RESULTS OF AFFINITY EXPERIMENTS

The compounds were divided into three groups according to their chemical structure and the binding results are presented in Tables 1-4. The affinity for the 5-HT₁₆ receptor for the series of benzol[d]isoxazole derivatives 1-10 is shown in Table 1. Except 2, all the new compounds displayed high to moderate affinity at 5-HT₁₆ (43–528 nM). Compound 1 with the 3-[3-(4-aminophenyl)pyridin-2-yl]isoxazole fragment revealed the moderate affinity (Kᵢ = 393 nM), but the substitution of the phenyl ring (3-Br) in position C-3, caused the increase of 5-HT₁₆ activity, and m-Br derivative 4 displayed Kᵢ = 62 nM. Analogically, ligand 7 with unsubstituted thiophen group was found less active then its 5,6-dimethoxy substituted derivative 8 which presented the highest affinity (Kᵢ = 43 nM).

Increasing the distance between the aromatic ring and the sulfonic acid functionality caused the increase of 5-HT₁₆ activity. For instance, compound 1 displayed about 10-fold higher affinity than its partly constrained analogue – compound 2.

The replacement of the isoxazole fragment revealed the moderate affinity for the 5-HT₁₆ receptor. When compared to their structural analogues in the series of benzo[d]isoxazole derivatives, all compounds were significantly less active. Among the tested derivatives, the highest activity to 5-HT₁₆ binding site was observed for the racemic mixture of compound 19 (Kᵢ > 625 nM) with the 5,6-dibromothienyl substituent. Its R isomer 20 revealed even higher affinity (Kᵢ = 397 nM) that was six-fold more active than its counterpart 20 with 5 conformation of the 2-ethylenepiperazinyl spacer.

In the third group of compounds 26-34 different modifications of terminal amine moiety were applied (Table 3). All compounds, i.e derivatives of perhydroquinoline (24) and N-cyclohexyl (27), N-acetyl (29) and N-methyl (31) piperazines, and secondary amines 28, 32, 33, as well as 1,2,3,4-tetrahydroadenosine (34), were practically inactive at 5-HT₁₆ (the Kᵢ > 5400 nM).

METHODS OF BINDING EXPERIMENTS

Membrane preparation and general assay procedures for 5-HT₁₆, 5-HT₃, 5-HT₅, 5-HT₂, 5-HT₃, 5-HT₂, 5-HT₁ and 5-HT₂ receptors were performed exactly as previously described. For binding experiments 7-9 sample concentrations, each run in triplicate, were used to determine inhibition constant (Ki) on the base of Cheng-Prusoff’s equation: Ki = IC₅₀ / (1 + L/R). Values are means of three experiments run in triplicate, SEM ± 16%.

DOCKING STUDIES TO THE SEROTONIN 5-HT₁₆ RECEPTOR HOMOLOGY MODEL

Models of 5-HT₁₆ receptor were generated as previously described for 5-HT₂ using β, adrenergic template. Selection of models was based on docking results of a set of 106 ligands (30 referenced ligands from publications) and 41 ligand-like compounds with Kᵢ > 1000 nM. The four models with the highest discrimination ratio were next used in docking of the whole set of 34 studied compounds. Majority of active ligands (Kᵢ < 1000 nM) were successfully docked to the best model (60%), which recognized only 5% of inactive compounds.

Figure 2. Representative binding mode - compounds 9 (A) and 10 (B) - within the 5-HT₁₆ receptor model.

CONCLUSIONS

The quality of terminal amine fragment is significant for affinity to 5-HT₁₆. The type of aryl fragment of phenylsulfonamide terminal is important for interactions with 5-HT₁₆ receptor. Ligands with R conformation of 2-ethylenepiperazinyl spacer are preferred at 5-HT₁₆ binding site.