# New Serotonin 5-HT$_7$ Receptor Ligands with 1,2,4-Oxadiazole Fragment

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## Introduction

The 5-HT$_7$R is the most recently discovered G-protein-coupled receptor from the serotonin family. Potential therapeutic application of 5-HT$_7$R ligands attracts the growing interest of researches. There are a lot of evidences that these receptors have a significant and unique role in many essential brain functions such as learning, memory, sleep, circadian rhythms, thermoregulation and mood regulation.[1] Many studies show involvement of brain 5-HT$_7$R in different neurological and psychiatric diseases, e.g. anxiety, obsessive compulsive disorder, migraine headaches, chronic pain conditions, schizophrenia. Moreover, the pharmacological blockade the 5-HT$_7$R is recently postulated as a new, fast acting, antidepressant strategy.[1,2] For these reasons, the 5-HT$_7$R has become an important target for the development of novel drugs.

As a part of our ongoing efforts to discover new 5-HTR ligands, we designed and synthesized a new series of compounds with 1,2,4-oxadiazole moiety. Such heterocyclic ring was used previously as structural component of high affinity 5-HTR agents (fig. 1), e.g. **1** (5-HT$_{1A}$R and 5-HT$_{1D}$R),[3] **2** (5-HT$_6$R),[4] **3** (5-HT$_{1A}$R and 5-HT$_{2A}$R).[5]

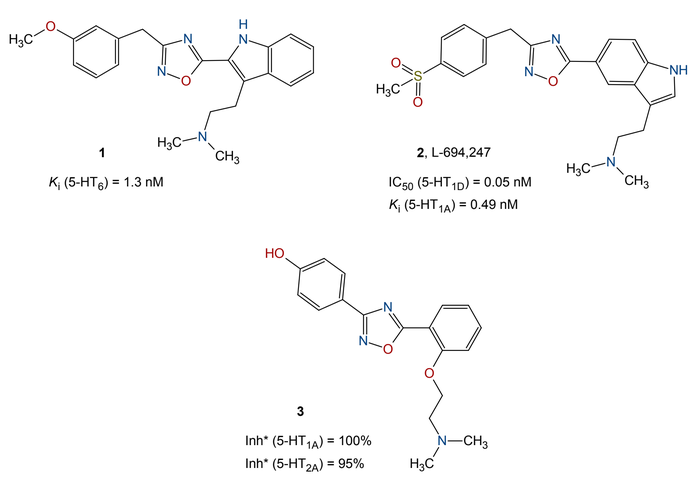


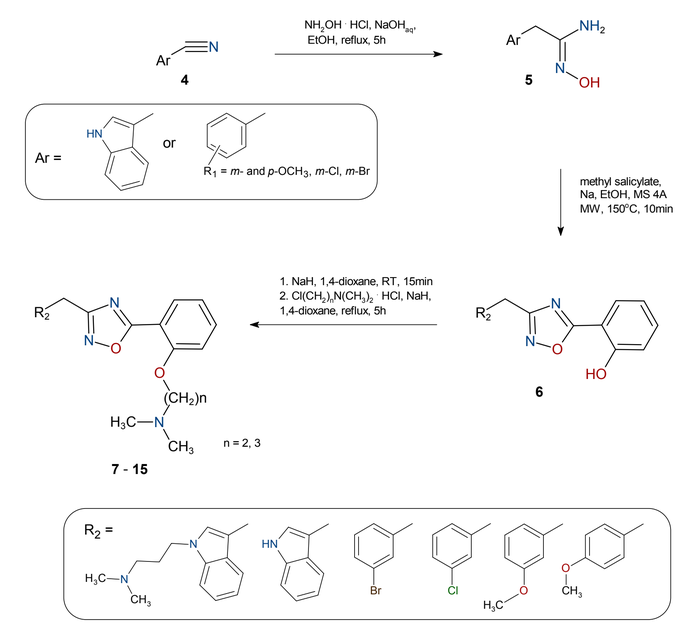

**Figure 1.** High affinity 5-HTR ligands with 1,2,4-oksadiazole fragment. * - percent of inhibition at 10μM concentration.

In the series of target compounds the 2-(1,2,4-oxadiazol-5-yl)phenol system was used as the central core. Different methylenearomatic substituents at position 3 of central moiety were applied, and the length of aliphatic linker between central core and terminal dimethylamine group was modified.

## Synthesis

The synthesis of the target 1,2,4-oxadiazoles **7** - **15** was performed as outlined in Scheme 1. The appropriately substituted nitriles **4** were treated with hydroxylamine hydrochloride, in the presence of sodium hydroxide, to give the amidoximes intermediates **5**. Condensation of **5** with methyl salicylate, in the presence of sodium ethanolate, provided the oxadiazoles **6**. In the last step of the synthesis the O-alkylation of phenols **6** with alkyl halides were carried out.

The structures of new derivatives **7** - **15** were confirmed by $^1$H NMR spectra and for pharmacological experiments free bases were converted into hydrochloride salts which molecular formulae and molecular weights were established on the bases of elemental analysis.




**Scheme 1.** Methods of new compounds **7** - **15** synthesis.

## Acknowledgments

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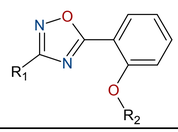
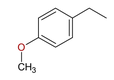
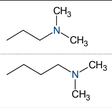
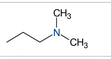
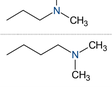
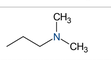
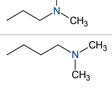
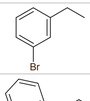
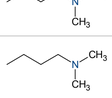
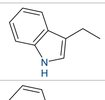
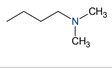
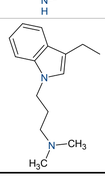
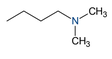
## Pharmacology

The new compounds **7** - **15** were tested in competition binding experiments for serotonin 5-HT$_7$, 5-HT$_6$ and 5-HT$_{1A}$ receptors. The affinity data are collected in Table 1.

**Table 1.** Structure and affinity data on serotonin (5-HT$_7$R, 5-HT$_6$ and 5-HT$_{1A}$) receptors of investigated compounds **7** - **15**




| Cmpd | $R_1$ | $R_2$ | $K_i$ [nM] 5-HT$_{1A}$ | 5-HT$_6$ | 5-HT$_7$ |
|---|---|---|---|---|---|
| **7** |  |  | 903 | 3433 | 1327 |
| **8** | |  | 1188 | 2308 | 227 |
| **9** |  |  | 372 | 3342 | 892 |
| **10** | |  | 711 | 633 | 111 |
| **11** |  |  | 602 | 1534* | 613* |
| **12** | |  | 1205 | 397 | 92 |
| **13** |  |  | 640 | 268 | 50 |
| **14** |  |  | 286 | 179 | 99 |
| **15** |  |  | 1024 | >10000* | >10000* |

**data from screening experiments*

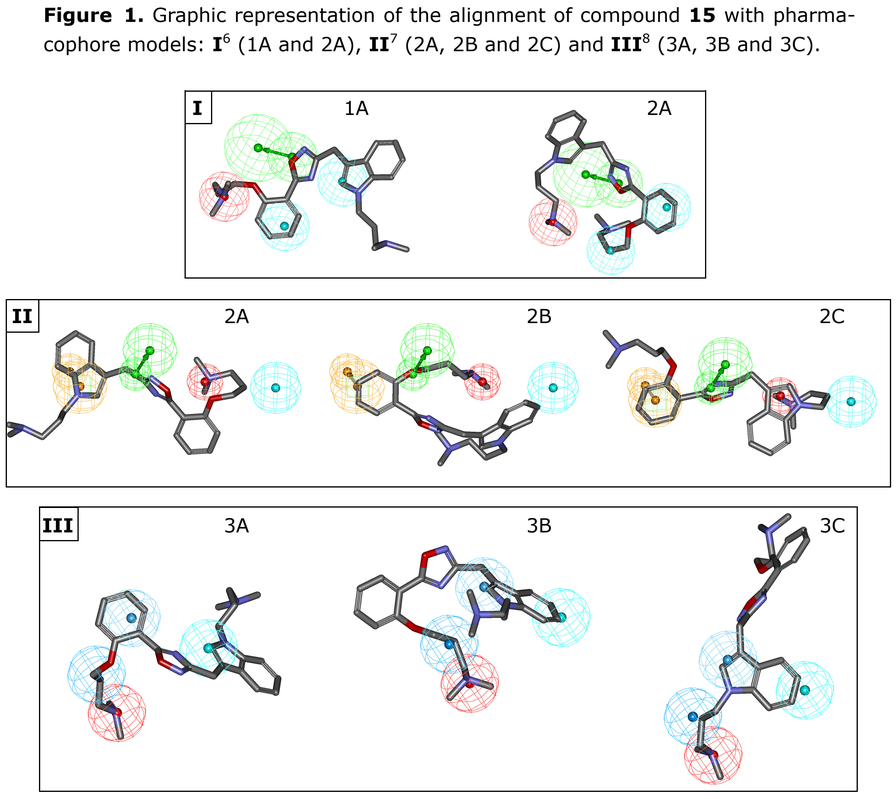
## New compounds *vs.* 5-HT$_7$R pharmacophore models

Based on the three previously reported four-point pharmacophore models for 5-HT$_7$R ligands **I** - **III**,[6-8] all new compounds were examined in protocol *'Ligand Pharmacophore Mapping'* which is implemented in program Discovery Studio (Accelrys). Conformations which were considered when mapping to each pharmacophore model were restricted within a 20 kcal/mol energy threshold to the lowest energy conformation.

| Cmpd | Pharmacophore models **I**[6] | **II**[7] | **III**[8] |
|---|---|---|---|
| **7** 130 conformers | ***FV*** = 0.95-0.58<br>***CF*** = 65<br>***FM***: all as on picture 1A | ***FV*** = 0.23-0.02<br>***CF*** = 7<br>***FM***: 5 as on picture 2A,<br>2 as on picture 2B | ***FV*** = 0.82-0.33<br>***CF*** = 42<br>***FM***: all as on picture 3B |
| **8** 164 conformers | ***FV*** = 0.89-0.41<br>***CF*** = 86<br>***FM***: all as on picture 1A | ***FV*** = 0.66-0.16<br>***CF*** = 9<br>***FM***: 4 as on picture 2A<br>5 as on picture 2B | ***FV*** = 0.83-0.29<br>***CF*** = 26<br>***FM***: 16 as on picture 3A<br>10 as on picture 3B |
| **9** 209 conformers | ***FV*** = 0.93-0.69<br>***CF*** = 107<br>***FM***: all as on picture 1A | ***FV*** = 0.09-0.01<br>***CF*** = 3<br>***FM***: 2 as on picture 2A<br>1 as on picture 2B | ***FV*** = 0.80-0.34<br>***CF*** = 49<br>***FM***: all as on picture 3B, |
| **10** 218 conformers | ***FV*** = 0.97-0.74<br>***CF*** = 102<br>***FM***: all as on picture 1A | ***FV*** = 0.42-0.09<br>***CF*** = 7<br>***FM***: 5 as on picture 2A<br>2 as on picture 2B | ***FV*** = 0.94-0.28<br>***CF*** = 69<br>***FM***: 26 as on picture 3A<br>43 as on picture 3B |
| **11** 147 conformers | ***FV*** = 0.90-0.62<br>***CF*** = 107<br>***FM***: all as on picture 1A | ***FV*** = 0<br>***CF*** = 0 | ***FV*** = 0.78-0.31<br>***CF*** = 28<br>***FM***: all as on picture 3B |
| **12** 189 conformers | ***FV*** = 0.93-0.30<br>***CF*** = 109<br>***FM***: all as on picture 1A | ***FV*** = 0.46-0.17<br>***CF*** = 5<br>***FM***: 3 as on picture 2A<br>2 as on picture 2B | ***FV*** = 0.76-0.28<br>***CF*** = 67<br>***FM***: 47 as on picture 3A<br>20 as on picture 3B |
| **13** 179 conformers | ***FV*** = 0.92-0.64<br>***CF*** = 67<br>***FM***: all as on picture 1A | ***FV*** = 0.30-0,001<br>***CF*** = 7<br>***FM***: 6 as on picture 2A<br>1 as on picture 2B | ***FV*** = 0.84-0.36<br>***CF*** = 52<br>***FM***: 17 as on picture 3A<br>35 as on picture 3B |
| **14** 149 conformers | ***FV*** = 0.95-0.45<br>***CF*** = 78<br>***FM***: all as on picture 1A | ***FV*** = 0.23-0.02<br>***CF*** = 5<br>***FM***: 4 as on picture 2A<br>1 as on picture 2B | ***FV*** = 0.87-0.40<br>***CF*** = 35<br>***FM***: 26 as on picture 3A<br>9 as on picture 3B |
| **15** 213 conformers | ***FV*** = 0.93-0.32<br>***CF*** = 56<br>***FM***: 33 as on picture 1A<br>23 as on picture 1B | ***FV*** = 0.46-0.03<br>***CF*** = 15<br>***FM***: 4 as on picture 2A<br>1 as on picture 2B<br>10 as on picture 2C | ***FV*** = 0.84-0.34<br>***CF*** = 32<br>***FM***: 20 as on picture 3A<br>6 as on picture 3B<br>6 as on picture 3C |

Models: **I** - Rault *et al.* (2004)[6]. **II** – Kołaczkowski et al. (2006)[7], **III** - Badarau, E.; et al. (2010)[8]
Abbreviations: ***FV*** – FitValue range,
***CF*** – Number of conformers fitted to model
***FM*** – Fit Modes of conformers fitted to model

**Figure 1.** Graphic representation of the alignment of compound **15** with pharmacophore models: **I**[6] (1A and 2A), **II**[7] (2A, 2B and 2C) and **III**[8] (3A, 3B and 3C).




## Results and discussion

***1. In vitro binding experiments***

- Compounds **8**, **10**, **12** with longer aliphatic linker between central core and terminal dimethylamine group revealed 1.5 - 8 times higher affinity for 5-HT$_7$R and 5-HT$_6$R then their shorter counterparts **7**, **9**, **11**. In contrast, the opposite relationship for these compounds was observed in the case of the affinity for the 5-HT$_{1A}$ receptors.
- In the series of compounds with methoxy substituent on the terminal phenyl fragment (**7** - **10**), the *meta* position was favored by all tested receptors. This was particularly evident in the case of compounds with longer alkyl chain (**8** and **10**).
- The chlorine substituent on terminal phenyl moiety of compounds **11** and **12** is much more beneficial in interactions with 5-HT$_7$ and 5-HT$_6$ receptors than methoxy in **9** and **10**. The opposite relationship was observed for the 5-HT$_{1A}$R.
- The increase in affinity for all tested receptors was observed when the bromine substituent in place of chlorine was used (compound **12** *vs.* **13**).
- The usage of indolyl substituent as terminal aromatic fragment (compound **14**) allowed to maintain a comparatively high affinity for 5-HT$_7$R as in the case of the compound **12** with *m*-chlorophenyl group, while the affinity for 5-HT$_{1A}$ and 5-HT$_6$ receptors were increased.
- Substitution of additional dimethylaminoethyl group in position 3 of indole ring (compound **15**) caused a significant reduction in affinity of all the studied receptors.

***2. In silico 5-HT$_7$R pharmacophore models mapping***

- The high values of FitValue range ***FV*** (table 2) in the fitting the generated conformations to pharmacophores were obtained for hypothesis **I** and **III**, while lower value of ***FV*** for model **II** was found.
- Similarly, higher percentage of the total population conformations for each compound adopted to models **I** and **III** in comparison to model **II** was observed (parameter ***CF***, table 2).
- Visual analysis of the mode types (***FM***) of fitting to model **I** revealed that generally one mode was preferred (fig. 1/1A). In the case of mapping the compound **15** to all three models **I** - **III**, due to presence of dimethylaminoethyl substituent of indol fragment (second positive ionizable group), the additional inverse modes were observed (fig. 1/1B, 2C and 3C).
- HBA feature of model **II** was adopted either by 1,2,4-oksadiazole ring or oxygen atom in alkyl chain which caused the occurrence of two major fitting modes (fig. 1/2A and 2B).
- In results of fitting to model **III** (hypothesis without HBA feature) the 1,2,4-oksadiazole ring was outside the pharmacophore features. The low-affinity compounds **7**, **9** and **11** (with shorter alkyl chain) fitted to model only in one mode as on fig. 1/3B, while the active ligands in two modes (fig. 1/3A and 3B). Generally, the mode as presented on fig. 1/3A the higher values of parameter FitValue were scored.

## Conclusions

At first, we have succeeded in getting new series of compounds with very different affinity for 5-HT$_7$R (50 - >10 000nM) and selectivity towards 5-HT$_{1A}$R and 5-HT$_6$R. The structure-affinity relationships of these compounds delivered useful hints for designing further derivatives.

It was found that 5-HT$_7$R affinity and selectivity depends on methylenearomatic substituent at position 3 of central 5-phenyl-1,2,4-oxadiazole fragment, as well as on the length of aliphatic linker between central core and terminal dimethylamine group.

Next, the results of fitting the presented compounds to three recent pharmacophore 5-HT$_7$R hypotheses revealed that each of them meet the structural requirements of the tested 5-HT$_7$R models. Visual analysis of the fitting mode types showed that for the tested compounds only model **III** may be useful to distinguish between active and inactive compounds.

Summing up, the new derivatives with 1,2,4-oxadiazole fragment were synthesized, next, their 5-HT$_7$, 5-HT$_6$ and 5-HT$_{1A}$ receptor affinity was measured, and, finally their analysis of the mapping on the recently published pharmacophore models was carried out.

**References:** [1] Pittalà V. et al. *Mini-Reviews in Medicinal Chemistry* **2007**, *7*, 945. [2] Mnie-Filali O. et al. *Neuropsychopharmacology* **2011**, *36*, 1275. [3] Beer M. S. et al. *Br. J. Pharmacol.* **1993**, *110*, 1196. [4] Russell M. G. et al. *J. Med. Chem.* **2001**, *44*, 3881. [5] United States Patent **2001**, US 6,277,872. [6] Lepailleur A. et al. *J.Chem. Inf. Comput. Sci.* **2004**, *44*, 1148. [7] Kołaczkowski M. et al. *J. Med. Chem.* **2006**, *49*, 6732. [8] Badarau E. et al. *Bioorg. Med. Chem.* **2010**, *1*, 1958.