Chapter 5
Inverse Virtual Screening in Drug Repositioning: Detailed Investigation and Case Studies

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Abstract Inverse virtual screening is a useful tool for drug repositioning or repurposing. The utility of this tool lies in the identification of potential targets for small-molecule ligands. With reference to drug repositioning, approved/existing small-molecule drugs can be processed by inverse virtual screening for the discovery of potential new molecular targets for such drugs. Both the ligand- and structure-based approaches can be used for the in silico screening. PharmMapper is a web-based tool for ligand-based inverse screening that employs pharmacophore mapping approach and identifies potential target candidates for small molecules. The present study demonstrates the usefulness of this approach for computational repositioning of approved/existing drugs. Here, query molecules belonging to protein kinase inhibitors, monoamine transporter inhibitors and G protein-coupled receptor antagonists were used. The results revealed potential novel molecular targets for the query molecules. Detailed literature search involving the query molecule-novel target pair led to interesting findings. The book chapter summarizes the interesting outcomes of the ligand-based inverse virtual screening.

Keywords Inverse virtual screening · Reverse pharmacophore mapping · Drug repositioning · PharmMapper

5.1 Introduction

Drug discovery and development processes include various phases till the new molecular entity (NME) is introduced in the clinic [1]. The initial discovery phase involves target discovery and validation, identification of lead by high-throughput screening (HTS) or related approaches, and lead optimization. Then comes the

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preclinical studies, where the compounds are tested in vitro and in animal models to test the pharmacological efficacy and toxicity. In the clinical development phase, the investigational new drug (IND) is tested in humans in Phase I, II, and III clinical trials. The failure or attrition rate of the IND due to adverse effects and lack of efficacy in humans during clinical trials were major concerns few years ago [2]. Even though the attrition rate is under control, the exorbitant cost of drug development (>2 billion US $) has led to reduced 'productivity' of the pharmaceutical industry across the globe. Therefore, the pharmaceutical scientists are in search of alternate drug development strategies. Of the several such lateral approaches, investigations involving approved/existing small-molecule drugs (drug repositioning or repurposing) or shelved compounds (drug rescue) seem to be attractive on time and money scale [3]. Repositioning of already existing drugs lead to substantial reduction in development cost and time compared to the traditional discovery efforts, due to availability of previously collected pharmacokinetic, toxicology, and safety data [4].

In recent times, the number of success stories of drug repositioning is increasing. Sildenafil (phosphodiesterase 5 inhibitor) is on top of the list. Another such story is thalidomide. It was recently repositioned for use in erythema nodosum leprosum and multiple myeloma [5]. Minoxidil (a potassium channel opener) was originally developed for hypertension and then repositioned to treat male baldness [6]. Raloxifene (selective estrogen receptor modulator) was approved in 2007 by USFDA for osteoporosis after early clinical trials for breast cancer [7].

Similar to clinical drug repositioning efforts described above, computational repositioning is an established field of research [8]. Computational repositioning generates potential new indications hypotheses for a query drug by design and validation of automated workflows. In contrast to clinical repositioning approaches, computational investigations analyze the data for several drugs and diseases simultaneously. Three computational repositioning methods namely, transcriptomic, side effects, and genetics-based methods, are described systematically in the literature [8]. Overall, these repositioning hypotheses were generated using extensive experimental and clinical data analyses.

On the same lines, inverse virtual screening—ligand-based or structure-based—can be potentially useful for computational repositioning. In the structure-based approach, the strategy, reverse docking, involves screening of small-molecule ligand against a database of clinically relevant macromolecular targets for its binding complementarity [9]. Similarly, ligand-based approaches include methods like reverse pharmacophore mapping, shape- and electrostatics screening. Reverse pharmacophore matching algorithms such as PharmMapper involve finding the best interaction mode between the potential target candidates and ligand molecule [10]. PharmMapper uses the pharmacophore mapping approach and carries a large, internal collection of pharmacophore databases, namely PharmTargetDB [11] annotated from all the targets information in BindingDB [12], DrugBank [13] and potential drug target database (PDTD) [14], including over 7000 receptor-based pharmacophore models (covering over 1500 drug targets information).

In this chapter, we extended our study to investigate reverse pharmacophore matching approach for identifying novel targets for the query molecules belonging to three

different target classes, namely, protein kinase inhibitors, antidepressants, and antimuscarinic agents. The query molecules used were well-established and/or blockbuster drugs. The overall aim of the study was to devise and validate computational workflows to generate hypotheses for new indications for a given drug candidate [15–19].

5.2 Materials and Methods

5.2.1 Hardware and Software

The molecular modeling studies described herein were performed on Lenovo UltraBook Laptop (Intel® Core™ i5-3317U CPU @ 1.70 GHz, RAM 4 GB) running Windows 7 Home Basic Operating System. Schrödinger Small-Molecule Drug Discovery Suite Release 2013-1 [20] and the products included therein were used for performing various molecular modeling operations described in the chapter.

5.2.2 Selection and Preparation of Query Molecules

Approved drugs belonging to three target classes, protein kinases inhibitors, antidepressants, and antimuscarinic agents (**1**–**4**, Fig. 5.1) were selected for computational repositioning using putational repositioning usin. The molecules were built and prepared using LigPrep 2.6 implemented in Schrödinger suite 2013-1. All the default settings were used. For PharmMapper search, .mol2 file was submitted on the server (59.78.96.61/pharmmapper/).

5.2.3 Potential Therapeutic Target Identification by PharmMapper

PharmMapper [11] uses pharmacophore mapping principles to match putative target candidates. The online server executed an automatic search using the small molecules to attain the 'best mapping poses' of the query molecule against the complete in-house database of PharmMapper. Parameters in the database were set as follows: 'Advanced Options' of 'Conformation Generation'; the option of "Energy Minimization" was set to "Yes" and the remaining options were used as available in the default settings. Under "Pharmacophore Mapping", "human protein targets only" was selected as the targets set. Similarly, under "Advanced Options", "Perform GA Match" was set to "Yes" to optimize the pharmacophore mapping, and the remaining options were used as such. The hit list included possible binding targets showing pharmacophore features similarity to the native ligand bound to the macromolecular target. The hit list was subjected to manual inspection coupled to

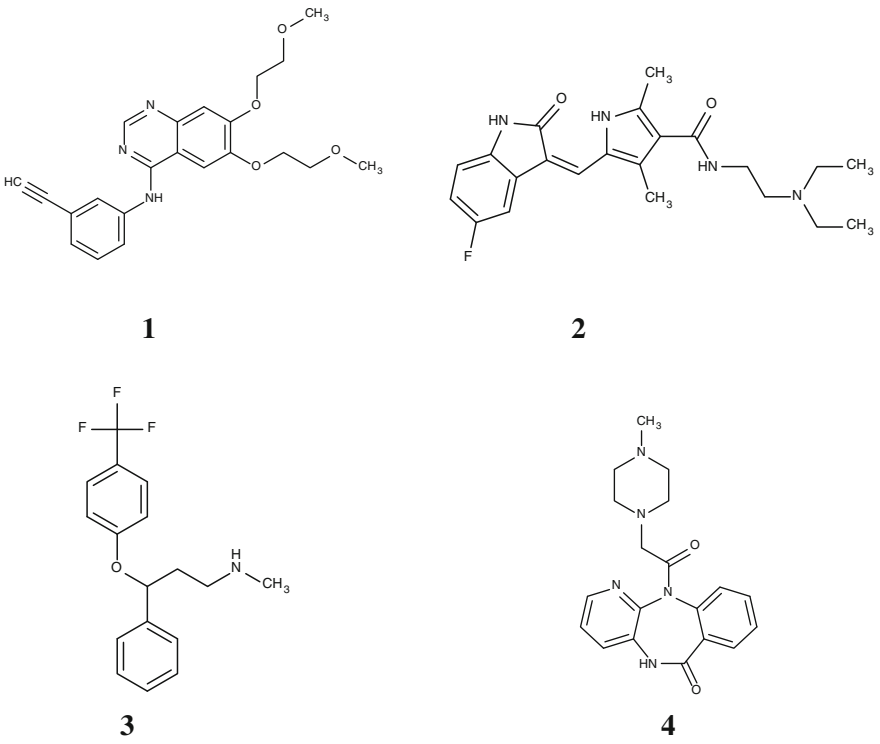

Fig. 5.1 Molecular structures of query molecules erlotinib (**1**), sunitinib (**2**), fluoxetine (**3**) and pirenzepine (**4**)

the extensive literature search. The aim was to look for any experimental evidence to the repositioning hypotheses generated by the inverse screening.

5.2.4 Docking Studies

Docking studies of the query molecules **1**–**4** were performed using Glide version 5.7, as implemented in Schrödinger suite 2013-1 [21–23]. Top 5 protein targets from the PharmMapper hit list, belonging to different target families (e.g., enzyme, receptors) presented for each molecule (Tables 5.1, 5.2, 5.3 and 5.4) were selected for the docking studies of the query molecule along with the native ligand for comparison.

The crystal structures of the targets were imported from Protein Data Bank (PDB) [24] in Maestro 9.4 [25] and subjected to Protein Preparation step. All the default settings were used as implemented. The prepared protein was then subjected to further minimization using Prime 3.2 (default settings). The refined protein structure was used for Receptor Grid Generation (Glide 5.9). The native ligand present in the target structure was used for placing the enclosing box. The grid was then used for docking the ligands in the ligand-binding site.

Table 5.1 Results of top 5 targets hypotheses of erlotinib generated by PharmMapper

Rank	Target	PDB ID	Fit score	Therapeutic area
1	Acetylcholinesterase	1HBJ	4.897	Cholinergics
4	Tetracycline repressor protein class D	2TCT	4.547	Antibiotics
5	Aldose reductase	2DUX	4.502	Antidiabetics
11	Cell division protein zipA	1Y2F	4.408	Antibacterial
12	Chorismate synthase	1QXO	4.401	Antifungal

Table 5.2 Results of top 5 targets hypotheses of sunitinib generated by PharmMapper

Rank	Target	PDB ID	Fit score	Therapeutic area
2	Cathepsin K	1TU6	5.078	Involved in bone resorption
3	Acetylcholinesterase	1HBJ	4.861	Cholinergics
5	HIV I protease	1MUI	4.567	Antiviral
6	Gag-pol polyprotein	1TL1	4.524	Antiviral
7	Gag-pol polyprotein	1D4I	4.398	Antiviral

Table 5.3 Results of top 5 targets hypotheses of fluoxetine generated by PharmMapper

Rank	Target	PDB ID	Fit score	Therapeutic area
2	Gag-pol polyprotein	1KZK	4.241	Antiviral
3	Retinoic acid receptor	1EXA	4.048	Retinoic acid receptors
5	3-hydroxy-3-methylglutaryl-coenzyme A reductase	3CDA	3.923	Hypolipidemics
6	Gag-pol polyprotein	1MSM	3.921	Antiviral
8	Acetylcholinesterase	1Q84	3.909	Cholinergics

Table 5.4 Results of top 5 targets hypotheses of pirenzepine generated by PharmMapper

Rank	Target	PDB ID	Fit score	Therapeutic area
1	Poly [ADP-ribose] polymerase 1	1PAX	5.435	Cellular processes involving mainly DNA repair and programmed cell death
3	Alcohol dehydrogenase class-3	1MC5	4.969	Alcohol dehydrogenase inhibitors
4	Alpha-mannosidase 2	1TQU	4.326	Enzyme involved in the cleavage of mannose
7	Protein-glutamine γ-glutamyltransferase E	1L9 N	3.915	Participates in complement and coagulation cascades
8	Penicillopepsin	2WEA	3.008	Antibacterials

For docking studies, the prepared ligands (query and the native) in the first stage were used. The ligands were docked in respective binding sites using ExtraPrecision (XP) mode with default settings. The docking results were analyzed using XP

Visualizer for the binding modes and associated statistics. The binding modes of the ligands (2D and 3D representations) are shown in Figs. 5.2b, 5.3b, 5.4b, 5.5b and Figs. 5.2c, 5.3c, 5.4c, 5.5c, respectively. The matched pharmacophoric features of

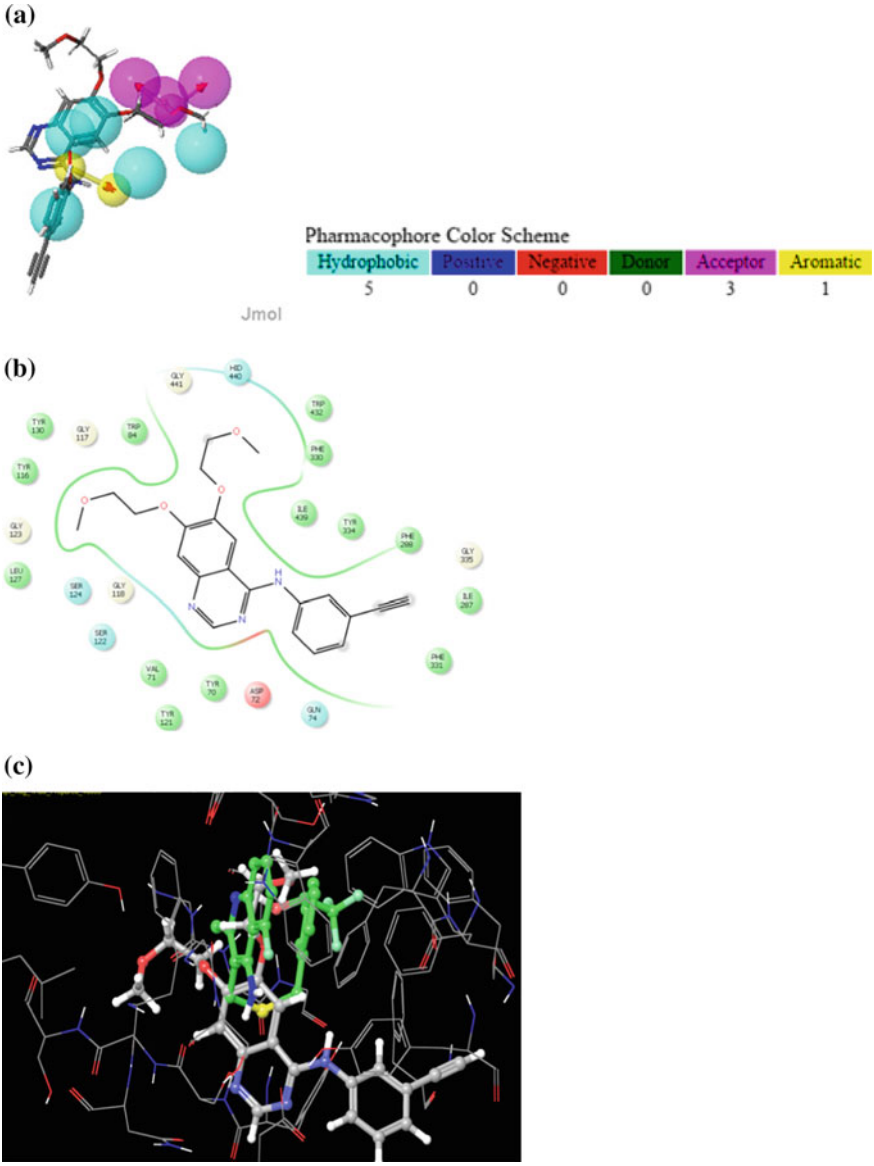

Fig. 5.2 **a** Pharmacophore features derived from AChE (PDB ID 1HBJ, Rank 1) mapped onto the molecular structure of query **1**. **b** 2D depiction of the binding mode of **1** with AChE. **c** 3D binding mode of **1** in the active site of AChE. The native ligand is shown as *green ball*-and-stick model and **1** is shown in element *color ball*-and-stick model

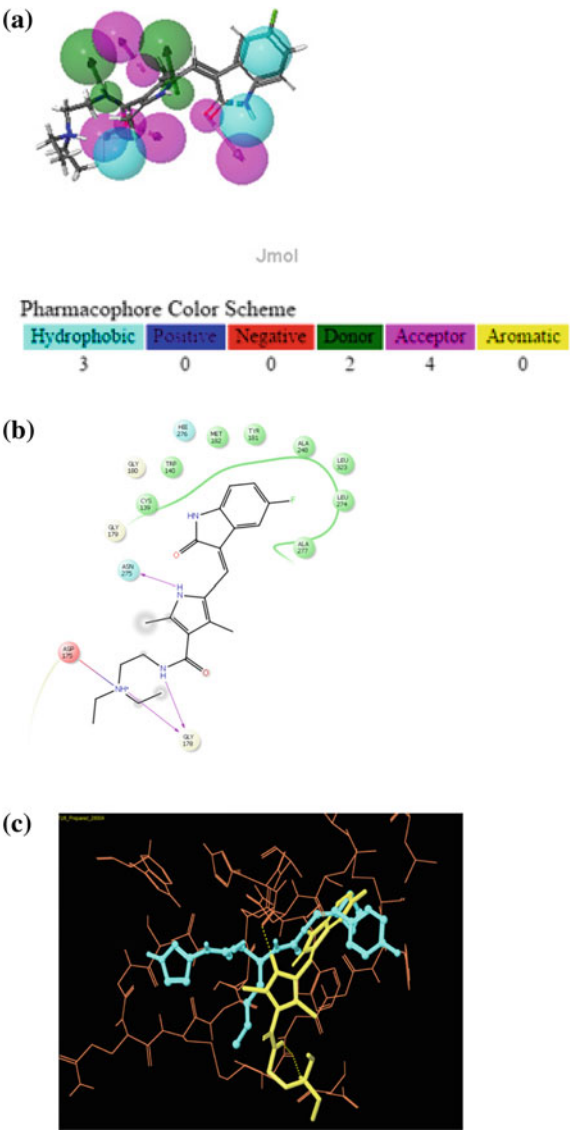

Fig. 5.3 **a** Pharmacophore features derived from cathepsin (PDB ID 1TU6, Rank 2) mapped onto the molecular structure of query **2**. **b** 2D depiction of the binding mode of **2** with cathepsin. **c** 3D binding mode of **2** in the active site of cathepsin. The native ligand is shown as *blue ball*-and-stick model and **2** is shown as *yellow ball*-and-stick model

each of the query molecules with the top target in the hit list are depicted for comparison in Figs. 5.2a, 5.3a, 5.4a and 5.5a.

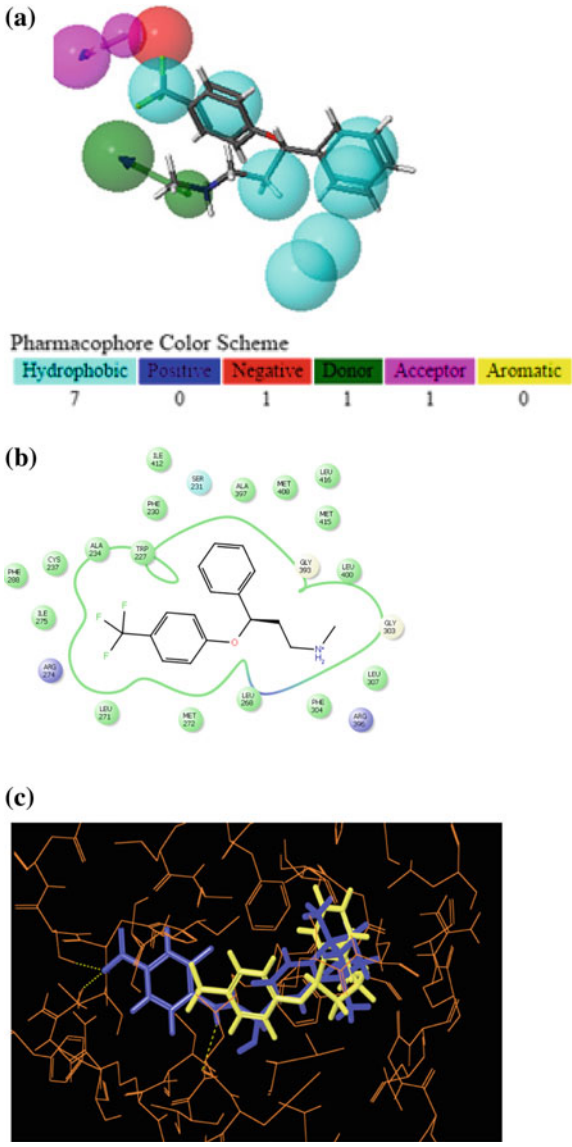

Fig. 5.4 **a** Pharmacophore features derived from retinoic acid receptor (PDB ID 1EXA, Rank 3) mapped onto the molecular structure of query **3**. **b** 2D depiction of the binding mode of **3** with retinoic acid receptor. **c** 3D binding mode of **3** in the active site of retinoic acid receptor. The native ligand is shown as *blue ball*-and-stick model and **3** is shown as *yellow ball*-and-stick model

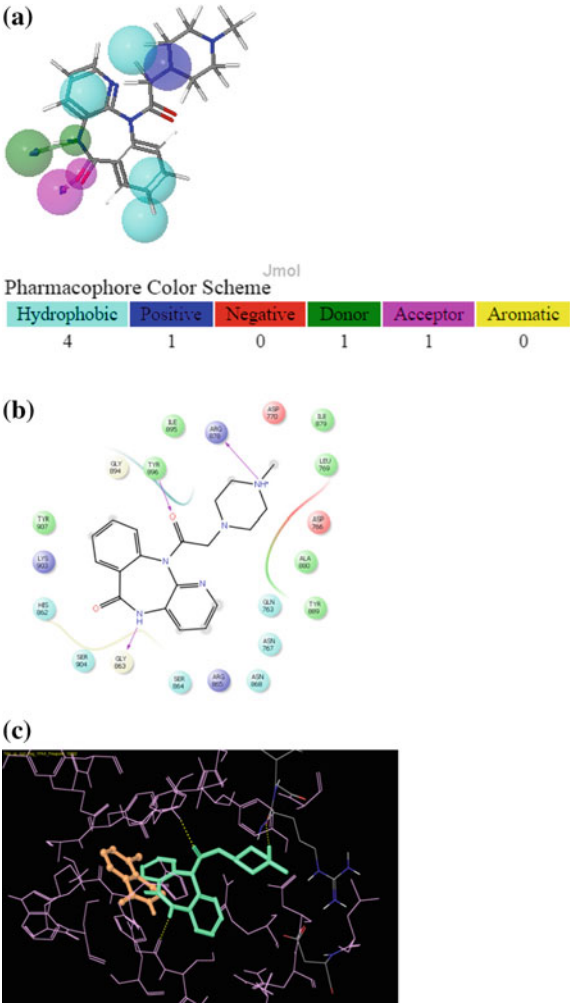

Fig. 5.5 **a** Pharmacophore features derived from poly [ADP-ribose] polymerase 1 (PDB ID 1PAX, Rank 1) mapped onto the molecular structure of query **4**. **b** 2D depiction of the binding mode of **4** with poly [ADP-ribose] polymerase 1. **c** 3D Binding mode of **4** in the active site of poly [ADP-ribose] polymerase 1. The native ligand is shown as *orange ball*-and-stick model and **4** is shown as *green ball*-and-stick model

5.3 Results and Discussion

In the present study, various computational approaches were used for the prediction of potential targets for approved/existing drugs listed in Table 5.1 (reverse pharmacophore mapping strategy). A simple methodology based on the ligand-based drug repositioning was used. The query molecules belonging to three different therapeutic classes—kinase inhibitors, antidepressants, and antimuscarinic agents, were used. The query molecules were selected based on the diversity of the molecular targets such as kinases, monoamine transporters and musarinic receptors along with their involvement in a variety of diseases and disorders. These target classes represent receptors and/or enzymes and transporter proteins. These targets

are localized peripherally as well as centrally. Overall, varied molecular targets formed the basis of query selection.

Table 5.1 depicts the top five diverse targets for query **1** (erlotinib, Fig. 5.1) identified by PharmMapper. The mapped pharmacophoric features are calculated to identify the optimal interaction modes in the target (Fig. 5.2a).

The pharmacophore model based on the chemical features serves as a guide in the screening of potential targets. The binding mode of **1** in acetylcholinesterase (AChE) binding site (PDB ID 1HBJ) is depicted as 2D interaction diagram (Fig. 5.2b), where the ligand is seen to occupy the binding cavity of the target. Figure 5.2c shows the 3D binding model of the native ligand and query **1** where the query molecule was found to partially overlap the native ligand-binding area. Since erlotinib is a bigger molecule than the native ligand, it showed additional hydrophobic interactions as seen in Fig. 5.2b. This may contribute additionally to the binding of erlotinib with the AChE. Intrigued by this observation, we went on searching the literature for possible experimental evidence. No reports were found relating erlotinib with AChE inhibition. Similar exercise was repeated for the remaining targets listed in Table 5.1. Query **1** showed good binding interactions with each of these targets (data not shown). Further literature search revealed interesting reports. One of the experimentally confirmed implications for erlotinib was inhibition of TNF-α, which in turn reduces insulin resistance, thus used for treating type I diabetes [26]. The results of PharmMapper also confirmed the presence of aldose reductase (Rank 5) as one of the targets for erlotinib, which is involved in antidiabetic action. But direct relation of query **1** with aldose reductase could not be found. Interested researchers may test the utility of erlotinib for the therapeutic areas listed in Table 5.1.

Similar study was done on query molecule **2** (sunitinib), which when analyzed by PharmMapper gave a list of potential targets (Table 5.2). Docking study of query **2** was done with cathepsin K, rank 2, a target involved in bone resorption. It is seen that a part of query **2** overlaps the native ligand of the receptor at its binding area. The interactions of pi-pi stacking of query **2** with the receptor are seen in Fig. 5.3b, that further assists its binding. The 3D binding mode (Fig. 5.3c) of sunitinib with the native ligand shows dotted lines representing H-bonds with the target protein. Further, very recent reports presented the use of sunitinib for renal cell carcinoma bone metastases [27], an activity related to bone resorption. This likeliness of the data made us search further for similarity in the acquired results and experimental results. It was also found that sunitinib suppresses antiviral immunity leading to oncolytic virotherapy [28]. The targets in the list of Rank 5, 6, 7 (Table 5.2) also refer to antiviral action. However, direct relation of query **2** with these targets could not be found.

The top hits for query **3** (fluoxetine) are listed in Table 5.3. Docking studies of query **3** with retinoic acid receptor (PDB ID 1EXA, Rank 3) are shown as 2D (Fig. 5.4b) and 3D (Fig. 5.4c) representations. It is seen that query **3** completely matches with the binding mode of the native ligand, since query **3** is a small molecule compared to the native ligand. These results were further matched by literature evidence. There are reports of query **3** (fluoxetine) for therapeutic uses

other than antidepressants. Fluoxetine has been found to inhibit Coxsackievirus Replication [29], as well as hepatitis C virus [30], that matches with the presence of an antiviral target as Rank 2.

Similarly, for query **4**, the hit list showed the presence of varied targets other than the original target (Table 5.4). The docking studies of the query with poly [ADP-ribose] polymerase 1 receptor (PDB ID 1PAX, Rank 1) are shown as 2D (Fig. 5.5b) and 3D (Fig. 5.5c) representations. The 3D binding modes of query **4** (Fig. 5.5c) showed presence of hydrogen bonds with the receptor, depicted by yellow-dotted lines that contribute greatly to the binding of query with the macromolecule. No literature reports were found for the use of pirenzepine against the top targets from the list. These hypotheses can therefore be explored further by curious scientists by drug repositioning approaches.

5.4 Conclusions

The overall aim of the chapter is the use of inverse virtual screening for generation of hypotheses for drug repurposing. This strategy is adapted within the drug discovery process in order to explore the usefulness of an active molecule. Ligand-based inverse technique using PharmMapper has gained increasing importance in the drug repositioning field by characterizing novel target proteins for already established drugs, which can be further validated by in vitro and in vivo bioassays. This can be used as an alternative computational strategy for quick identification of potential therapeutic targets. The application of this approach is the prediction of activity of unknown ligands, natural products and drugs with side effects for known targets involved in the development of many life threatening diseases like cancer and could be applied to other libraries of ligands and different panels of targets. With the new paradigm in drug discovery process, inverse virtual screening will play a fundamental role in discovering leads with desired therapeutic profiles and systematic data mining to reveal the synergistic effects and side effects of therapeutics.

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