SCREENING of SMALL MOLECULES BINDING to GPCR RECEPTORS.

Human genome sequencing has revealed about 1,000 sequences known or likely to be G-protein coupled receptors (GPCRs). More than half of these have sensory functions and are not generally relevant for drug discovery. About 400 GPCRs might bind endogenous ligands, already identified for approximately two thirds of these receptors.

A common classification system refers to Class A, B, and C receptor families. Class A includes hormone, neurotransmitter and light receptors that bind mainly to amines and peptide ligands.

Class B receptors bind to endogenous proteins, and Class C to a variety of ligands including several important neurotransmitters. 125 out of 371 known GPCR types and subtypes are targeted by compounds on the market or in development and 37 out of these 125 represent targets for which very few compounds have reached the development stage.

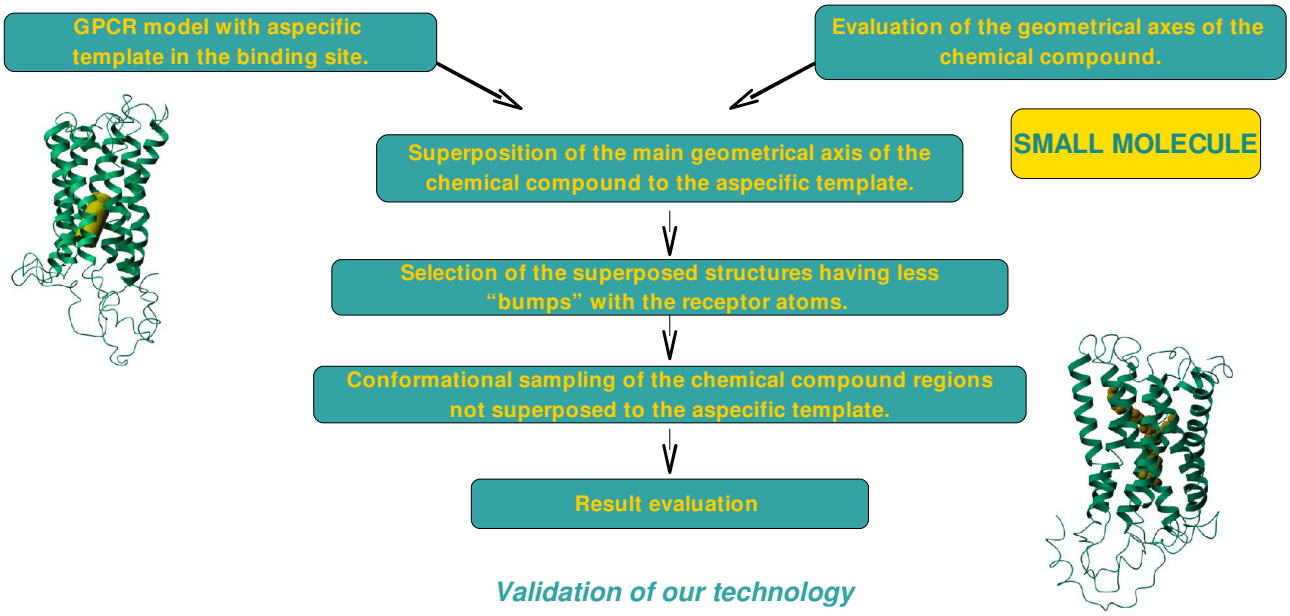
Having selected a target, companies can either rely on diversity screening only or combine screening with structure-based drug discovery.

Structure-based drug discovery and design, which previously had to rely mostly on ligand structures, received a tremendous boost with the publication of the β2-adrenergic, β1-adrenergic and Adenosine A2A receptor high-resolution x-ray structures and, furthermore, the crystal structure of an active GPCR opsin in complex with a C-terminal peptide derived from the Galpha subunit of transducin was deposited in 2008.

S.A.F.AN. BIOINFORMATICS developed a new technology based on the different structures of active and inactive GPCRs, that allows us to predict quickly and efficiently the affinity of new ligands to a GPCR receptor.

Based on our technology we developed our focused library, which contains 330,000 specifically classified compounds.

Our technology hallmarks

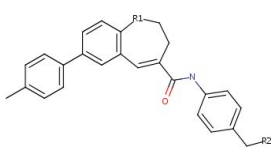


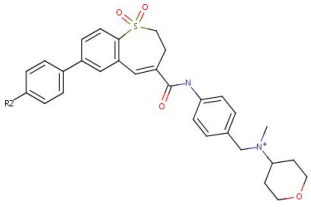
Validation of our technology

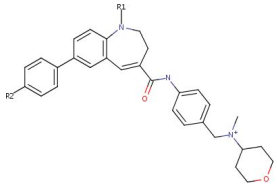
CCR5 is a GPCR having RANTES, macrophage inflammatory protein (MIP)-1a and MIP-1b as natural ligands. The group of Seto M. (*Proc. Natl. Acad. Sci. U.S.A., 96, 5698-6703 (1999)*, *J.Med. Chem., 43, 2049-2063 (2000)*) discovered that benzocycloheptene compounds exhibit a highly potent CCR5 antagonistic action and reported that the replacement of the benzocycloheptene ring with the 1-benzothiepine 1,1-dioxide or 1-benzoazepine ring moieties enhanced the activity. They performed this chemical modification on orally active compounds selected as new leads, in order to enhance their activity and improve their pharmacokinetic profiles. Papers *Chem. Pharm. Bull. 52(5) 577-590 (2004)* and *Chem. Pharm. Bull. 52(2) 254-258 (2004)* report the inhibitory effects of these compounds on the binding of RANTES to CCR5-expressing CHO cells.

We used their experimental data for validation purposes. Our results are shown in the following page.

Compound scaffolds:

A 

B 

C 

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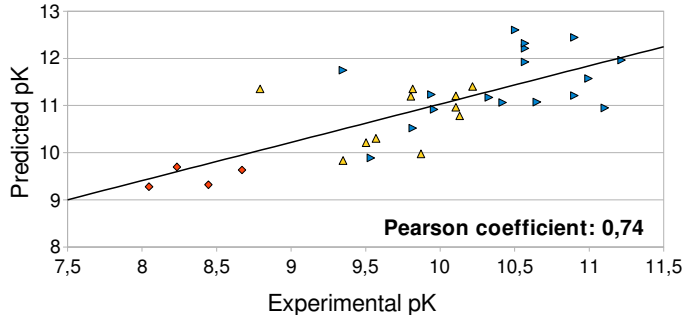
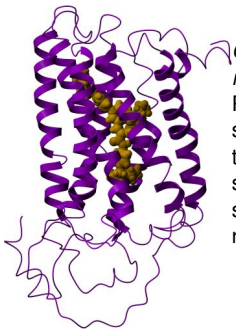
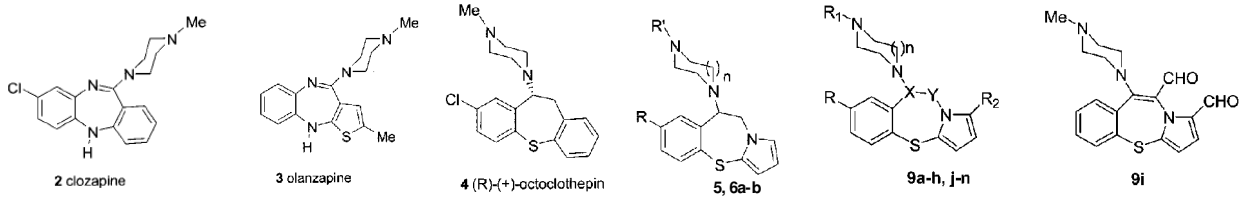




Correlation between the experimental and the computed data: Points related to the compounds of scaffold A are shown in yellow, those related to scaffold B are shown in red and those concerning scaffold C are cyano. The regression line is plotted in black.

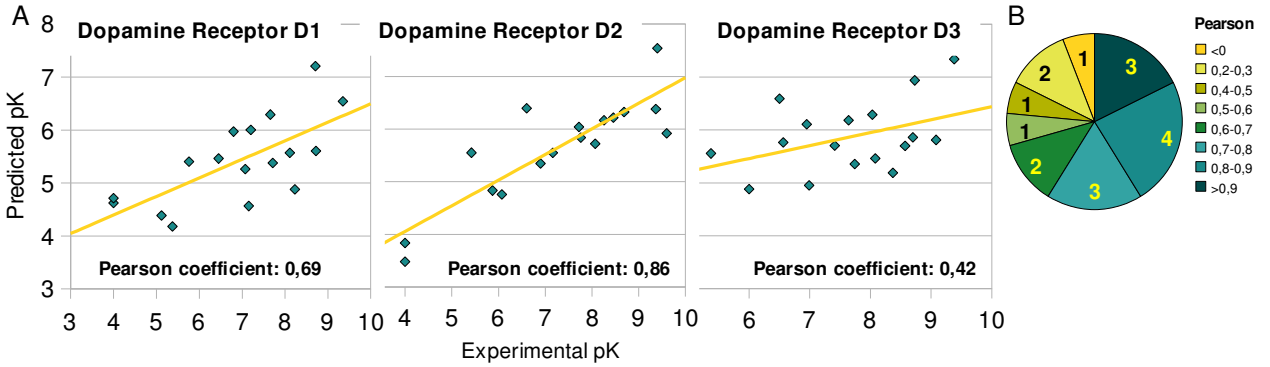
To further validate our technology we check how it predicts the specificity of various compounds for different members of the same GPCR subfamily.Campiani et al (J. Med. Chem. 2002, 45, 344-359 and J. Med. Chem. 2004, 47, 143-157) studied the binding and antipsychotic properties of a series of compounds binding to the Dopamine receptor subclass. We used their experimental data for validation purposes. Our results are shown next.

Compound scaffolds:



The strength of the linear relationship between the experimental and computed pK is measured by the Pearson coefficient. It can take on the values from -1.0 to 1.0. Where -1 is a perfect negative (inverse) correlation, 0 is no correlation, and 1 is a perfect positive correlation. The Pearson coefficient has been previously used to evaluate prediction methods such as for example NetMHCpan, a predictor of peptide binding to HLA-A and -B Locus proteins, in which the average performance in terms of Pearson coefficient is 0.77 (*Nielsen M, et al. (2007) PLoS ONE 2(8): e796.*).

Results for the different receptors are shown below:



Correlation between computed and experimental data. A: All the compounds complexed to a receptor. B: Each compound bound to the three receptors: the number of compounds featuring the Pearson coefficient interval described in the legend is shown in each sector.

Figure A shows the correlation between the experimental and computed data for the three different receptors. Figure B shows the distribution of Pearson coefficients between the experimental pKs and our predictions for 17 small molecules bound to the three dopamine receptors (DRD). 12 molecules have high Pearson coefficients (>0,6), validating the method also as a predictor of the ligand specificity for receptors belonging to the same family.

The validation studies presented above are the argument of a paper in preparation (Pugliese L and Mennini T "Validation of a virtual screening procedure of ligands targeting GPCR receptors.").

S.A.F.AN. BIOINFORMATICS offers:

1. Screening of 30.000 clustered molecules per day of:
   a. customer small molecule libraries against Class A GPCR receptors,
   b. S.A.F.AN. BIOINFORMATICS library against Class A GPCR receptors,
2. detailed analysis of the small molecule:receptor interactions,
3. detailed and personalized report describing analyses and results.

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