# Investigation of ligand binding mode at 5-HT$_6$R with the use of bioisosterism

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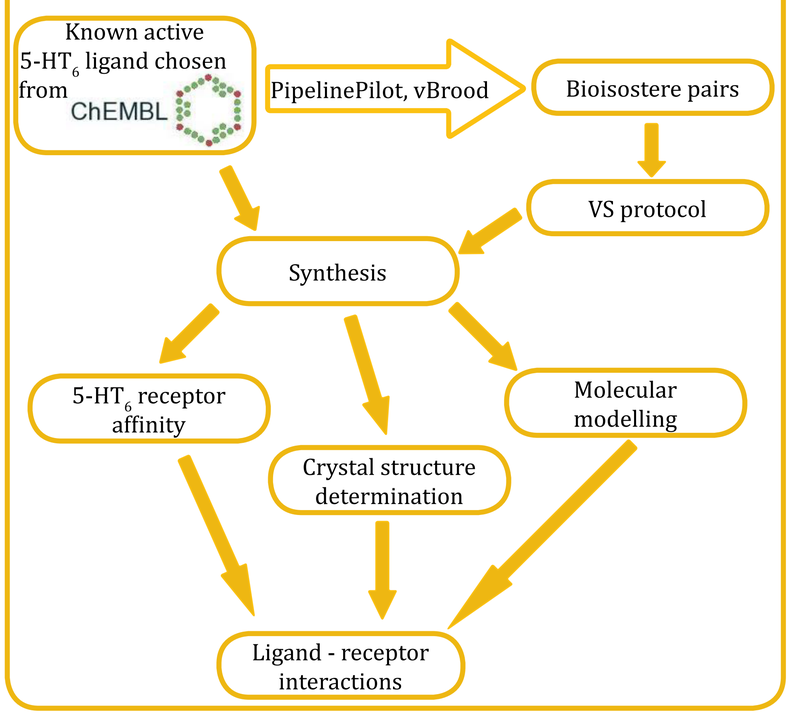
## Introduction

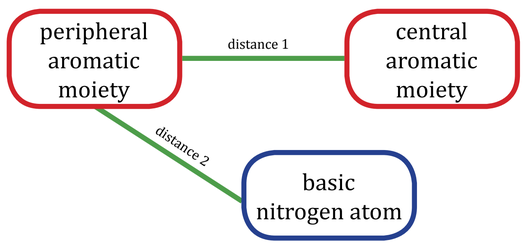
One of the most recently identified serotonin receptor subtypes – the 5-HT$_6$ receptor, localized practically only in the brain, is a very promising target for different new psychotropic drugs.[1,2,3] These receptors are supposed to be responsible mainly for motor control, memory and learning and its ligands can be used to treat cognitive impairments and also as an antiobesity drugs.[4-8] So far, several thousand of ligands have been synthesized and their structural diversity makes consensus binding mode very difficult to be defined. Isosterism is the most common technique used by medicinal chemists to design and synthesize new series of compounds. An isosteric replacement can change compound activity, bioavailability, pharmacokinetics and metabolism. If isosteric replacement doesn't substantially change biological properties of a substance, it is called bioisosteric replacement. Besides altering compound properties, bioisosterism can be used to get insight into interactions of ligand with the receptor. By carefully planning isosteric replacements it is possible to probe certain regions of receptor binding pocket.

## Concept




## Crystallographic studies

Up to date crystal structures of several analysed compounds were obtained. Two distances in crystal structures were measured and compared: between cetral and peripheral aromatic moiety and between peripheral aromatic moiety and basic nitrogen atom.




| Compound | distance 1 [Å] | distance 2 [Å] |
|---|---|---|
| 1 | 5.332 | 9.138 |
| 2 | 6.452 | 9.785 |
| 6 | 5.448 | 8.146 |
| 7 | 6.540 | 7.344 |
| 8 | 6.01 | 7.829 |
| 10 | 5.059 | 6.771 |
| 11 | 6.209 | 10.004 |
| 12 | 6.427 | 9.956 |
| 13 | 5.524 | 7.335 |
| 21 | 6.633 | 8.527 |
| | average | |
| active ($K_i$ <100 nM) | **5.792 ± 0.488** | **7.870 ± 0.751** |
| inactive ($K_i$ >100 nM) | **6.363 ± 0.109** | **9.915 ± 0.094** |

## Docking studies

In order to measure the position of a ligand in a binding pocket of 5-HT$_6$R, distances between ligand and different amino acid residues were calculated for 100 best scored complexes.

| Anchor points for measurement: Amino acid residue | Ligand | Average distance [Å] for active ligands ($K_i$ < 100 nM) | Average distance [Å] for inactive ligands ($K_i$ > 100 nM) |
|---|---|---|---|
| W6.48 | Nearest ligand atom | 6.220 ± 1.185 | 6.572 ± 1.325 |
| Nearest aa. in a loop between 2nd and 3rd helix | Nearest ligand atom | 2.021 ± 0.182 | 2.016 ± 0.204 |
| D3.32 | Ionised basic nitrogen atom | 6.832 ± 3.874 | 6.320 ± 3.437 |
| F6.51 | Nearest aromatic moiety | 5.859 ± 0.816 | 6.735 ± 1.056 |
| F6.52 | Nearest aromatic moiety | 6.623 ± 1.079 | 7.202 ± 1.807 |

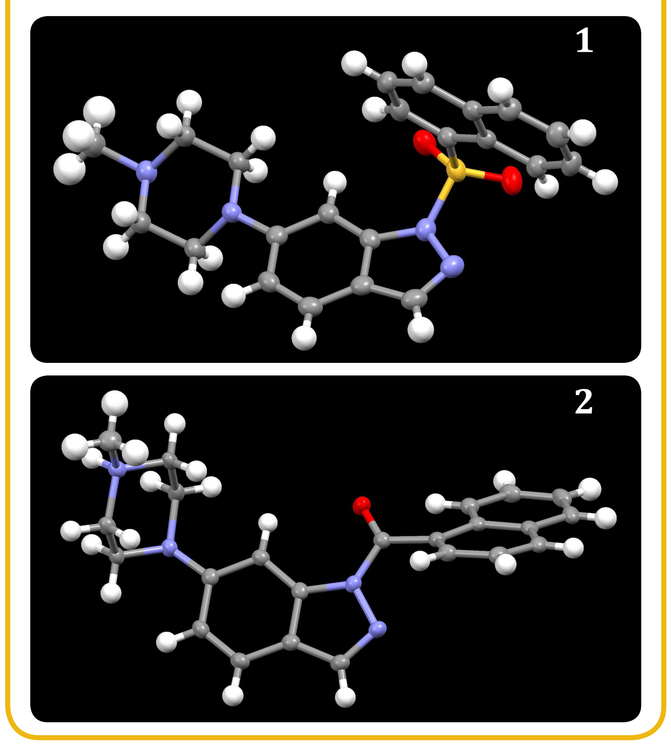
## SIFt representation

For each compound, only the best docking pose per receptor model was considered and 100 the best scored complexes were transformed into bitstring applying SIFt formalism statistically describing interactions between ligand and receptor.[9,10]

| Residue | [%] compounds interacting with residue: active $K_i$ < 100 nM | inactive $K_i$ > 100 nM | average SIFt: active $K_i$ < 100 nM | inactive $K_i$ > 100 nM |
|---|---|---|---|---|
| W3.28 | 42 | 50 | 0.69 | 0.72 |
| T3.29 | **83** | 75 | 0.68 | 0.62 |
| D3.32 | **100** | 83 | **0.88** | 0.78 |
| V3.33 | 100 | 100 | **0.90** | 0.83 |
| C3.36 | **100** | 83 | 0.81 | 0.76 |
| L4.61 | 50 | 50 | 0.62 | 0.61 |
| G146 | 0 | **25** | - | **0.60** |
| R162 | 0 | **17** | - | **0.57** |
| L163 | 100 | 92 | 0.98 | 0.92 |
| L164 | 67 | 67 | 0.71 | 0.76 |
| A165 | 100 | 92 | **0.82** | 0.74 |
| F5.38 | 100 | 100 | 0.81 | 0.76 |
| V5.39 | 100 | 100 | 0.85 | 0.83 |
| A5.42 | 92 | 83 | 0.72 | 0.70 |
| S5.43 | 33 | **50** | **0.82** | 0.64 |
| T5.46 | **75** | 42 | 0.61 | 0.65 |
| W6.48 | **75** | 50 | **0.64** | 0.57 |
| F6.51 | 100 | 100 | 0.99 | 0.95 |
| F6.52 | **100** | 83 | 0.79 | 0.73 |
| N6.55 | 100 | 92 | 0.85 | 0.82 |
| V6.58 | **58** | 17 | 0.66 | 0.71 |
| F7.35 | 100 | 100 | 0.99 | 0.99 |
| D7.36 | 17 | **50** | 0.62 | **0.72** |
| T7.39 | 100 | 100 | **0.92** | 0.84 |
| Y7.43 | 100 | 83 | 0.73 | 0.70 |

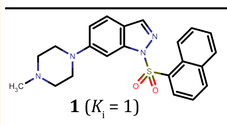
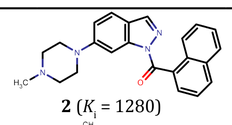
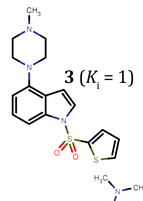
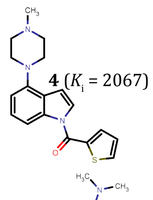
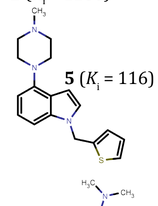
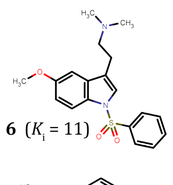
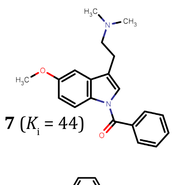
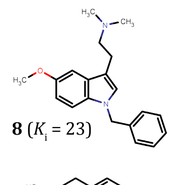
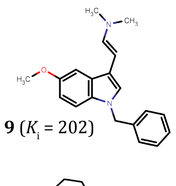
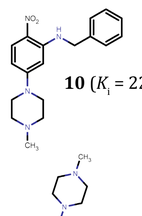
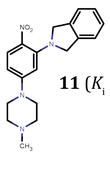
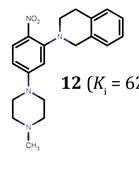
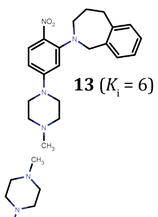
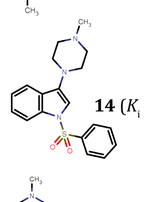
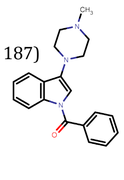
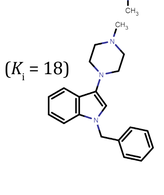
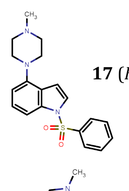
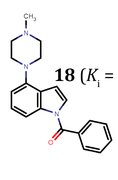
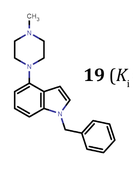
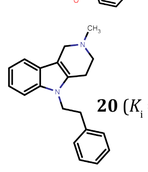
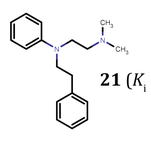
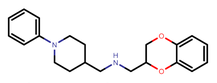
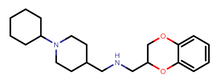
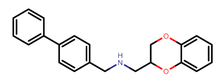
## Crystal structures

Crystal structures of **1** and **2**. Noticable is different mutual orientation of both aromatic moieties.



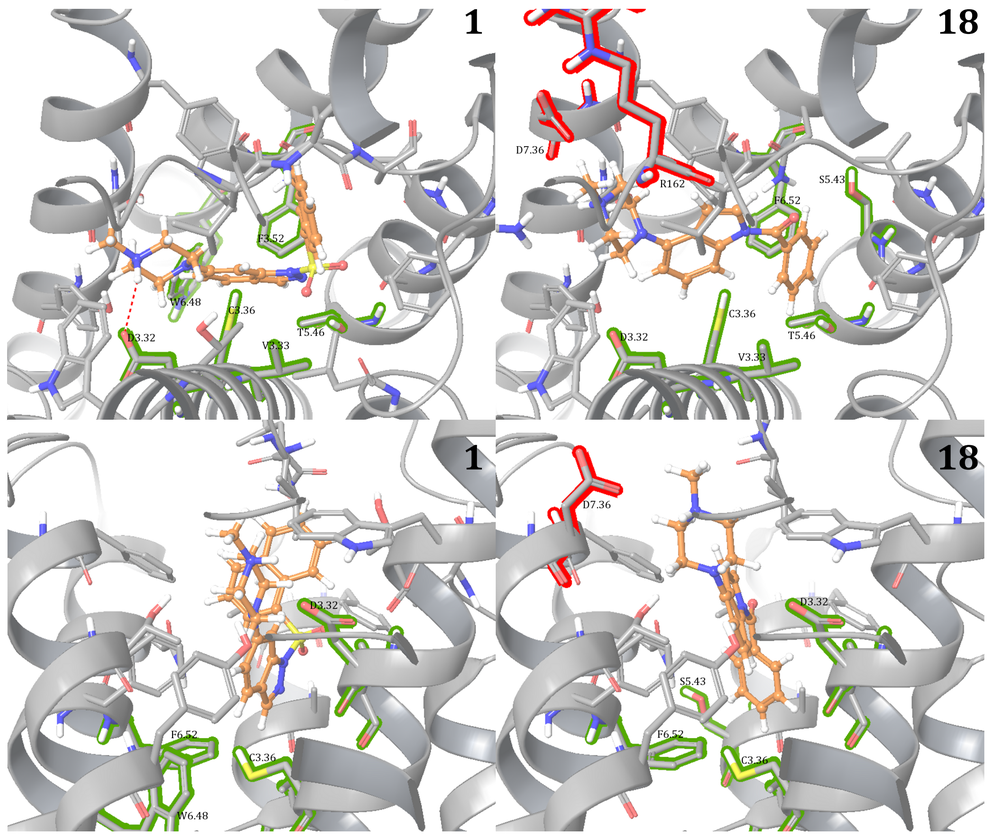

## Bioisosteric pairs

Affinity values ($K_i$) for 5-TH$_6$R in [nM] measured in our laboratory.

| Ligand | Bioisostere | Type of substitution |
|---|---|---|
|  **1** ($K_i$ = 1) |  **2** ($K_i$ = 1280) | sulphonyl - carbonyl |
|  **3** ($K_i$ = 1) |  **4** ($K_i$ = 2067) |  **5** ($K_i$ = 116) | sulphonyl - carbonyl, methanediyl |
|  **6** ($K_i$ = 11) |  **7** ($K_i$ = 44) |  **8** ($K_i$ = 23) |  **9** ($K_i$ = 202) | sulphonyl - carbonyl, methanediyl |
|  **10** ($K_i$ = 22) |  **11** ($K_i$ = 245) |  **12** ($K_i$ = 62) |  **13** ($K_i$ = 6) | noncyclic - cyclic ring expanding |
|  **14** ($K_i$ = 4) |  **15** ($K_i$ = 187) |  **16** ($K_i$ = 18) | sulphonyl - carbonyl, methanediyl |
|  **17** ($K_i$ = 1) |  **18** ($K_i$ = 2204) |  **19** ($K_i$ = 24) | sulphonyl - carbonyl, methanediyl |
|  **20** ($K_i$ = 22) |  **21** ($K_i$ = 63) | cyclic - noncyclic |
|  **22** ($K_i$ = 2280) |  **23** ($K_i$ = 2675) |  **24** ($K_i$ = 3760) | phenyl - cyclohexane piperidine - phenyl |

## Receptor-Ligand Complexes

Representative complexes with one of receptor conformation for active (**1**) and inactive (**18**) compounds. Aminoacid residues selected as important for active ligands interaction with receptor are marked in green. Aminoacid residues responsible for interaction with inactive compounds are marked in red.




## Conclusions

The goal of research was to investigate ligand-receptor interactions using designed and synthesized bioizosteric pairs. As a result, several amino acids from receptor binding pocket were highlighted as significant for binding ligands with high affinity for 5-HT$_6$ receptor. These amino acids are: D3.32, V3.33, C3.36, S5.43, T5.46, W6.48, F6.52 and V6.58. Especially, the phenylalanine cluster of F6.51 and F6.52 was selected as most important due to statistically closer position of potent ligands to these amino acids.

Additionally, crystal structures revealed significant differences in intermolecular distances between active and inactive compounds, being much shorter for ligands with high affinity.

Conducted research provided useful introduction to additional studies of the ligand-receptor interactions based on wider group of compounds. What more, developed methodology can be implemented to examine interactons of ligands with another receptors.

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