present in natural or semisynthetic ergoline derivatives and plays a noticeable role in determining the pharmacological profile [5].

With the aim of identifying selective 5-HT_{1A} ligands structurally unrelated to the classes of azapirones and aminotetralines, our attention was focused on the 5-HT_{1A} component present to different extents in simple ergoline compounds.

From a structural point of view, the ergoline skeletons can be envisioned as a set of rigid serotonin conformers.

With this in mind, a set of ergoline structures with different ring size, degree of unsaturation and stereochemistry was designed considering these parameters important for the interaction with the receptor.

The different degree in constraint and structural homogeneity make these compounds valid tools for probing the spatial requirements for 5-HT_{1A} binding.

There is, in fact, a clear advantage in using rigid compounds for the definition of a pharmacophore since the conformation of a flexible molecule, when bound to a receptor, is almost always experimentally unknown and, when resorting to theoretical approach, the calculated minimum energy conformation of the molecule might not be the same as when it is bound to the receptor.

Many structure-activity relationship (SAR) and structure-affinity relationship (SAFIR) studies of 5-HT_{1A} receptor ligands have been reported in the literature.

These articles have been recently reported and examined in some reviews, one of the most comprehensive of which is the paper by R.A.Glennon [3] where 86 references were examined in detail.

Some of these studies led to the definition of a pharmacophore and a three dimensional map of the 5-HT_{1A} recognition site.

Hilbert and coworkers [6,7] proposed two separate pharmacophore models for 5-HT_{1A} agonist and antagonist ligands based on agents of different chemical classes.

The pharmacophore proposed in the above mentioned articles consists of a basic nitrogen at a distance “d” from the centroid of an aromatic ring and at a height “h” from the plane of the same aromatic ring.

The values “d” and “h” were determined to be 5.3 Å and 0.2 Å for the agonistic model, 5.6 Å and 1.6 Å for the antagonistic model.

As stated by the authors, these pharmacophore models represent the minimum necessary structural features for recognition of the receptor, but the presence of these features might not be sufficient for an effective interaction with the $5HT_{1A}$ recognition site.

The present work aims at improving on the definition of existing pharmacophore model for the affinity to the 5-HT_{1A} receptor to the extent of possibly being able to discriminate between compounds with good affinity for the 5-HT_{1A} receptor from compounds with low or no affinity.

We believe that the issue of defining an agonist and antagonist model of the 5-HT_{1A} receptor is not settled yet since the models that have appeared in the literature were constructed in some cases employing partial agonists such as buspirone. The ergolines and abeoergolines used in this study proved to be agonists, even though some of these compounds presented just those “d” and “h” values reported as characteristic of an antagonist pharmacophore model [7].

The pharmacophoric distances of the rigid templates used in this work did not support the conclusions of the 3-D QSAR CoMFA study of A.Agarwal and E.W.Taylor [8], where the differences between 5-HT_{1A} agonist and antagonist pharmacophores were described as consistent with the models proposed by Hibert [7].

Materials and Methods

The compounds considered in this study were prepared according to the following procedures: compounds 9, 1, 2, and 7, 8, were obtained by $NaBH_4$ reduction of 1-methyl-10α-methoxy-dihydrolysergic acid methyl ester, 10α-methoxy-dihydrolysergic acid methyl ester, dihydrolysergic acid methyl ester and lysergic acid methyl ester, respectively.

In the latter case the basicity of the medium led to a partial epimerization of the substrate affording isolysergol compound 8 besides lysergol 7.

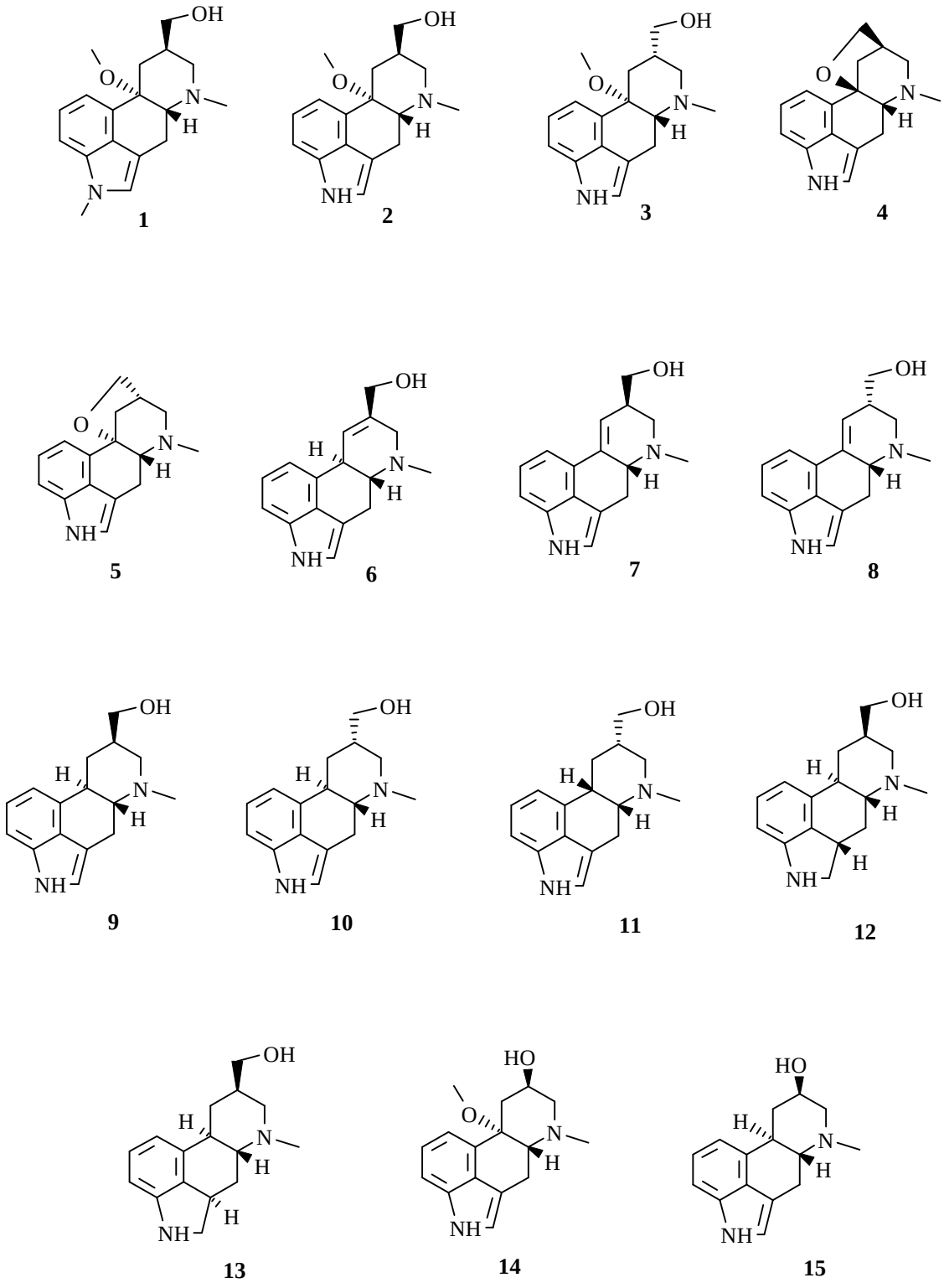
Compounds 3, and 10 were obtained by $LiAlH_4$ reduction performed on the corresponding dihydroisolysergic acid methyl ester, obtained by epimerization of the corresponding dihydrolysergic acid methyl ester, by means of lithium diisopropylamide at low temperature and subsequent quenching with methanol.

Compound 4 and 5 were obtained by photochemical ring closure of compounds 7 and 8.

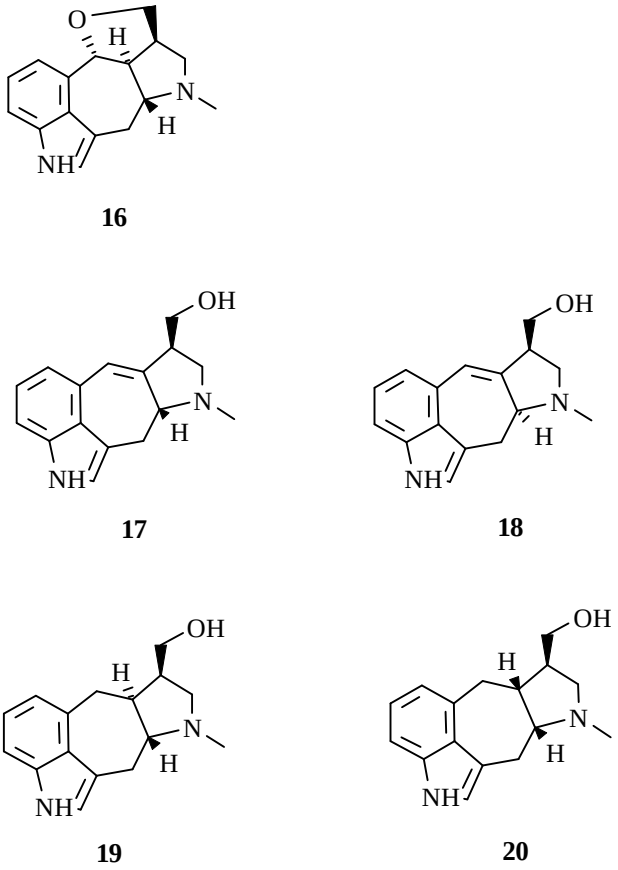
Compounds 12 and 13 were obtained by reduction of compound 9 by means of $NaBH_4$ in trifluoroacetic acid.

Compound 11 was obtained in highly diastereoselective manner by catalytic hydrogenation of 8 in acidic medium.

Oxidative hydroboration of lysergic acid methyl ester afforded with a high degree of regio- and stereoselectivity a diol, which was monoacetylated at the primary hydroxyl group.



Scheme 1. *Ergoline derivatives.*



Scheme 2. *Abeoergoline derivatives.*

Subsequent treatment of the latter with $POCl_3$ in pyridine gave rise through a Grob fragmentation followed by a Cope rearrangement to the (5R,S) abeo derivatives 17 and 18 which structures and absolute stereochemistry was established by chemical correlation supported by spectroscopic and chiroptical data [9].

Catalytic hydrogenation of compound 17 gave rise to compounds 19 and 20 whilst photochemical ring closure led to compound 16.

The three-dimensional structures of the compounds were built using the Builder Module of the modeling program InsightII version 2.3 (Biosym) and then minimized with molecular mechanics calculation performed with Discover version 2.9 (Biosym). The minimizations were performed with Discover because of its better performance in reproducing the ergolinic ring scaffold when compared to the x-ray crystal structures of known ergolinic compounds.

In particular the structures of compounds 7 and 9, minimized with the molecular mechanics program of Sybyl (Tripos) and Discover (Biosym) and with the semiempirical MNDO method, were compared with the x-ray crystal structures of Bromocriptine and 8β-(benzyloxycarbonyl-aminomethyl)-6-methyl-10α-ergoline (these latter structures were obtained from the Cambridge Structural Database).

All the calculations and modeling were performed on Silicon Graphics workstations.

The affinity was measured by displacement of [3H]-8-OH-DPAT, the prototype of selective 5-HT_{1A} ligands [10].

The functional 5-HT_{1A} activity of the ergoline derivatives considered in this study has been assessed by measurements of cAMP levels in transfected HeLa cells.

Serotonin 5-HT_{1A} receptors are negatively coupled with adenylyl cyclase through a Gi protein and the decrease in cAMP levels is detected when an agonist stimulates these receptor e.g. it will inhibit the production of cAMP, whereas antagonists are able to counteract this effect.

The data obtained clearly showed that all the compounds considered exert a functional 5-HT_{1A} agonistic activity that correlate with the different affinity.

Moreover, for compounds displaying 5-HT_{1A} nM affinity a noticeable 5-HT syndrome was revealed in vivo fully confirming the biochemical result [11].

The set of compounds reported in Schemes 1 and 2 was divided into two subsets in accordance with their affinity values, subset 1 formed by compounds with good affinity: IC_{50} μM <0.5 and subset 2 formed by compounds with low or no affinity: IC_{50} μM >1.

Results and Discussion

The minimized structures of subset I and II were superimposed taking compound 7 (lysergol) as the reference molecule because of its high affinity to 5-HT_{1A} and because it is one of a family of natural clavines.

The criterion for superposing the molecules in order to compare their structural features was chosen to be the least square fitting procedure, considering, for the matching process, the normal to the aromatic ring (a 2 Å long vector centred on the phenyl ring centroid), the basic nitrogen atom and the lone pair of the nitrogen itself.

This fitting procedure reflects the fundamental pharmacophore features required for the binding to the 5-HT_{1A} receptor, which are an aromatic ring and a basic nitrogen with its lone pair.

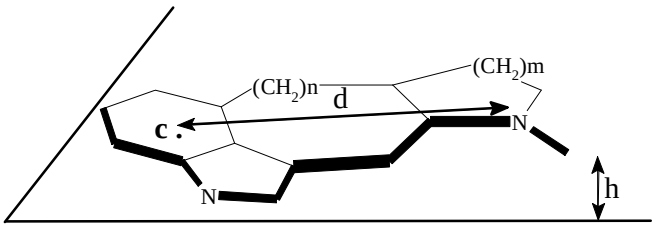


Fig.1. *Schematic drawing of ergoline and abeoergoline skeleton. "d" represents the distance of the basic nitrogen to the centroid of the aromatic ring. "h" the distance of the nitrogen atom to the plane of the aromatic ring.*

The minimized structures of the molecules were superposed calculating the corresponding RMS values and deriving the values of the descriptors that define the general 5-HT_{1A} pharmacophore model: the distance "d" of the basic nitrogen to the centroid of the aromatic ring and the distance "h" of the nitrogen atom to the plane of the aromatic ring (see Fig.1)

In Table 1 the values of "d" and "h" and the RMS deviations are reported for each compound along with the 5-HT_{1A} affinity expressed as IC_{50} (μM).

From the data of Table 1 it is possible to note that the compounds of subset 1 have values for the distance "d" in the range 5.2-5.8 Å, and height "h" in the range 0.9 below the plane to 1.5 Å above the plane.

It is interesting to observe that, with the exception of compound 11, also the compounds of subset 2 (low or no affinity) meet the fitting requirements found for the subset 1.

From this observation, it was deduced that the pharmacophore model based on the two vectors "d" and "h" was not sufficiently precise to discriminate, within this set of molecules, the active from the inactive compounds in terms of affinity.

An attempt was made to find other possible geometrical indicators, such as the distance of the nitrogen's lone pair to the indole nitrogen or to the centroid of the benzene ring, in order to explain the result obtained, but no correlation was found among these indicators and the binding data.

The observation of the superimposed three dimensional structures of the active and inactive compounds led us to hypothesize that the compounds of subset 2 would have the same pharmacophore characteristics as the active compounds, but occupy a volume required by the receptor.

Table 1. *Affinity is expressed as IC_{50} (fM). "d" represents the distance of the basic nitrogen to the centroid of the aromatic ring; "h" represents the distance of the nitrogen atom to the plane of the aromatic ring. Positive and negative values refer to distances above and below the plane respectively. For each compound it is reported the RMS deviations taking lysergol as reference compound.*

Compound	Subset	IC_{50} (fM)	"d"(A°)	"h"(A°)	RMS
1	2	>10	5.31	0.76	0.1159
2	2	2.33	5.31	0.78	0.1159
3	2	>10	5.31	0.76	0.1478
4	2	8.56	5.32	0.44	0.2798
5	2	6.9	5.34	0.41	0.0401
6	1	0.05	5.30	0.30	0.1418
7	1	0.03	5.33	0.48	—-
8	1	0.021	5.34	0.46	0.0084
9	1	0.25	5.30	0.38	0.1383
10	1	0.23	5.31	0.42	0.1456
11	2	>10	4.55	-1.84	0.7982
12	1	0.43	5.27	- 0.90	0.3081
13	1	0.32	5.22	1.55	0.1561
14	2	3.41	5.31	0.78	0.1142
15	1	0.49	5.31	0.39	0.1343
16	1	0.03	5.82	-0.23	0.2317
17	1	0.02	5.75	1.02	0.2042
18	2	>10	5.77	-1.08	0.6605
19	1	0.029	5.82	0.36	0.2282
20	2	1.36	5.74	1.49	0.2253

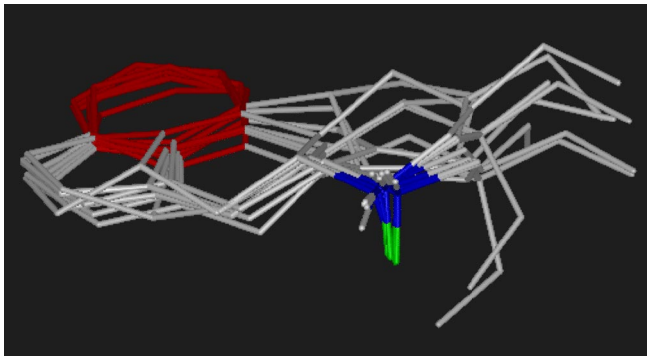


Fig.2. *Superposition of compounds of subset 1 (active) . The lone pair electrons of the basic nitrogen are shown in green, the nitrogen in blue and the aromatic ring in red.*

Figure 2 depicts the superimposition of compounds of subset 1, where the lone pair electrons of the basic nitrogen are also reported while, for clarity, all the hydrogens have been removed.

In this picture it can be noticed that the nitrogen's lone pair of all the compounds points in a direction almost perpendicular to the plane of the aromatic ring.

In figure 3 is reported the superimposition of all compounds of subset 1 and subset 2 (compound 11 and 18 are shown respectively in violet and yellow).

Also in this picture it can be noticed that the nitrogen's lone pair of all the compounds points in a direction which is almost perpendicular to the plane of aromatic ring with the exceptions of compound 11, whose lone pair is found to be apart from the lone pair of the other compounds, and compound 18 whose lone pair points in a direction opposite to the plane. This latter case can be explained by invoking a conformational preference: it was found, in fact, that when the hydrogens of the protonated basic nitrogen were modeled downwards, the energy would increase of about 9 kcal/mole with respect to the corresponding compound with the hydrogens modeled upwards.

In addition minimization by MNDO semiempirical computations [12] starting from both conformers always gave the compound with the lone pair pointing upwards.

For these reasons the two compounds, 11 and 18, have high values of RMS.

In order to verify the hypothesis that some compounds might occupy some space required by the receptor, the union volume formed by all the superimposed compounds of subset 1 was derived. This volume could be defined as the space accessible to ligands in the 5-HT_{1A} recognition site.

By subtracting the volume of each of the inactive compounds from the above defined accessible volume it was found that all the inactive compounds had an excess volume.

The union of all the thus far obtained excess volumes defines locations of space potentially occupied by the receptor, while the mapping of the receptor-essential volume is derived determining the intersection of all the excess volumes.

Figure 4 shows the union of the excess volumes produced by compounds of subset 2, with the exception of the already discussed compounds 11 and 18, while the intersection of the excess volumes of the same compounds is reported in figure 5. This latter volume, which is shared by the all inactive compounds, is of great value and represents the volume with the highest probability of being occupied by the receptor.

Compound 1 even shows an excess volume that corresponds to the CH_3 of the indole nitrogen, in accordance with the fact that compound 1 has a binding value lower than compound 2.

Compound 11, whose "d" and "h" values are not within the range of the pharmacophore feature, also has numerous excess volumes.

We observed that the height "h" is not a very strict requirement for the affinity. Indeed the active compounds 12

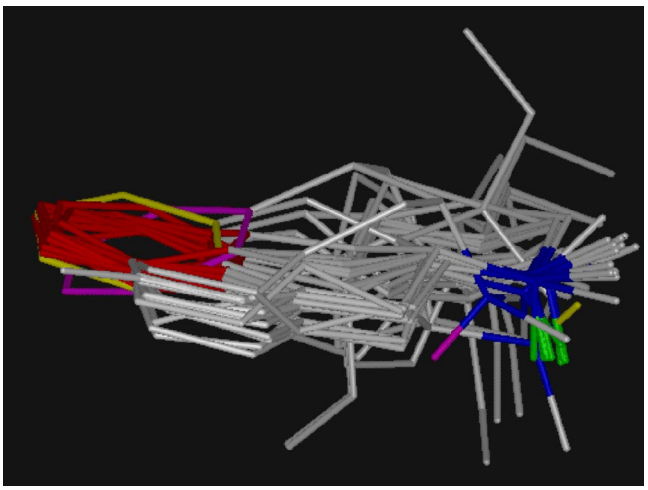


Fig.3. *Superposition of all compounds (subset 1 and subset 2). Compound 11 and 18 are shown respectively in violet and in yellow.*

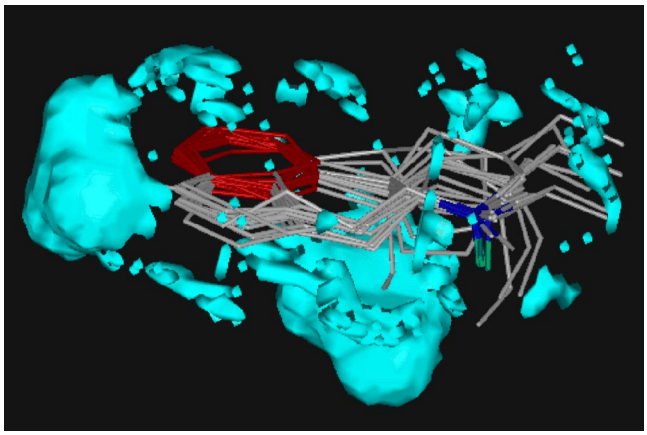


Fig.4. *Union of the excess volumes produced by compounds of subset 2, with the exception of compounds 11 and 18.*

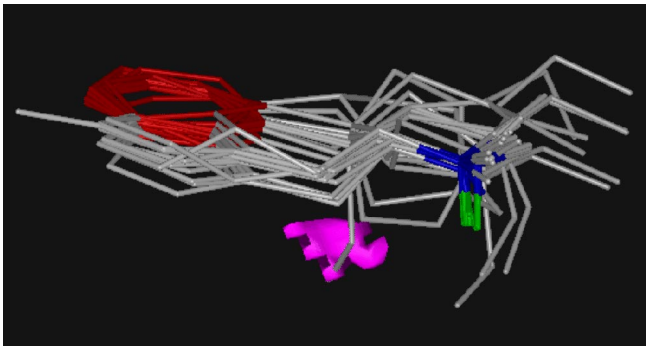


Fig.5. *Intersection of the excess volumes of the compounds of subset 2 (with the exception of compounds 11 and 18).*

and 16 have the basic nitrogen on the opposite side of the plane with respect to the nitrogen of other compounds.

The reduction of indole ring to indoline, exemplified by compounds 9, 12 and 13, leads to a great variation of height "h" but does not produce a meaningful variation in affinity.

Analysing the structure of the three compounds 4, 5, and 16 characterized by an oxygen bridge shows that compound 16, displaying an affinity 200-300 times higher than the other two, is clearly the flattest. The flatness implies a diminished steric hindrance to the surface of the receptor above and below the aromatic ring plane.

The pharmacophore model thus obtained was also validated by superimposition of some non-ergolinic $5HT_{1A}$ ligands. In particular the 5-HT, 8OH-DPAT, and Buspirone were superimposed to our pharmacophore model with good RMS values (< 0.22 Å).

Conclusions

The pharmacophore features necessary for the binding to the $5\text{-}HT_{1A}$ receptor resulting from this study (summarized by the model reported in figure 6) are a basic nitrogen placed at a distance of 5.2-5.8 Å from the centroid of an aromatic ring and at an height from the plane of the ring included in the range of 0.9 Å below the plane to 1.5 Å above the plane.

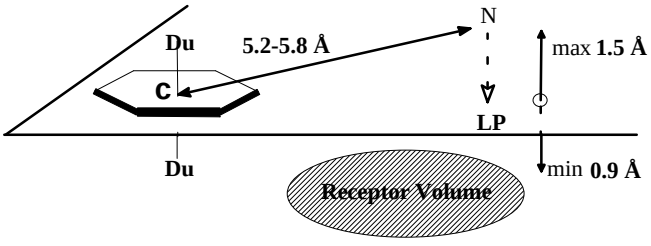


Fig.6. *Basic pharmacophore for the binding to the 5-HT$_{1A}$ receptor.*

The direction of the lone pair must be quasi-perpendicular to the plane of aromatic ring.

In addition the model is characterized by the definition of a localized receptor volume that must not be occupied by the ligand.

The centre of this volume (the intersection of the excess volume of inactive compounds) is localized approximately at 4.0 Å from the basic nitrogen and 3.8 Å from the centroid of the benzene ring and at a distance of 2.2 Å from the plane of benzene ring.

With this model of pharmacophore we are able to verify that among the compounds of subset 2 that satisfy the constraints of "d" and "h" the modulation of activity was due to various degree of occupation of the above mentioned forbidden volume of space. Indeed, the planarity of the molecule seems to be one of the requirements for high affinity.

The broad range of height "h" suggests some degrees of flexibility in the binding site of the receptor.

The model obtained in our work using ergolinic compounds may be considered a general model for $5\text{-}HT_{1A}$ affinity since non-ergoline $5\text{-}HT_{1A}$ ligands can be fitted into it.

The present set of rigid compounds did not confirm the reported $5\text{-}HT_{1A}$ antagonist models because the structures that show the correct pharmacophoric features for the antagonism described in the literature are agonists. The features that define the difference between the $5\text{-}HT_{1A}$ agonist and antagonist pharmacophores must be more subtle than those reported in the literature and work along these lines is currently being conducted in our department.

The model obtained in this SAFIR study will be employed for a rationale design of potential $5\text{-}HT_{1A}$ ligand.

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