A new approach for the development of tracers: data base screening and in silico modeling for the identification of new ligands for SSTR2

Abstract

New ligands are needed to improve diagnostics and treatment of SSTR2 expressing tumors. *We implemented a procedure* to identify ligands based on computer processing methods. A multistep procedure was used. Search entries were taken from National Cancer Institute database. Application of criteria defined by the Lipinski rules reduced the initial data set. Then a pharmacophore criterion including Lys and Trp residues was the next step of the hierarchical filtering, and the ligands considered were transformed from 2D to 3D. Finally, dedicated software was applied for docking ligand studies. *Our results* have shown that by virtual screening and trial docking, we identified novel ligands with better scores of docked poses compared with previously reported ligands. *In conclusion*, the use of a focused library that incorporates an initial probe, improved the possibility of a successful virtual screening as compared with random screening and is cost efficient by further combination of trial docking.

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Introduction

Virtual screening, using a computational approach to assess the interaction of an in silico library of small molecules and the structure of a target macromolecule, has been introduced for the rapid identification of new drug leads [1]. This typical knowledge-driven approach starts with the 3D structure of the target protein, which is then exploited to discover new leads by computer searches in large compound libraries. Recently, several successful examples have been reported [2-4]. A prerequisite for the success of this method is a detailed understanding of the structural properties of the target protein and the criteria that determine the binding of ligands.

Up to now, five somatostatin receptors (SSTR1-5) have been cloned and characterized [5, 6]. All five receptors are members of the superfamily of receptors having seven transmembrane segments [7]. Structure-function studies with a large number of peptidal analogues have shown that the Trp^8-Lys^9 dipeptide of somatostatin is necessary for high affinity binding [8], and the ligand-binding domain for SST ligands is made up of residues in TMs III-VII with a potential contribution by the second extracellular loop [9]. In an effort to discover novel small molecule somatostatin receptor modulators, database-searching techniques for the Merck compound sample collection and combinatorial chemistry, were previously employed [8].

In our group, positron emission tomography (PET) with Ga-68-1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid-[Tyr^3]-octreotide (DOTATOC) has been investigated in endocrine tumors expressing SSTR2 [10, 11]. It was known from this work that the effect of treatment was primarily dependent on the receptor binding capacity of the tracer. Furthermore, yttrium-90-DOTATOC provides the possibility to perform radioisotope treatment [12]. In general, new ligands for SSTR2 are needed for both improved diagnostics and radioisotope treatment. Herein, based on existing drugs binding to the SSTR2, dedicated chemical libraries are created with virtual screening, and then used to select and assess possible substances with higher binding capacity via trial docking by Flexible Rapid Exhaustive Docking (FRED) (OpenEye Scientific Software, Santa Fe, NM, USA).

The aim of this study was to create a pipeline-structured procedure for the efficient identification of new ligands for SSTR2, and in the future further confirm the new procedure by a bioassay of these new ligands.

Caixia Cheng,
Leyun Pan,
Antonia Dimitrakopoulou-Strauss,
Ludwig G. Strauss

Clinical Cooperation Unit Nuclear Medicine, German Cancer Research Center, Heidelberg, Germany

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Correspondence address:
Caixia Cheng, PhD
Clinical Cooperation Unit Nuclear Medicine,
German Cancer Research Center,
Im Neuenheimer Feld 280,
D-69120 Heidelberg, Germany
Email: c.cheng@dkfz.de

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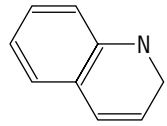
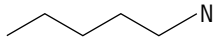
Materials and methods

The National Cancer Institute (NCI) database composed of 250251 open structures, is available for searching. All ligands considered in this study were transformed from 2D to 3D via ChemBio3D Ultra 11.0. Definition of a search pharmacophore was accomplished through PubChem Server Side Structure Editor V1.21. The 3D structure of the target protein was retrieved from the PDB database (accession number: 1boj). FTree (BioSolveIT GmbH, Sankt Augustin, Germany) was employed as a similarity analysis engine. Parallel docking was done with Fred running on a Mac OS X.

Results

Starting from the NCI database composed of 250251 open structures as mentioned above, we applied a protocol of consecutive hierarchical filters (Fig. 1). These filters were selected to discover compounds that may fit into the binding pocket of 1boj. In our virtual screening, we firstly applied the criteria defined by Lipinski et al. (1997) and removed all compounds with a molecular weight larger than 500 Da and containing more than 10 rotatable bonds, in order to retrieve more lead like structures [13]. This resulted in a reduction of the original data set to 2116 entries. Subsequent to this 2D connectivity search, we performed a 3D search based on a predefined pharmacophore hypothesis. This hypothesis was directly extracted from the requirements imposed by the binding site [14]. Based on this query, a residual set of 68 compounds was retrieved. Among these hits, nearly 98% of the considered molecules contained a terminal tryptophan residue.

Table 1. *Amino acid residue selected as potential binding pocket anchor groups.*

Residue	SLN Code
	C1=CC2=C(C=C1)[N]C=C2
	CCCCCN

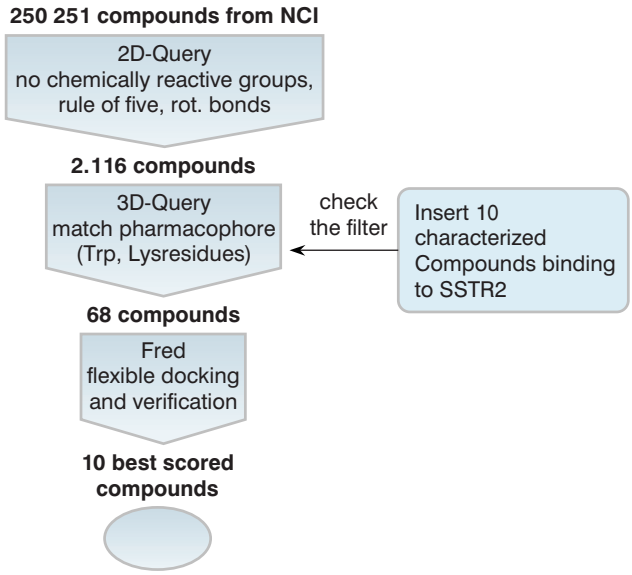


Figure 1. The protocol of consecutive filters applied in the virtual screening.

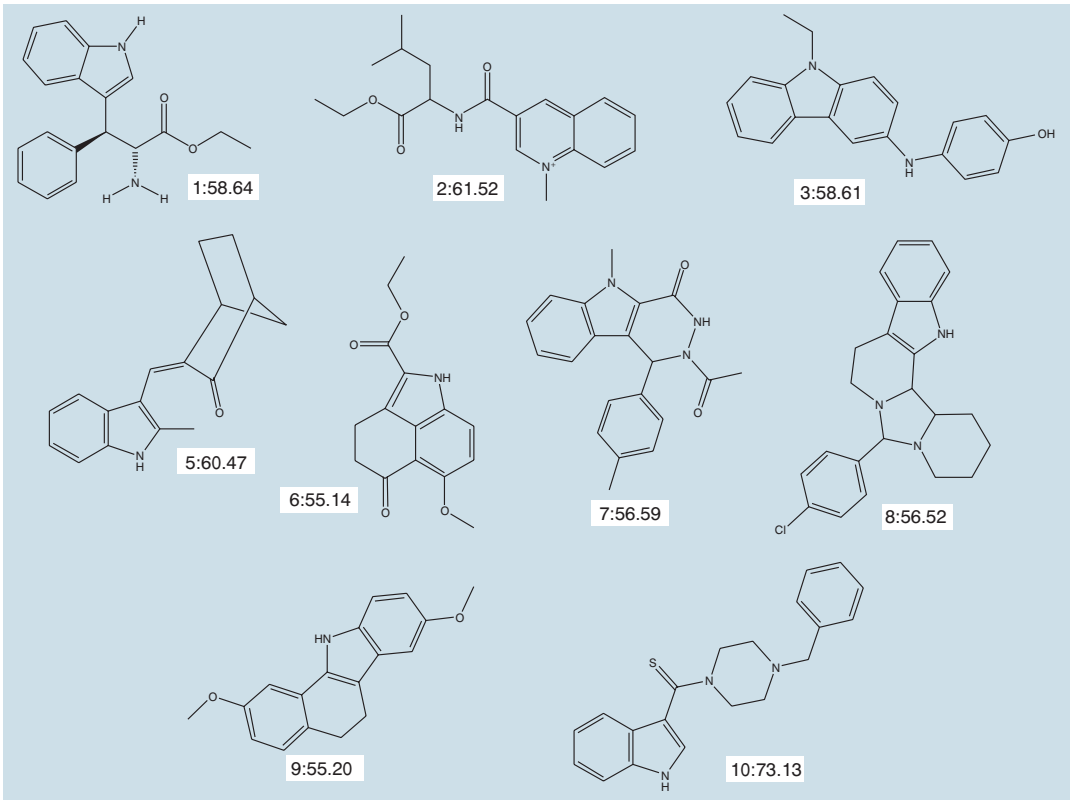


Figure 2. The chemical structure of potential ligands discovered by virtual screening and their docked score with FRED.

Nowadays, we face a vast pool of different docking and scoring methodologies. Often, a selection of an appropriate combination for a particular target becomes necessary since no generally applicable tool that offers robust and accurate solutions to a majority of various docking problems has been found so far. Among FLEXX (a docking software from BioSolveIT GmbH), DOCK (a docking software from University of California, San Francisco), FRED and GOLD (a docking software from the Department of Chemistry of Cambridge University), FRED, which was used in this study, is the fastest tool, and allows protein flexibility in virtual screening [15]. During the process of trial docking with FRED, Chemgauss 3 was selected to score possible poses and for optimization.

In addition, diversity is an important issue in virtual screening because the purpose of lead discovery is to identify hits covering different regions of chemical space, in order to increase the chances of developing a drug candidate with an acceptable pharmacological profile. Herein, FTree (BioSolveIT GmbH, Sankt Augustin, Germany) was employed as similarity analysis engine to select the compounds with positive docking results. As a result, 10 of the 68 compounds considered after the hierarchical filters were chosen for the bioassay on the basis of docked score and similarity analysis to Trp^{8}-Lys^{9} dipeptide. The structure of them and the corresponding docking score with FRED, are shown in Figure 2. Among representative docked pose of compound 1, binding to SSTR2 is shown in Figure 3.

Discussion

PET is a promising method for the evaluation of the cellular metabolism, tumor perfusion and expression of receptors in endocrine tumors. Recently, tracers for specific tumors used in oncological PET examinations have been developed. Recent studies in our lab were focused on the pharmacokinetics of ^{68}Ga-DOTATOC in patients with tumors expressing SSTR2. It was known from this work that DOTATOC uptake in neuroendocrine tumors (NETs) is mainly depended on k_1 (receptor binding) [10, 16]. Therefore, there is an urgent need for substances with an improved binding to receptors, to achieve an improved therapeutic effect. Obviously, in silico search for such substances based on existing chemical libraries, namely virtual screening is a universal and fast approach, and how to set the filters in virtual screening, is the key point.

In order to test the validity of the pharmacophore structure of Trp residue and Lys residue (Table 1), we inserted 10 characterized nonpeptide ligands for SSTR2, containing glucose-based nonpeptide mimetic, 3-functionalized proline, lactam peptidomimetics and its derivatives into the search sample

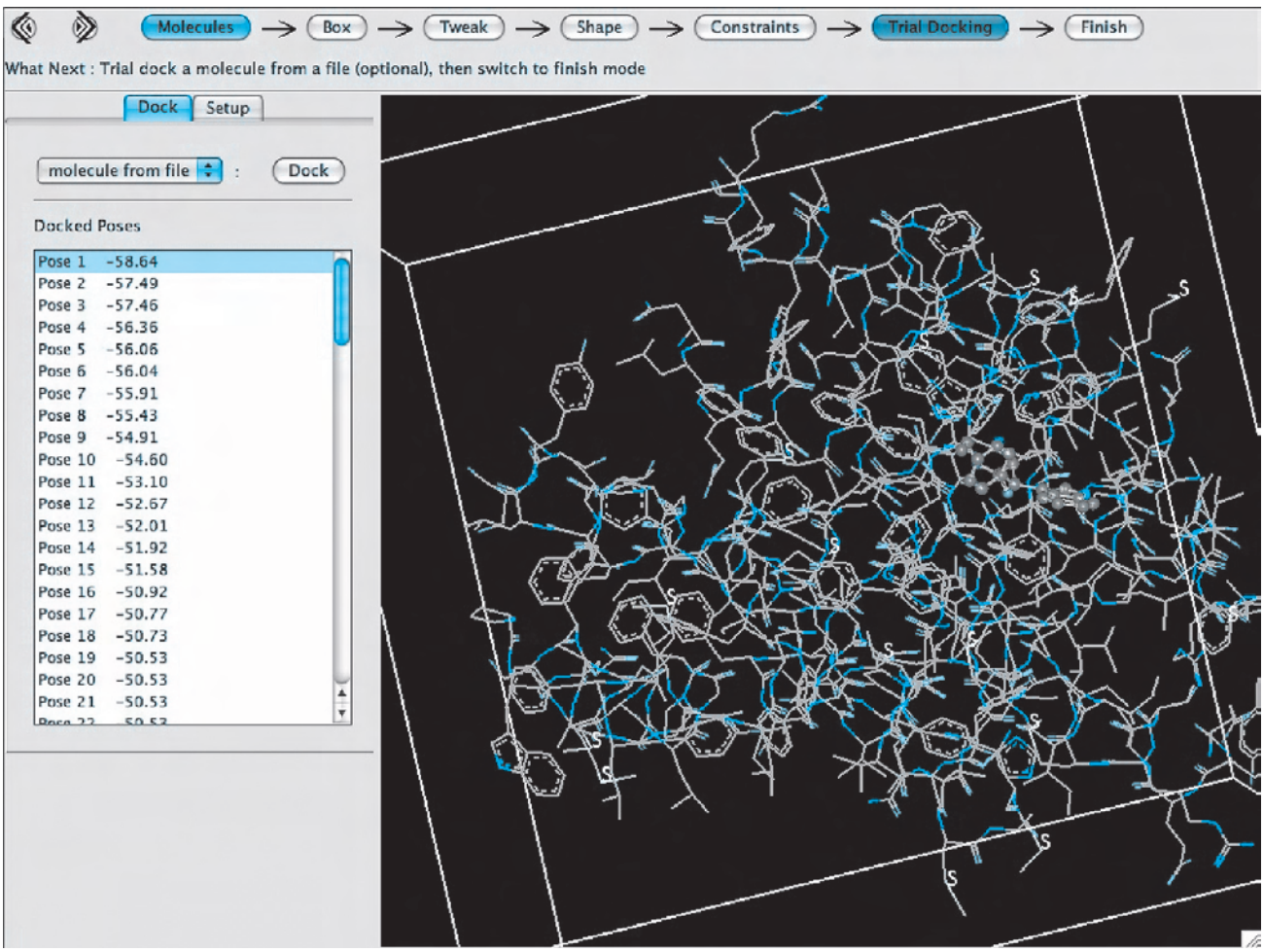


Figure 3. Representative docked pose of compound 1 binding to SSTR2.

[14,17,18]. The 10 characterized ligands were kept after this filter, which demonstrated that the structure of pharmacophore was effective.

Previously, database-searching techniques were employed to screen the Merck compound sample collection. The database probe was based on a modeled conformation of the cyclic peptide c(Pro^6-Tyr^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}) [8]. Subsequently, Mosley et al. (1995) used the side chains of the Tyr^7-D-Trp^8-Lys^9 in the modeled hexapeptide as the probe to search a database of 3D models of compounds (in the Merck compound sample collection), combining with 3D similarity search engine and selected 75 compounds [19]. However, the peptide backbone is not required for activity, which was illustrated in carbohydrate and benzodiazepine-based somatostatin ligands [17]. In addition, non-peptides with SST4 receptor affinity, based on a query consisting of two aromatic groups for virtual screening, have been reported [20]. Herein, splitting the peptide backbone to keep the residues, two simple queries not only mimicking the Trp but also extending the query to contain all sorts of nitrogen, were employed. Furthermore, the 5 carbons backbone was verified more active [18] and also contained all sorts of carbon. Doubtlessly, this screening strategy improved the possibility of finding new ligands. In addition, comparatively, combinatorial chemistry proved very useful for rapid refinement of new lead compounds. Meanwhile virtual screening was preferred in order to enrich the diversity of ligands.

It should be noted, that Mosley et al. (1995) identified a new ligand L-264, 930 with high affinity, by testing 75 compounds in the murine SSTR2 assay, while trial docking was further employed by us to reduce the finally considered compounds in the bioassay. This will may save much time and money, which is very important in the field of new drugs design.

In the future, in our group, the final compounds for biological assay will be performed (in our group). Furthermore, considering the use for PET, the compounds 1, 2 and 3 will be investigated regarding the possibility of binding to DOTA. *In conclusion*: one of the most important conclusions from this study is that the use of a focused library that incorporates an initial probe, improved the possibility of a successful virtual screening as compared with random screening and also saved time and money for the bioassay by further trial docking. These new ligands enrich the diversity of non-peptide ligands for SSTR2 and are promising to be a new lead compound.

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