EXHAUSTIVE COMFA AND COMSIA ANALYSES AROUND DIFFERENT CHEMICAL ENTITIES: A LIGAND-BASED STUDY EXPLORING THE AFFINITY AND SELECTIVITY Profiles OF 5-HT1A Ligands

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ABSTRACT

The 5-hydroxytryptamine (5-HT1A) receptors represent an attractive target in drug discovery. In particular, 5-HT1A agonists and partial agonists are deeply investigated for their potential role in the treatment of anxiety, depression, ischaemic brain disorder and more recently, of pain. On the other hand, 5-HT1A antagonists have been revealed promising compounds in cognition disorders and, lately, in cancer. Thus, the discovery of 5HT1A ligands is nowadays an appealing research activity in medicinal chemistry. In this work, Comparative Molecular Fields Analysis (CoMFA) and Comparative Molecular Similarity Index Analysis (CoMSIA) were applied on an in-house library of 5-HT1A ligands bearing different chemical scaffolds in order to elucidate their affinity and selectivity for the target. Following this procedure, a number of structural modifications have been drawn for the development of much more effective 5-HT1AR ligands.
Introduction

Serotonin (5-hydroxytryptamine, 5-HT) represents a key neurotransmitter playing a fundamental role especially at the central nervous system (CNS), being involved in numerous behavioural processes, such as anxiety, mood, aggression, addictive behaviours, as well as memory and learning processes, sleep, thermoregulation, appetite, emesis, thalamic blood pressure, nociception and migraine headaches. Up to now, at least 14 receptor subtypes (5-HT1 to 5-HT7) have been identified and therefore classified in terms of amino acid sequence and biological function. Among them, the 5-HT1A receptor was the first one to be identified and pharmacologically investigated, relying on a number of ligands employed as pharmacological tools, such as BMY-7378 and WAY-100635 (Figure 1). From a pharmacological point of view, the most interesting therapeutic potential of 5-HT1A agonists and partial agonists revolves around the treatment of anxiety, depression, ischaemic brain disorder and, more recently, in pain. On the contrary antagonists of this receptor proved to be promising in cognition disorders, like Alzheimer's disease therapy and, lately, in cancer.

On the other hand, due to the high pairwise similarity percentage between 5-HT1A and 1-adrenoreceptor (ADRs), a number of 5-HT1A ligands proved to efficiently bind also to ADRs, at the expense of selectivity, as shown for instance in Figure 1 around the references BMY-7378 and WAY-100635. The 1-adrenergic receptors (1-ADRs) are deeply involved in the modulation of the activity of the sympathetic nervous system, becoming relevant druggable targets for many therapeutic agents. 1-ADRs are classified into at least three subtypes named 1A, 1B and 1D. Antagonists of these receptors have been initially introduced for hypertension management, being later explored also for the treatment of benign prostatic hyperplasia (BPH).

During the last years, we focussed our attention on the development of new classes of potent 5-HT1A receptor ligands, including 1,3-dioxolane-, 1,3-oxathiolane-, 1,3-dithiolane-, spiro-dioxolane-tetrahydrofuran-, cyclopentanone- and cyclopentanol-based derivatives, whose 5-HT1A versus ADRs selectivity profiles were variable being in particular overall lower those towards the 1D subtype. Here, with the aim of further exploring the key requirements to enhance the serotoninergic affinity and selectivity over 1D ADR, a three-dimensional quantitative structure–activity relationship (3D-QSAR) ligand-based computational protocol was applied to the aforementioned in-house library of compounds. In particular, we performed Comparative Molecular Fields Analysis (CoMFA) and a Comparative Molecular Similarity Index Analysis (CoMSIA), which represent useful tools to investigate the affinity as well as the selectivity profiles of compounds.

The role played by steric, electrostatic, H-bond acceptor and donor and hydrophobic features with respect to the 5-HT1A affinity and selectivity trends observed within the library of ligands suggested some structural modifications which could be useful for the development of new chemical scaffolds with an improved affinity and selectivity profile for 5-HT1A receptor. The reliability of the final models was consistent with the effectiveness of an external series of 5-HT1A ligands, giving a further validation of the 3D-QSAR studies here discussed. Moreover the results allowed us to derive robust statistical models to be used to predict the affinity of new analogues prior to synthesis and therefore to pave the way for the further design of more promising derivatives.

Methods

Dataset

In this work, the employed dataset included 81 compounds already synthesised by some of us (compounds 1–91). These molecules were divided into two groups according to the different linker between the five-membered ring and the terminal aromatic moiety of the scaffold, as shown in Tables 1 and 2. Group A (1–42; Table 1) included flexible open linker derivatives, while Group B (43–91; Table 2) contained all the piperazine ones. Tables 1 and 2 are also listed the 5HT-1A pKi and the 1-ADR pKs. Any details concerning the biological assays performed to evaluate the compounds affinity towards 5HT-1A receptors and ADRs have been reported in the previously cited works.

Any compound was built, parameterised (Gasteiger-Hückel method) and energy minimised within MOE using MMFF94 force field.

Notably, since all the analysed compounds displayed at least one chiral carbon atom, the selection of the most proper enantiomer was required for the further ligand-based studies. As far as we knew from our previous experimental studies on enantiomeric resolution of compound 1, the stereoselectivity of the derivative was poor being (S)-1 5-HT1A pKi = 8.42 and (R)-1 5-HT1A pKi = 8.52.

For this reason, we considered to rely on these data and therefore to select for the in silico design (and alignment) those enantiomers along the whole dataset which proved to be also recommended by our docking results, as we previously detailed.

Briefly, within both Group A and B diphenyl-substituted dioxolane compounds were selected as 1R enantiomers (as suggested by the enantiomeric resolution of (1) as well as for the tetrahydrofurans and cyclopentanones, while the cis and trans mono-phenyl substituted ones followed the preferred 1S,1R and 1R,1S conformers. The cis and trans isomers of the cyclopentanol-based derivatives of Group A were recommended as 2R,2S and 2S,2R enantiomers. Those belonging to Group B were selected as 3S,3R and 3R,3S enantiomers. Lactam- and imide-based molecules were
Table 1. Chemical structures of compounds 1–42 (Group A).

| Comp. | R₁ | n | R₂ | Y | R₃ | 5-HT₁A pKᵢ | α₁-D pKᵢ |
|-------|----|---|----|---|----|-------------|-----------|
| 1     |     | 1 | H  | -CH₂CH₂O- |   | 8.45        | 8.37      |
| 2     |     | 1 | H  | -CH₂CH₂O- |   | 9.22        | 8.65      |
| 3     |     | 1 | CH₃| -CH₂CH₂O- |   | 6.32        | 6.06      |
| 4 cis|     | 1 | H  | -CH₂CH₂O- |   | 5.93        | 7.09      |
| 5 trans|    | 1 | H  | -CH₂CH₂O- |   | 5.70        | 6.94      |
| 6     |     | 1 | H  | -CH₂CH₂S-  |   | 7.03        | 6.87      |
| 7     |     | 1 | H  | -CH₂CH₂NH- |   | 6.56        | 6.49      |
| 8     |     | 1 | H  | -CH₂CH₂CH₂- |   | 8.56        | 6.28      |
| 9     |     | 1 | H  | -CH₂CH₂CH₂- |   | 8.10        | 6.86      |
| 10    |     | 1 | H  | -CH₂CH₂O-  |   | 7.33        | 6.49      |
| 11    |     | 1 | H  | -CH₂CH₂O-  |   | 7.34        | 6.69      |
| 12    |     | 1 | H  | -CH₂CH₂O-  |   | 8.58        | 6.61      |
| 13    |     | 1 | H  | -CH₂CH₂O-  |   | 7.01        | 6.90      |
| 14    |     | 1 | H  | -CH₂CH₂O-  |   | <6          | 6.77      |
| 15    |     | 1 | H  | -CH₂CH₂O-  |   | 8.12        | 6.64      |
| 16    |     | 1 | H  | -CH₂CH₂O-  |   | 8.99        | 8.68      |

(continued)
| Comp. | R₁ | n | R₂ | Y          | R₃      | 5-HT₁₅ pKᵢ | α₁-DA pKᵢ |
|-------|----|---|----|------------|---------|-------------|------------|
| 17    |    | 1 | H  | CH₂CH₂O⁻  |         | 8.55        | 7.37       |
| 18    |    | 1 | H  | -CH₂CH₂⁻ |         | 8.72        | 6.66       |
| 19    |    | 1 | H  | -CH₂O⁻  |         | 9.89        | 7.50       |
| 20    |    | 1 | H  | CH₂⁻  |         | 8.61        | 7.05       |
| 21    |    | 1 | H  | -CH₂CH₂⁻ |         | 8.00        | 6.36       |
| 22    |    | 1 | H  | -CH₂O⁻  |         | 8.66        | 6.10       |
| 23    |    | 1 | H  | -CH₂O⁻  |         | 7.54        | <5         |
| 24    |    | 1 | H  | -CH₂O⁻  |         | <6          | 5.08       |
| 25    |    | 1 | H  | -CH₂O⁻  |         | 9.05        | 7.91       |
| 26    |    | 1 | H  | -CH₂O⁻  |         | 8.08        | 6.77       |
| 27    |    | 1 | H  | -CH₂O⁻  |         | 8.77        | 7.59       |
| 28    |    | 1 | H  | -CH₂O⁻  |         | 7.98        | 6.30       |
| 29 cis|    | 1 | H  | -CH₂O⁻  |         | 8.03        | 6.88       |
| 30 trans| | 1 | H  | -CH₂O⁻  |         | 8.02        | 6.86       |
| 31    |    | 1 | H  | -CH₂O⁻  |         | 8.46        | 6.54       |
| 32 trans| | 1 | H  | -CH₂O⁻  |         | 9.49        | 8.42       |
| 33    |    | 1 | H  | -CH₂O⁻  |         | 9.08        | 8.09       |
chosen as \(\alpha R, \alpha S\) isomers, while spiro-derivatives were preferred as \(\alpha R, \alpha R\).

Consequently, in order to apply a common and homogeneous protocol, for all the dataset compounds we assigned the 5-HT\(_{1A}\) p\(K_i\) of the racemic mixture to the preferred enantiomers.

The followed alignment process considered the existing differences in conformation due to the various flexibility of Group A and B molecules. Initially, the most probable conformers of compounds 2 and 43 (taken as references for Group A and B, respectively) were superimposed using the rigid body alignment protocol implemented in MOE. Therefore, the flexible alignment approach of MOE was applied to guide a proper alignment of all the compounds of each group on the already aligned derivatives, used as rigid templates. Successively, the dataset was submitted to 3D-QSAR analyses, including CoMFA\(^{25}\) and CoMSIA\(^{26}\) studies, by Sybyl-X 1.0 software\(^{27}\).

### 3D-QSAR analyses

CoMFA and CoMSIA approaches were performed to better understand especially how the steric and electrostatic parameters as well as the hydrophobic, H-bond acceptor and H-bond donor descriptors could modulate the selectivity over \(\alpha_{1D}\) ADR (Model I) and the affinity for 5-HT\(_{1A}\) receptor (Model II) of the whole dataset here proposed. Both the Models I and II were built considering the selectivity-based weighted 5-HT\(_{1A}\) p\(K_i\) (Model I) and the experimental 5-HT\(_{1A}\) p\(K_i\) (Model II) as the dependent variables while the different specific CoMFA and CoMSIA descriptors were taken into account as independent ones.

In particular, for Model I, the ligand affinity towards the 5-HT\(_{1A}\) receptor has been re-calculated by taking into account the selectivity over \(\alpha_{1D}\) ADR, following a procedure we recently applied around the development of selective PDE4B\(^{16}\) and PDE7 inhibitors\(^{21}\).

In this case, we considered the difference in p\(K_i\) between the 5-HT\(_{1A}\) and \(\alpha_{1D}\) ADR and the related ratio, obtaining a selectivity-based weighted 5-HT\(_{1A}\) p\(K_i\), which are described as follows. Equation (1) was applied for those compounds displaying higher or comparable \(\alpha_{1D}\) ADR and 5-HT\(_{1A}\) p\(K_i\) while Equation (2) for the most selective 5-HT\(_{1A}\) ligands.

\[
\text{Weighted 5-HT}_{1A} \, pK_i = (5-HT_{1A} \, pK_i \times \text{MR}) + \left(\frac{\text{5-HT}_{1A} \, pK_i - \alpha_{1D} \, pK_i}{\text{MD}}\right)
\]

MR is the mean of the ratio between the 5-HT\(_{1A}\) and \(\alpha_{1D}\) p\(K_i\) values observed within all the molecules characterised by MR values \(<1.0\) and MD the mean of the difference between the 5-HT\(_{1A}\) and the \(\alpha_{1D}\) p\(K_i\) values of the same set of compounds.

\[
\text{Weighted 5-HT}_{1A} \, pK_i = 5-HT_{1A} \, pK_i \times \text{MR} + \left(\frac{\text{5-HT}_{1A} \, pK_i - \alpha_{1D} \, pK_i}{\text{MD'}}\right)
\]

MR is the mean of the ratio between the 5-HT\(_{1A}\) and the \(\alpha_{1D}\) p\(K_i\) values observed within all the molecules characterised by MR spanning from 1.0 to 1.2 and MD’ the mean of the difference

| Comp. | \(R_1\) | \(n\) | \(R_2\) | \(Y\) | \(R_3\) | 5-HT\(_{1A}\) p\(K_i\) | \(\alpha_{1D}\) p\(K_i\) |
|-------|--------|------|--------|------|--------|----------------|----------------|
| 34 E  | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)CH=CH- | | 7.63 | 6.68 |
| 35    | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)CH\(_2\)- | | 7.32 | 6.53 |
| 36    | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)- | | <6  | 6.04 |
| 37    | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)CH\(_2\)CH\(_2\)- | | 7.37 | 6.46 |
| 38    | \(\alpha R, \alpha R\) | 2    | H      | -CH\(_2\)CH\(_2\)- | | 7.24 | 6.65 |
| 39    | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)O- | | 7.43 | 6.54 |
| 40    | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)O- | | 6.90 | 7.31 |
| 41    | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)O- | | 7.22 | 7.68 |
| 42    | \(\alpha R, \alpha R\) | 1    | H      | -CH\(_2\)O- | | 6.96 | 7.12 |
Table 2. Chemical structure of compounds 43–91 (Group B).

| Comp. | R₁ | pKi | Com | R₁ | pKi |
|-------|----|-----|-----|----|-----|
| 43    | ![Structure](image1.png) | 7.64 | 8.14 | 68  | ![Structure](image2.png) | 6.93 | n.d. |
| 44 cis| ![Structure](image3.png) | 7.52 | 8.11 | 69  | ![Structure](image4.png) | 7.09 | 7.20 |
| 45 trans| ![Structure](image5.png) | 7.63 | 7.86 | 70  | ![Structure](image6.png) | 6.80 | 6.89 |
| 46 cis| ![Structure](image7.png) | 7.31 | 7.54 | 71  | ![Structure](image8.png) | 7.84 | 8.60 |
| 47 trans| ![Structure](image9.png) | 8.75 | 8.16 | 72  | ![Structure](image10.png) | 7.80 | 7.43 |
| 48 cis| ![Structure](image11.png) | 7.73 | 7.80 | 73  | ![Structure](image12.png) | 8.05 | 6.87 |
| 49 trans| ![Structure](image13.png) | 8.22 | 7.55 | 74  | ![Structure](image14.png) | 7.23 | 7.45 |
| 50    | ![Structure](image15.png) | 8.26 | 7.52 | 75  | ![Structure](image16.png) | 7.30 | 7.29 |
| 51    | ![Structure](image17.png) | 8.18 | 7.33 | 76  | ![Structure](image18.png) | 7.69 | 7.02 |
| 52    | ![Structure](image19.png) | 8.46 | 6.43 | 77  | ![Structure](image20.png) | 7.44 | 6.71 |
| 53 cis| ![Structure](image21.png) | 9.25 | 7.21 | 78  | ![Structure](image22.png) | 7.37 | 7.38 |
| 54 trans| ![Structure](image23.png) | 9.10 | 6.82 | 79  | ![Structure](image24.png) | 7.19 | 6.48 |
| 55 trans| ![Structure](image25.png) | 7.95 | 7.43 | 80  | ![Structure](image26.png) | 6.49 | 7.50 |

(continued)
| Comp. | R₁ | pKᵢ | αᵢ₁D | pKᵢ | αᵢ₁D |
|-------|----|-----|-------|-----|-------|
| 56 cis | 8.16 | 6.79 | 8.18 | 7.18 |
| 57 trans | 8.14 | 7.75 | 8.28 | 7.05 |
| 58 cis | 8.25 | 7.57 | 7.57 | 6.96 |
| 59 cis | 8.29 | 6.42 | 7.36 | 7.98 |
| 60 trans | 7.59 | 7.78 | 7.61 | 8.02 |
| 61 cis | 8.50 | 6.19 | 7.16 | 7.36 |
| 62 trans | 7.35 | 7.57 | 7.43 | 7.37 |
| 63 cis | 8.58 | 6.78 | 7.22 | 7.27 |
| 64 trans | 8.90 | 8.51 | 7.48 | 7.26 |
| 65 | 8.29 | 7.88 | 7.10 | 7.29 |
| 66 | 7.33 | 7.33 | 7.06 | 6.53 |
| 67 | 8.03 | 6.84 | | |
between the 5-HT1A and the \( \alpha_{1D} \) pKi values of the whole dataset. For molecules displaying MR > 1.2 this term is approximated to 1.0.

Following this procedure, the variation of the weighted pKi values within the dataset concerns 5 log orders, being most of the selective compounds endowed with a weighted pKi spanning from 8.00 up to 10.00. Conversely, the unselective (or reversed selective) molecules fall in the range of 5.00–8.00.

Notably, it is expected that such a weighted parameter could be much more predictive of the ligand selectivity profile over the \( \alpha_{1D} \) ADR subtype observed within the chemical space disclosed around the whole dataset here investigated.

### Training set and test set

Concerning Model I, the overall dataset was divided into a training set (68 compounds), for model generation, and in a test set (22 compounds), for model validation, being compound 68 excluded since it was not evaluated for \( \alpha_{1D} \) ADR activity. For the development of Model II, 73 and 18 molecules were assigned to the training set and the test set, respectively.

For both the models, compounds were divided (in training and the test sets) manually in order to include a representative range of biological activities and structural variations of the starting dataset and including in the test set at least 25% of the number of training set molecules. Binding affinity values (pKi) range of the compounds covered at least 4 log orders of magnitude.

### CoMFA and CoMSIA models and statistical evaluation

CoMFA and CoMSIA methods are widely used 3D-QSAR techniques which allow to relate any variation of an experimental parameter (dependent variable) within a series of compounds with respect to specific descriptors (independent variables). In details, the steric and electrostatic fields and also the steric, electrostatic, hydrophobic, H-bond donor, H-bond acceptor ones were employed for CoMFA and CoMSIA analyses, respectively. Starting from a proper molecule alignment within a 3D cubic lattice (with a 2 Å grid spacing), the descriptor was calculated, using the standard Tripos force field method. Successively, the derived model goodness and reliability were evaluated using specific statistical tools, such as partial least square (PLS) analysis and cross-validation methods.

Finally, the predictive ability concerning those compounds belonging to the test set \( r^2_{\text{pred}} \) was also calculated, by means of the following equation:

\[
 r^2_{\text{pred}} = \frac{(SD - PRESS)}{SD}
\]

being SD and PRESS the sum of the squared deviations between the biological activities of the test set molecules and the mean activity of the training set compounds, and the squared deviation between the observed and the predicted activities of the test set compounds, respectively.

Any further detail concerning the (standard) CoMFA and CoMSIA protocols and the related statistical and predictive evaluation we applied were previously reported in a number of our works.

### Results

CoMFA and CoMSIA analyses here reported were used to explore, through quantitative methods, the main features responsible of the activity of an in-house library of 91 compounds as 5-HT1AR ligands, and also of their related selectivity profile over \( \alpha_{1D} \) ADR.

As shown in Figure 2, the whole dataset included molecules displaying a basic nitrogen, incorporated in a linear (in particular ethanolamine, cysteamine, ethylenediamine, methylamine, ethylamine, propylamine, butylamine, allylamine) or piperazine moiety, connecting a five-membered heterocycle or carbocycle (in particular dioxolane, 1,3-oxathiolane, 1,3-dithiolane, tetrahydrofurane, cyclopentanone, cyclopentanol and spiro-dioxolane) bringing at least a phenyl ring or a spiro appendage. The members of the library were divided into two groups, A and B, depending on whether the basic nitrogen is part of the flexible chain or is incorporated into the piperazine ring (Tables 1 and 2, respectively).

Energy minimisation and alignment of the dataset highlighted a relevant difference in the preferred conformation between Group A and B. In particular, for the molecules of Group A, a U-shaped conformation was the energetically favoured one, while a much more linear disposition was found for Group B compounds (Supplemental data S1). On the overall alignment, we performed two series of 3D-QSAR analyses.

In detail, the first CoMFA and CoMSIA study (Model I) was performed around the selectivity profile over \( \alpha_{1D} \) using a weighted pKi (as described in the experimental section) while the second one analysed 5HT1A potency trend using the ligands experimental 5-HT1A affinity values (Model II).

### 3D-QSAR analyses

Concerning Model I CoMFA and CoMSIA analyses were performed by dividing compounds 1–91 excluding 68 into a training set for...
model generation and into a test set for model validation (see Methods section). A graphical distribution of the selectivity (green dots) and affinity profile (blue dots) of the dataset compounds is shown in Figure 3.

Model II CoMFA and CoMSIA studies were calculated including sixty-eight derivatives into the training set and choosing the other molecules for the test set.

All statistical parameters supporting the two series of 3D-QSAR analyses (Models I and II) are reported in Table 3 and detailed as follows.

The final Model I CoMFA was generated by employing non-cross-validated PLS analysis with the optimum number of components (ONC = 7) to give a non-cross validated $r^2 (r^2_{ncv}) = 0.94$, a test set $r^2 (r^2_{pred}) = 0.80$, standard error of estimate (SEE) = 0.278, steric contribution = 0.678 and electrostatic contribution = 0.322.

The related CoMSIA analysis was derived using a statistical PLS analysis leading to the following results: ONC = 6, a non-cross validated $r^2 (r^2_{ncv}) = 0.92$, a test set $r^2 (r^2_{pred}) = 0.78$, SEE = 0.173, electrostatic contribution = 0.139, hydrophobic contribution = 0.358, H-bond acceptor = 0.213 and H-bond donor = 0.117.

An overall overview of the predictive ability of Model I study can be obtained from graphical distributions of the predicted weighted pKi values of the training set (blue dots) and test compounds (red dots), as shown in Figure 4. The corresponding experimental-based and predicted pKi values are reported as Supplemental data S2 and S3.

The selected CoMFA Model II was generated by employing non-cross-validated PLS analysis with the ONC = 6 to give a non-cross validated $r^2 (r^2_{ncv}) = 0.93$, a test set $r^2 (r^2_{pred}) = 0.79$, SEE = 0.312, steric contribution = 0.173, electrostatic contribution = 0.139, hydrophobic contribution = 0.358, H-bond acceptor = 0.213 and H-bond donor = 0.117.

The related CoMSIA analysis was derived using a statistical PLS analysis leading to the following results: ONC = 6, a non-cross validated $r^2 (r^2_{ncv}) = 0.91$, a test set $r^2 (r^2_{pred}) = 0.77$, SEE = 0.280, steric contribution = 0.175, electrostatic contribution = 0.155, hydrophobic contribution = 0.352, H-bond acceptor = 0.214 and H-bond donor = 0.104.

The derived distributions of the predicted pKi values of the training set and test compounds are depicted in Figure 5 while the specific experimental and predicted affinity values are reported as Supplemental data S4 and S5.

### Discussion

**CoMFA and CoMSIA contour maps**

Notably, for both the Models I and II, the CoMFA steric map descriptors underline through green polyhedra those areas which prove to be favourable in terms of steric hindered substitutions, while yellow maps highlight regions where bulky decorations impaired the 5-HT1A selectivity (for Model I) or affinity (for Model II) profile. The CoMFA electrostatic descriptors are shown as blue
areas around regions which tolerate electropositive groups, while red polyhedra occupy any area where electronegative groups enhance the selectivity (or affinity) towards the target.

Concerning CoMSIA analyses, the yellow and white hydrophobic contours suggest the insertion of lipophilic and polar groups, respectively.

Finally, the introduction of H-bond acceptor and H-bond donor moieties is encouraged or discouraged by magenta and cyan and green (or red for Model II) and purple polyhedral, respectively.

**Model I (5HT \textsubscript{1A} ligand selectivity over \textit{a} \textsubscript{1D} ADR)**

Based on an overall analysis of the CoMFA steric map, it could be observed that those compounds belonging to Group A much more properly fit the contour map, rather than the Group B analogues (see Figure 6(a) and (b)). Indeed, the presence of a flexible linker allows to arrange R1 of Group A compounds in proximity of a large favoured green area, while the piperazine ring of Group B moves this substituent to a quite switched positioning.

As a consequence, for Group A the diphenyl-substituted derivatives are adequately projected towards the aforementioned large green area, and also close to a second small one, while the spiro-derivatives 40–42 fall in a disfavoured yellow area (Figure 6(a)).

Accordingly, most of the diphenyl-substituted compounds 1–38 are characterised by an acceptable selectivity profile, while spiro-derivatives led sometimes to a reversed potency trend [see 40 (5-HT \textsubscript{1A} pKi \textit{a} = 6.90, \textit{a} \textsubscript{1D} pKi \textit{a} = 7.31) 41 (5-HT \textsubscript{1A} pKi \textit{a} = 7.22, \textit{a} \textsubscript{1D} pKi \textit{a} = 7.68), 42 (5-HT \textsubscript{1A} pKi \textit{a} = 6.96, \textit{a} \textsubscript{1D} pKi \textit{a} = 7.12)]. Nevertheless, spiro-compounds could be optimised in selectivity by proper further
decoration, such as those described within Group B (see compounds 68–78) including bulky H-bond acceptor moieties, gaining new contacts with the green map.

Furthermore, the presence of a proper linker Y, as a propyl chain, optimises the overall flexibility of the molecule and allow the terminal phenyl ring and also R1 to better overlap the favoured steric contours. Consequently, compounds 8 (Y = CH₂CH₂CH₂; 5-HT₁A pKi = 8.56, ₁D pKi = 6.28), 9 (Y = -CH₂CH₂CH₂; 5-HT₁A pKi = 8.10, ₁D pKi = 6.86) and 18 (Y = -CH₂CH₂CH₂; 5-HT₁A pKi = 8.72, ₁D pKi = 6.66) result to be much more selective than the analogues 1 (Y = -CH₂CH₂O; 5-HT₁A pKi = 8.45, ₁D pKi = 8.37), 2 (Y = -CH₂CH₂O; 5-HT₁A pKi = 9.22, ₁D pKi = 8.65) and 17 (Y = -CH₂CH₂O; 5-HT₁A pKi = 8.55, ₁D pKi = 7.37), respectively.

Interestingly, the ligand selectivity does not take advantage from the introduction of any decoration onto the terminal phenyl ring ortho positions. Indeed, moving from 8 (R₃ = phenyl; 5-HT₁A pKi = 8.56, ₁D pKi = 6.28) and 20 (R₃ = phenyl; 5-HT₁A pKi = 8.61, ₁D pKi = 7.05) to the 2-methoxy-substituted congeners 9 (R₃ = 2-methoxyphenyl; 5-HT₁A pKi = 8.10, ₁D pKi = 6.86) and 19 (R₃ = 2-methoxyphenyl; 5-HT₁A pKi = 9.89, ₁D pKi = 7.50), the ligand affinity trend reverses or increases towards both the receptors. This information is also supported by the biological results obtained for 26 (R₃ = 2-methylphenyl; 5-HT₁A pKi = 8.08, ₁D pKi = 6.77), 25 (R₃ = 2-ethoxyphenyl; 5-HT₁A pKi = 9.05, ₁D pKi = 7.91) and 27 (R₃ = 2-isoproxyphenyl; 5-HT₁A pKi = 8.77, ₁D pKi = 7.59).

Concerning the Group B compounds, the piperazine ring constrains the R1 substituent to be oriented towards a disfavoured yellow area, leading most of the ligands far from the green one (Figure 6(b)). In particular, the spiro-substitution is the one that much more deeply impairs the selectivity within the group, as exemplified by 75–77 (5-HT₁A pKi = 7.30–7.44, ₁D pKi = 6.71–7.29), with a poor selectivity profile for 5HT₁-R over ADR-₁D whilst sometimes it results also reversed [see 69–71 (5-HT₁A pKi = 6.80–7.84, ₁D pKi = 6.89–8.60) and 84–86 (5-HT₁A pKi = 7.16–7.61, ₁D pKi = 7.37–8.02)].

Then, only the (potentially small) mono-substituted derivatives are those that appear fitting a green polyhedron. On the other hand, it should be noticed that the choice of introducing a lactam or imide moiety linked to the R1 five-membered ring could provide for a better overall flexibility. In particular, the related cis isomers, rather than the trans ones, achieve a conformation to efficiently contact the steric map. The reliability of this information can be verified comparing the selectivity profile of the cis conformers 56, 58, 59, 61, 63 (5-HT₁A pKi = 8.16–8.58, ₁D pKi = 6.19–7.57) with those of trans ones 55, 57, 60, 62, 64 (5-HT₁A pKi = 7.35–8.90, ₁D pKi = 7.43–8.51).

As shown in Figure 6(c) and (d), the electrostatic contour map reveals favoured region with the introduction of electronegative groups (red polyhedral) in the area surrounding the spiro-derivatives and the diphenyl-substituted ones of Group A, also suggesting the presence of electropositive portions for bulkier groups in R1 (blue area). Interestingly, these results allow to point out the introduction of electro-withdrawing groups onto the spiro-derivatives or the development of
analogs bearing lactam or imide moieties, as displayed within the Group B series.

On the other hand, a small blue area placed around oxygen atom of the linker Y in compounds 12 and 39 (Figure 6(c)) strongly confirms the beneficial role played by the propyl chain at this position (rather than the ethoxy one).

A comparable electrostatic preferences profile can be described for compounds belonging to Group B (see Figure 6(d)). Notably these results are in good agreement with the privileged role played by a lactam or imide moiety at the R1 five-membered ring, as revealed by the steric map.

The steric and electrostatic maps obtained from CoMSIA analysis were in good agreement with those derived from the previous CoMFA study.

Hydrophobic moieties are beneficial (yellow map) around the oxygen atom of tetrahydrofuran and near the corresponding heteroatom of the dioxolane, dithiolane and oxathiolane derivatives, while one disfavoured region (white map) falls around the cyclopentanone or cyclopentanol functional moiety (Figure 7(a)). Consequently, any kind of these five-membered rings appears to have the proper polarity balance to encourage 5HT1A selectivity of action. Mono-substituted or diphenyl-substituted compounds of Group B better surround the hydrophobic yellow map, than the spiro-derivatives, which partially overlap the disfavoured white region (Figure 7(b)). This contour map proves to be reached properly by those compounds endowed with a flexible lactam or imide moieties, being the cis isomers the most favoured (see also the information coming from the steric map).

Magenta areas, favourable for H-bond acceptor moieties, are located in proximity of the two phenyl rings of the disubstituted derivatives of Groups A and B, being also highly overlapped by any lactam and imide moieties in R1, and are placed also in proximity of the spiro-derivatives of Group B (Supplemental data S6). Finally, H-donor favoured regions (cyan polyhedral) are fulfilled by the secondary amine nitrogen atom of the linker displayed within Group A, but not by the piperazine tertiary one (Supplemental data S7). Thus, the flexible linker is confirmed as the better choice to ameliorate the selectivity issue.

**Model II (SHT1A ligand affinity)**

Since the first aim in the development of new SHT1A ligands is the design of compounds with an improved affinity profile, the description of Model II results was organised in four sections, based on the various substitutions displayed by the molecules of interest: (i) part 1, including all the substitutions on the quaternary carbon atom of the five-membered ring; (ii) part 2, consisting of the five-membered ring (R1); (iii) part 3, formed by the linker Y; (iv) part 4, represented by the terminal aromatic ring (R3).

The derived Model II CoMFA and CoMSIA maps were reported in Charts 1 and 2, respectively. Only the CoMSIA hydrophobic, H-bond acceptor and H-bond donor descriptors were reported, since the steric and the electrostatic ones were highly similar to those shown for the related CoMFA study. In any case, the contour areas were depicted around compounds 19, 39 and 53 cis, 67 to represent Group A and B ligands, respectively.

**Part 1**

A favourable steric and hydrophobic polyhedra is detected in the centre of the area occupied by part 1 of the scaffold, which proved to be differently fitted by Group A and B derivatives because of the different conformer positioning of the flexible or the rigid linkers, respectively.

Thus, the affinity profile of mono-substituted, di-substituted and spiro-substituted compounds follows a different trend within the two groups. Indeed, within Group A, the mono-phenyl substitution is the most detrimental with respect to the di-phenyl and spiro-analogues, poorly satisfying the aforementioned contour maps. In addition, the insertion of a spiro or (spiro)anellated cycle results to be tolerated, being these compounds able to occupy partially the favoured steric areas and fully the hydrophobic map. Nevertheless, the (spiro)anellated 40–42 (pKi = 6.90–7.22) display a lower SHT1A affinity profile, if compared with the di-substituted derivatives. Indeed, the di-substituted derivatives of Group A successfully overlap the aforementioned steric and hydrophobic regions, as confirmed by the adequate affinity profile of most of them (see 25–33; pKi = 7.98–9.49), pointing out this kind of substitution as the most effective within Group A. In particular, this conclusion can be confirmed especially comparing compound 1 (pKi = 8.45) to 42 (pKi = 6.96).

Finally, a consistent H-bond acceptor favourable area surrounds the part 1 of all these derivatives, suggesting a beneficial role potentially due to the introduction of proper H-bonding functions at this molecule portion.

Concerning Group B, the rigid linker constrains the ligand conformation to be arranged in a different and overall more effective positioning, which allows the part 1 of the molecule to better contact the contour maps. Thus, the di-substituted compounds
maintain an adequate ability to fit the CoMFA steric map, as previously discussed within the Group A analogues. In addition, also the mono-substituted- and the spiro-derivatives of Group B are much more properly oriented towards favoured steric and hydrophobic areas, as confirmed by the high affinity values of compounds 44–49 (p$_{Ki}$ = 7.31–8.75) and 67 (p$_{Ki}$ = 8.03).

Nonetheless, only small decorations on the spirocycle part 1 are tolerated, as shown by 71–91 (p$_{Ki}$ = 6.49–7.84), which have lower 5HT$_{1A}$ affinity than 67 (p$_{Ki}$ = 8.03).

In addition, for the mono-substituted analogues, a privileged trend involving the trans isomers rather than the cis ones is highlighted [compare 48 cis (p$_{Ki}$ = 7.73) with 49 trans (p$_{Ki}$ = 8.22)].

Regarding the di-substituted compounds, CoMFA maps promoted the insertion of a hydrophobic and electronegative decoration in part 1 in the case of trans conformers, while electropositive and polar moieties are recommended for the cis ones. Accordingly, for the imide and lactam derivatives of the dataset the cis isomer was preferred rather than the trans one, even if both showing good p$_{Ki}$ value [e.g. compare compounds 55 trans (p$_{Ki}$ = 7.95), 57 trans (p$_{Ki}$ = 8.14), 60 trans (p$_{Ki}$ = 7.59), 62 trans (p$_{Ki}$ = 7.35), with the related isomers 56 cis (p$_{Ki}$ = 8.16), 58 cis (p$_{Ki}$ = 8.25), 59 cis (p$_{Ki}$ = 8.29), 61 cis (p$_{Ki}$ = 8.50)]. In addition, an overall positive role played by the imide and lactam moieties is confirmed by an extended H-bond acceptor favoured area (magenta polyhedral) involving part 1.

**Part 2**

As regard the five-membered ring of the scaffold, 3D-QSAR analyses identify several defined features related to 5HT$_{1A}$ binding affinity. For both the Groups A and B, CoMFA maps promote the introduction of substituent onto the atom connecting part 1 and part 3, in particular bearing electronegative groups, while the CoMSIA contours discourages any putative H-bond acceptor moiety around the whole five-membered ring. Interestingly, these information prove to be consistent with the higher affinity trend.
Chart 2. Contour maps of Model II CoMSIA model hydrophobic, H-bond acceptor and H-bond donor descriptors are shown. Compounds 19, 39 and 53 cis, 67 are depicted.
observed within the cyclopentylloxy derivatives rather than that about the cyclopentanone ones

Notably, the five-membered ring of Group A derivatives highly fit the CoMSIA hydrophobic map, while the Group B counterparts are arranged in a slightly different conformation due to the rigid piperazine linker, preventing to fully overlap the aforementioned contour.

Based on an overall perspective of these results, the dithiolane ring appears to be the most adequate one for Group A, fully satisfying the aforementioned maps, as confirmed by higher affinity values of 19 ($pK_i = 9.89$) and 20 ($pK_i = 8.61$) if compared with 12 ($pK_i = 8.58$) and 1 ($pK_i = 8.45$), respectively. Concerning Group B, the replacement of the dioxolane core with the oxathiolane or dithiolane ones is anyway allowed, [see the higher affinity values of 50 ($pK_i = 8.26$) and 51 ($pK_i = 8.18$) with respect to 43 ($pK_i = 7.64$)]. Finally, the encouraging effect of the secondary amine rather than the tertiary one is described by the presence of a close favoured H-donor contour that matches one of the two hydrogen of the amine linker of Group A derivatives.

Part 3
The most relevant result of the 3D QSAR analysis about this part of the scaffold is the most positive steric effect of the open linker rather than the piperazine on 5HT1A affinity. Bulky substitution at the linker level as well as the presence of an additional substitution on the secondary amine seems to be poorly and not allowed by the contours, as confirmed by the different affinity profiles of compounds 1 ($pK_i = 8.45$) and 43 ($pK_i = 7.64$). In particular, the propylamine linker is tolerated, while longer or shorter carbon chains are embedded in an electronegative region at different levels, lowering the compound affinity for the target [see 35 ($Y = -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$; $pK_i = 7.32$), 36 ($Y = -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$; $pK_i < 6$) and 37 ($Y = -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$; $pK_i = 7.37$)]. Finally, the encouraging effect of the secondary amine rather than the tertiary one is described by the presence of a close favourable H-donor contour that matches one of the two hydrogen of the amine linker of Group A derivatives.

Part 4
All the fields considered by CoMFA and CoMSIA studies reveal a positive role played by the 2-methoxyphenyl ring around the Group A derivatives. More in details, an electronegative contour (red polyhedral) close to one of the two ortho positions of the phenyl ring in R3 denotes the alkoxy decoration as a suitable choice to improve the affinity for the SHT1A receptor. This conclusion is also underlined by the presence of polar, unfavourable H-bond donor and favourable H-bond acceptor polyhedra in proximity of part 4.

On the other hand, a favourable steric polyhedron, surrounded by unfavourable contours, suggested that the insertion of bulkier substitutions than the methoxy one could impair the ligand affinity, making the methoxy group the most effective alkoxy moiety to be exploited.

This result may be verified by the decreasing affinity trend moving from compounds 2 ($R3 = 2$-methoxyphenyl; $pK_i = 9.22$), 25

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**Chart 3.** Superimpositions of the in-house derivative 63 cis and the external set compounds 93, 95 and 97 are depicted. The chemical structure and the 5-HT1A affinity values for 92–97 are also shown.
(R3 = 2-ethoxyphenyl; pKi = 9.05) and 27 (R3 = 2-isopropoxyphenyl; pKi = 8.77) to the biaryl analogue 22 (pKi = 8.66).

Notably, the introduction of further groups in meta and para positions of the R3 phenyl ring was discouraged by the steric field. In particular, any structural modification including fused ring systems such as naphthyl moieties, or meta and/or para (di)substituted derivatives led to less active compounds.

For Group B compounds, less information were available since no example of compound with an un-substituted phenyl ring was collected by our “in-house” library of molecules.

Taking into account an overall perspective of Model I and II studies, the selection of an open linker rather than the piperazine seems to be the most effective for both affinity and selectivity, while piperazine derivatives could be further optimised through the introduction of a longer chain (than the methylene) connecting with the terminal phenyl ring.

In details, the selectivity issue seems to be related to an adequate flexibility profile of the molecule.

Thus, the design of novel ligands bearing a flexible linker combined with a diphenyl substitution or a proper branched decoration enriched with polar moieties such as the lactam and imide moieties onto the five-membered ring may derive new more selective compounds. Spiro-derivatives may be optimised by linking them to linear or to cyclic amine spacers and selecting additional polar functions as decoration for the spiro-group or longer linker including the basic core.

Notably, selectivity does not take advantage from the introduction of decorations onto the terminal phenyl ring, conversely to the results obtained around the 5-HT1A affinity profile. In addition, while the presence of cyclopentanone and of dithiolane as five-membered rings is overall encouraged by Model II, only the dithiolane one (or generally a hydrophobic group) is the most recommended for selectivity (Model I).

It should be observed that the derived data are in harmony with the affinity and selectivity of reference compounds BMY-7378 and WAY-100635, which exhibit longer aliphatic chains connected to the piperazine ring in tandem with a 2-methoxy substitution onto the terminal phenyl ring. In particular, our observations about the decorations on the terminal phenyl ring are consistent with the high affinity but poor selectivity of the two.

Finally, in order to gain a qualitative validation of the results here discussed, we also evaluated the ability of Model II contour maps to rationalise the effectiveness of an external series of novel (pyperazinyl)alkyl-based 5-HT1A ligands. In particular, these compounds included a 1,3-dimethyl-purine-2,6-dione heterocycle as acceptor and hydrophobic features of a number of chemical scaffolds included in an in-house library of 5-HT1A ligands. The results coming from Model I and II CoMFA and CoMSIA studies reveal useful suggestions for the further design of new chemical entities, and are also able to predict their affinity and selectivity profiles prior to synthesis.

In particular, despite the fact that the two group compounds showed different conformations and occupation of the 3D-QSAR contour maps, the introduction of lactame and imide decorations attempted only for Group B resulted to be beneficial (also in terms of selectivity) within the whole dataset. This kind of substitution is highly encouraged as well as the choice of a proper flexible linker connecting the terminal aromatic ring with an hydrophobic core enriched with polar and H-bond acceptor functions.

Disclosure statement

The authors declare that they have no conflict of interest.

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