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Discovery of cannabinoid-1 receptor antagonists by virtual screening

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ABSTRACT

In this work, we tried to find a new scaffold for a CB1 receptor antagonist using virtual screening. We first analyzed structural features for the known cannabinoid-1 receptor antagonists and, then, we built pharmacophore models using the HipHop concept and carried out a docking study based on our homology CB1 receptor 3D structure. The most active compound, including thiazole-4-one moiety, showed an activity value of 125 nM IC_{50}, with a good PK profile.

The CB1 receptor belongs to the rhodopsin (Rho) subfamily of G protein-coupled receptors (GPCRs), which make up approximately 30% of clinically marketed drugs.[1] The CB1 receptor antagonists/inverse agonists represent a promising new approach for reducing body weight and decreasing the co-morbidities associated with excessive adiposity.[2] To find novel CB1 receptor antagonists, we used ligand- and structure-based molecular modeling tools. For structure-based modeling approaches, because of the absence of a reliable CB1 receptor crystal structure,[3] we built a homology model based on the X-ray structure of rhodopsin. Also, pharmacophore models such as the ligand-based modeling tool have been suggested for a collection of structurally diverse compounds that are known to bind to the same active site. Generations of pharmacophore and docking models have been reported in detail in our other works in the literature.[3] Here, we performed virtual screening to discover a novel CB1 receptor antagonist using the previous predictive models. The filtered compounds derived by the prediction models were proved experimentally using CB1 receptor-37 cells. In Figure 1, the seven novel compounds discovered in this work are shown to be CB1 receptor antagonists.

All the compounds for virtual screening were filtered by applying the physicochemical properties of the known CB1 receptor antagonists. An analysis of the physicochemical properties of CB1 receptor antagonists resulted in the following filter criteria: molecular weight $\leqslant$500, H-bond donors $\leqslant$3, H-bond acceptors $\leqslant$6, *A* log *P* $\leqslant$7, and number of rings including aromatic rings $\leqslant$4. These filters helped to reduce by about 56% the number of compounds from an in-house chemical library, including 80,000 compound species.

Using HipHopRefine,[4] we generated a highly restricted pharmacophore model[3] with an excluded volumes algorithm in the Accelrys software. This model was validated by the test set of known CB1 receptor antagonists. We performed compound mapping in the pharmacohpore features and filtered the mapped compounds with fit values that computed by comparing a compound and a pharmacophore. The mapped compounds in the pharmacophore model (Fig. 2) were fitted to at least four features; their fit value was over 2.4. Through this method, we chose 160 compounds among pre-filtered compounds.

We also built a CoMFA[5] model with the validated Partial Least Square (PLS) statistics from the previously-mentioned paper.[3] The results of the contour maps corresponded with the generated pharmacophore features. Using our existing CoMFA model, we performed CoMFA prediction for the filtered compounds, and then selected 171 compounds with pIC_{50} >5.

Finally, to do virtual screening by docking, we also used the CB1 receptor homology model reported in our previous work.[3] To virtually screen out the selected compounds, we employed the Surflex-Dock method[6] in Sybyl 8.0. We docked rimonabant[7] and taranabant[8] into the CB1 receptor homology model and obtained a high correlation between the docking score and the CB1 receptor antagonist activities. The docking scores for rimonabant and taranabant were 11.5 and 12.1, respectively. Also, we identified the K192 site forming a salt bridge with D366 and three hydrophobic sites with aromatic ring residues on helices 5, 6 and 7 as key interaction sites of CB1 receptor.[9,10] We filtered the docked compounds with docking scores >5 considering the docking score of the reference compounds, and then selected 256 compounds through visual inspection. The binding modes for some of the final selected compounds are shown in Figure 3.

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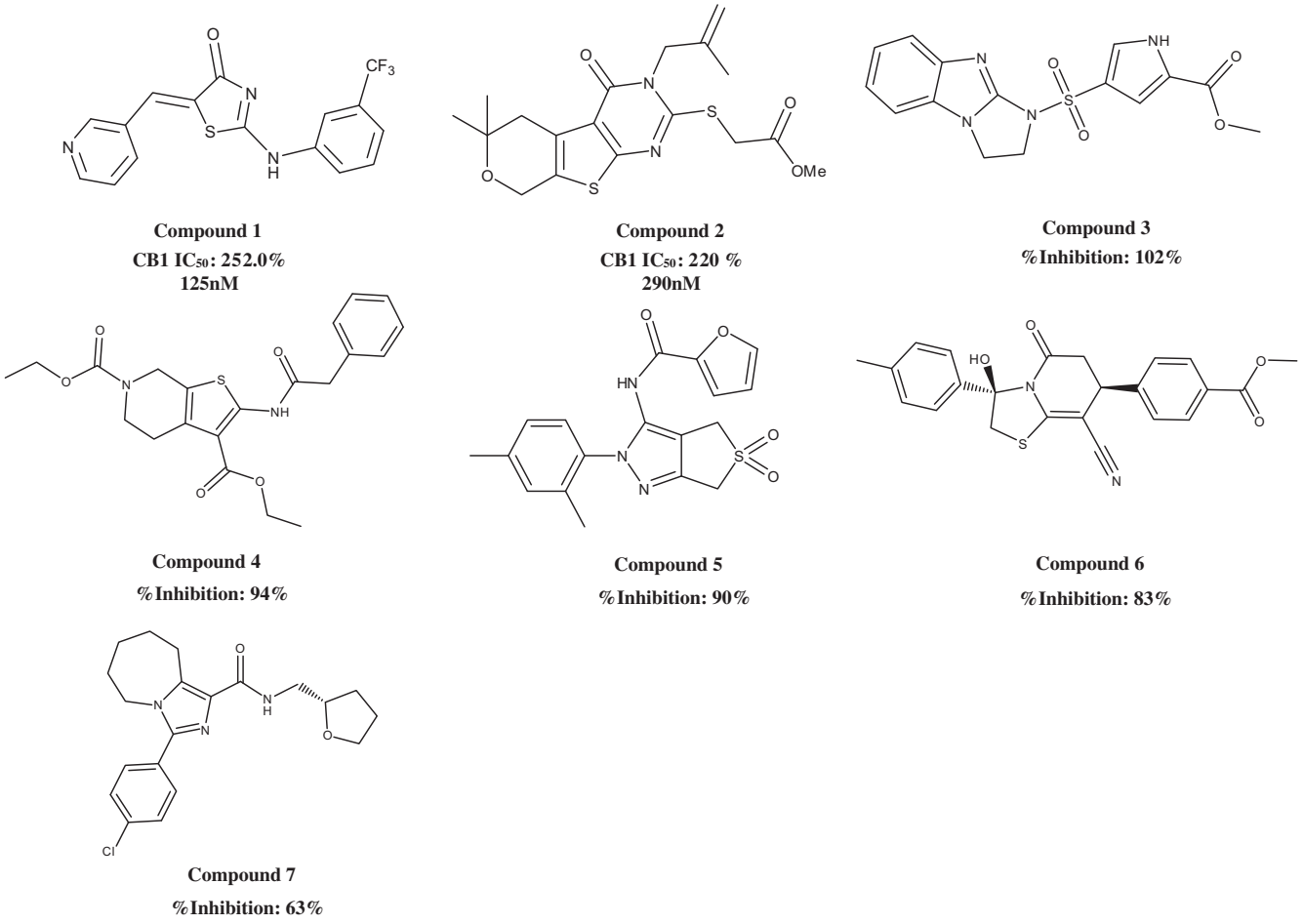

Figure 1. CB1 receptor antagonists discovered by virtual screening process.

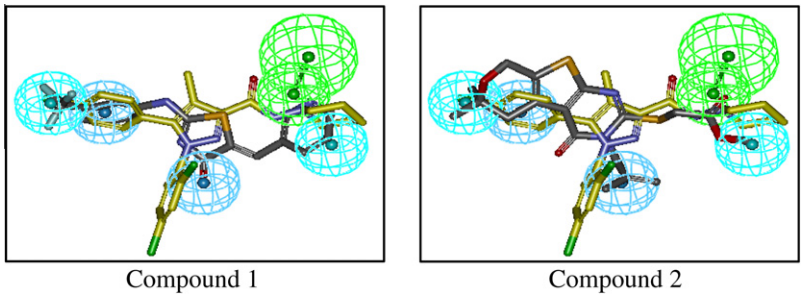

Figure 2. The pharmacophore mapping of discovered compounds. H-bond acceptor, hydrophobics and hydrophobic aromatics are shown in green, cyan, and blue spheres. Yellow stick compound is rimonabant. Compounds have fit value between 3 and 3.5 values.

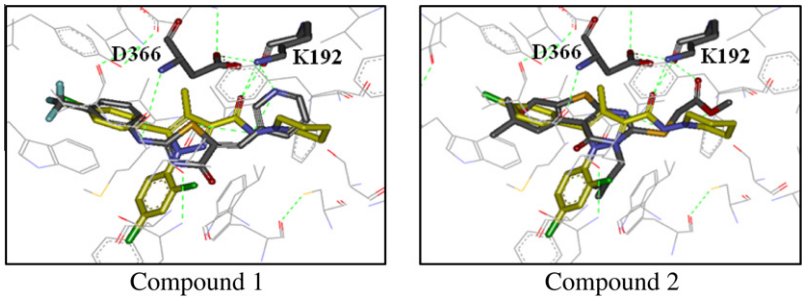

Figure 3. Docked pose of compounds discovered in receptor homology model. This homology model shows key interaction points in the active site. The H-bond interactions are displayed in dotted lines and yellow stick compound is rimonabant. Compounds have total score between 5 and 7.5 values.

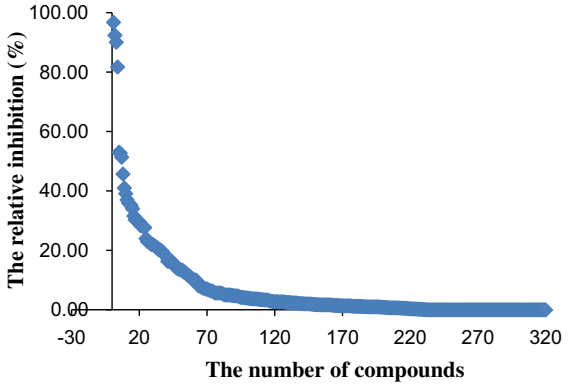

Figure 4. CB1 receptor antagonistic activities for the selected 320 compounds. IC_{50} for the reference compounds, rimonabant and taranabant are 25.0 and 1.0 nM, respectively.

Table 1
The results of CB1 receptor antagonistic activity for the selected 320 compounds according to the prediction models

	Docking model	CoMFA model	Pharmacophore model
>50%[a]	35 (35)[c]	39 (35)	35 (35)
<50%	141	83	78
Hit rate[b]	24.8%	46.9%	44.8%

[a] The relative inhibition % compared to rimonabant.
[b] The ratio of the number of compounds in 50% above and below.
[c] The number of overlapped compounds among the prediction models is shown in parenthesis.

We yielded 587 compounds that contained the duplicated compounds, which matched pharmacophore, CoMFA prediction, and docking score criteria. Then, 320 of the duplicated 587 compounds were obtained from visual inspection and were prepared (as four plates in 96-wells) for biological assay of CB1 receptor antagonistic activity. To be more suitable for fast screening, CB1 receptor antagonist assay was modified based on the methods described previously.[11] CB1 receptor-37 cells contain the human CB1 receptor and CRE-luciferase stably integrated into the CHO-K1 cell line. The cells were maintained with RPMI (Gibco) containing 10% FBS (Gibco) and 100 μg/ml G418 (Gibco). The cells were placed on a white 96-well plate (Greiner) by 2×10^4 cells/well and incubated for 24 h in 10% FBS RPMI. Then, the cells were treated with compounds in 5% FBS RPMI and incubated at 37 °C. After 4 h, the cells were treated with cell lysis buffer (Promega) and luciferase assay solution (Promega). Luciferase activity was detected by Luminometer (Molecular Device). The data analysis was performed with Prism4 software (GraphPad). The reference compounds, rimonabant and taranabant, showed activity of 25.0 and 1.0 nM IC_{50}.

The results of CB1 receptor antagonistic activity by virtual screening using various prediction models are shown in Figure 4 and in Table 1. The hit rate for each prediction model shown in Table 1 was highest in the pharmacophore model; also, the most active compounds, **1** and **2**, resulted in this model. Especially, structures of the seven active compounds selected by considering structural novelty are shown in Figure 1. The most active compound, which included thiazole-4-one moiety (compound **1**), showed an activity value of 125 nM IC_{50}. For this compound, we examined the PK profiles to determine whether the compound has the properties of a drug hit or not. For the pharmacokinetic profiling of compound **1**, in vitro liver microsomal stability and plasma protein binding ratio were studied (Table 2). For the case of human and rat liver microsomal stability, compound **1** was stable (>87%) after NADPH dependent oxidative metabolism by triplicates of time-dependent manner for 30 min. The reaction mixtures consisted of human and rat liver microsomes (0.5 mg/ml; BD Gentest), NADP+, glucose 6-phosphate, and G-6-PDH in 100 mM potassium phosphate buffer (pH 7.4). The final concentration of compound **1** was 2 μM. After the pre-incubation of the reaction mixture at 37 °C for 5 min, the reaction was initiated and time-dependent samples were collected at 0, 5, 15, and 30 min. The reaction was terminated by adding three times volume of ice-cold acetonitrile with imipramine (80 ng/ml) as internal standard. After pretreatment of the biological samples with vortex and centrifuging, the samples were analyzed by LC/MS/MS (AB Sciex 2000 Qtrap). For the protein binding assay, compound **1** (2 μg/ml) was tested with rat and human plasma using an ultrafiltration method. The plasma stability of compound **1** was studied and this compound was found to be very stable in human and rat plasma (>99%). After pre-incubation of the mixture at 37 °C for 30 min, an aliquot of 400 μl was transferred to the Amicon microCon with 30 k dalton MWCO YMT membrane (Millipore). The samples were centrifuged at 37 °C, 5000 rpm for 20 min and remaining and filtered aliquots were analyzed by LC/MS/MS (AB Sciex 2000 Qtrap). Non-specific binding was tested in water, and there was no significant non-specific binding between compound **1** and the ultrafiltration membrane of microCon (<1%). Compound **1** has a medium-high protein binding ratio to human and rat plasma protein. Therefore, we selected compound **1** as the early hit compound for CB1 receptor antagonist development.

Table 2
In vitro pharmacokinetic profiles of compound **1**

Parameters	Human	Rat
Liver microsomal stability[a]	87.0 ± 7.6	96.1 ± 7.8
Plasma stability[a]	>99%	>99%
Plasma protein binding ratio	94.2 ± 1.3	96.6 ± 2.1

[a] The stability was tested during 30 min.

Further biological evaluation pf compound **1** and the other hit compounds are now underway and will be reported.

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