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Pharmacophore-based virtual screening: a review of recent applications

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Importance of the field: In research relating to the development of new drugs, hit identification and validations are critical for successful optimization of candidates. To achieve rapid identification of new lead compounds, high-throughput screening assays have been employed in many pharmaceutical companies and laboratories. However, their success depends on the assay system relevant to *in vivo* conditions and they are physically limited by the repertoire of compounds. As an alternative or complementary approach to high-throughput screening assays, virtual screening is an efficient method to identify drug candidates *in silico* from large chemical compound databases. Its usefulness has been verified by current applications that successfully retrieved hit and lead identifications against various disease targets. However, for better application, the scoring functions for distinguishing possible active and inactive compounds must be improved.

Areas covered in this review: In this review, we provide an overview of pharmacophore-based virtual screening methods with a special focus on their successful application towards finding hits against various disease targets.

What the reader will gain: Readers will rapidly gain insight into the recent successful applications of pharmacophore-based virtual screening. They will acknowledge that this technique is a powerful and cost-effective alternative to high-throughput assays.

Take home message: Although there are many hurdles yet to be resolved, virtual screening techniques will emerge as essential infrastructure and as a prerequisite for developing new lead compounds with therapeutic applications.

Keywords: anticancer, antivirus, drug discovery, pharmacophore, virtual screening

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1. Introduction

Research on the development of new drugs generally starts with target selection followed by hit identification, hit-to-lead confirmation, lead optimization and clinical candidate selection. Although hit identification is an early stage in the whole drug discovery program, the availability of a pool of structurally diverse hit compounds is crucial for successful optimization of candidates. For rapid identification of new lead compounds, screening assays in high-throughput format have served as the major tool in many laboratories. However, the difficulties in establishing or accessing collections of existing compounds have hampered efficient identification of new lead compounds. In parallel to the high-throughput screening technologies, virtual screening has emerged as an

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Article highlights.

- Inhibitors of tubulin, cellular kinases and aromatase identified by pharmacophore-based virtual screening can be useful drug candidates for cancer therapy.
- Inhibitors of several viral and cellular proteins that participate in viral replication and reproduction, including viral enzymes and ligands as well as host counterparts, exhibit promising antiviral activities.
- Enzymes and receptors associated with metabolic diseases, such as 11β-HSD1, DPP-IV, CB1, PPARs and FFAR, are challenging targets for pharmacophore-based virtual screening to develop antimetabolic drugs.
- Pharmacophore-based virtual screening has proved to be successful for the identification of inhibitors of tau polymerization into filaments.

This box summarizes key points contained in the article.

efficient tool for the early identification of potential hit structures. Contrary to a physical library of chemical compounds required for high-throughput screening, virtual screening requires a database of chemicals and searches this database *in silico* for identifying drug candidates.

When the three-dimensional (3D) structure of a target is unknown, ligand-based virtual screening can be applied as a compound selection filter for the identification of biologically active compounds [1]. Ligand-based virtual screening involves two different methods: i) flexible alignment of molecules by considering only the atomic contributions; and ii) use of other chemical features that are unrelated to 3D pharmacophore representations, such as hydrogen bonding and lipophilicity, as the input data for flexible alignment [2]. In contrast, if the 3D structure of a target protein is available, both high-throughput docking and receptor-based pharmacophore virtual screening can be applied for identifying new drug candidates.

Pharmacophore is defined as an arrangement of molecular features or structural elements related to biological activity [3]. Recently, this term has become one of the most popular icons in drug discovery. As a useful tool for drug designing, pharmacophore-based virtual screening has proved useful for hit identification and lead optimization in the initial phase of new drug development programs. The applications of such virtual screening for various disease targets have been reviewed in recent articles [2,3].

The main advantage of this approach is that virtually millions of compounds can be screened for hit identification. Its disadvantage is that systematic approaches are not accomplished: important interactions may not be well represented in a specific chemical feature model, increasing the possibility of loss of meaningful information in the resulting 3D pharmacophore; as a result, it is impossible to estimate the binding free energy contributions of particular chemical features [2]. In this review, we focus on the studies that successfully applied 3D pharmacophore-based virtual screening by employing the ligand- or receptor-based method for hit identification.

2. Pharmacophore-based virtual screening

Pharmacophore features are generally represented by points in 3D space. A pharmacophore feature could be composed of functional groups, such as hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), cations, anions, aromatics and hydrophobic area (Hyp) [4,5]. The extraction of a pharmacophore feature from a set of bioactive compounds does not require the 3D structure of the target protein. Indeed, the extraction of common chemical features supposedly interacting with the target proteins is a critical step for pharmacophore generation. Caution should be exerted while handling the conformational flexibility for pharmacophore generation where the active conformation of molecules is postulated. There are several commercially available programs for automatic generation of pharmacophore models, including Catalyst [6], DISCO [7], GASP [8], Phase [9], MOE [10] and LigandScout [11]. These programs have their own algorithms for alignment and pharmacophore generation as well as for handling the conformational flexibility.

For pharmacophore-based virtual screening, it is necessary to generate a 3D structural database of ligands, define pharmacophore features and search for biologically active conformations. The generation of a 3D structure is generally achieved by using automated software programs to translate 2D structures into 3D formats such as SMILE strings, SLN strings and MDL SD connection tables. The most widely used 3D conformer-generation programs are CONCORD [12] and CORINA [13]. These programs allow rapid identification of reliable conformers with outputs in various file formats. In pharmacophore-based virtual screening, the flexibility of small molecules is handled and guided by multiple conformations for each molecule in the database. A critical validation of the quality of such multiconformers would be their ability to reproduce previously known bioactive conformations. There are several commercial programs available for multiconformer generation of ligands, including Omega (http://www.eyesopen.com/products/applications/omega.html), Catalyst [6], Macro-Model [14] and Confort [15].

The process of ligand- or receptor-based pharmacophore virtual screening is demonstrated in **Figure 1**. The process encompasses a variety of sequential computational steps: target selection, database preparation, pharmacophore model generation, 3D screening and prioritization of compounds for final confirmation of biological activity. Comprehensive reviews have already covered each step [1,2,5,16-23], outlined the theoretical background and described prominent examples of 3D pharmacophore-based virtual screening strategies. To avoid unnecessary overlaps, we have excluded detailed descriptions of the theoretical background from this review.

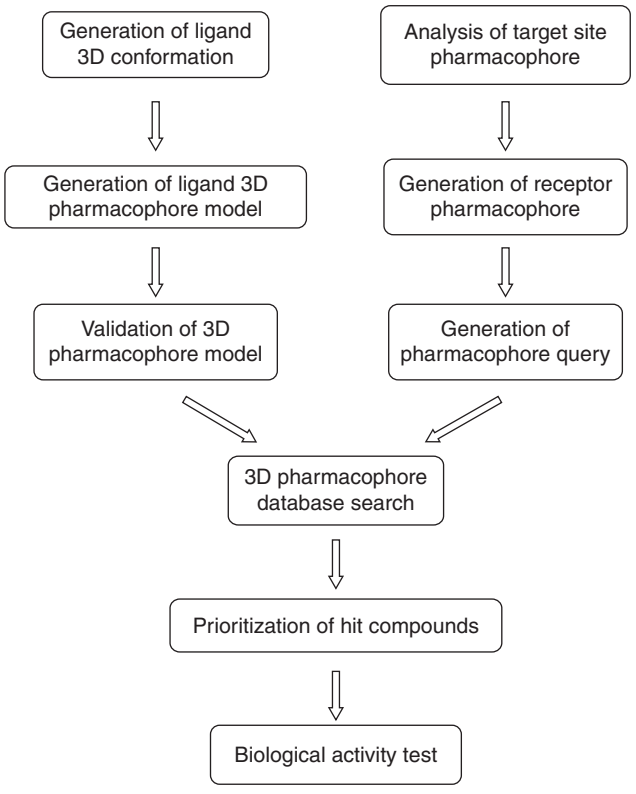


Figure 1. Process for the ligand and receptor pharmacophore-based virtual screening. A typical process consists of the preparation of pharmacophore model, three-dimensional database screening and selection of hit compounds.

3. Recent applications of pharmacophore-based virtual screening

In recent years, the number of studies on pharmacophore-based virtual screening for various disease targets has dramatically increased. Advances in the screening tools that rely on chemical feature-based pharmacophore models have significantly influenced the rational design of new molecular entities. Catalyst, a leading software for chemical feature-based pharmacophore modeling, is arguably the most widely used program. A number of previous studies focused on the validation of pharmacophore-based virtual screening – by evaluating pharmacophore models, by pharmacophore identification and by the associated methodologies for screening – with respect to the reproduction of the binding affinity or enrichment of hit lists from a database of decoys [24-32]. In this section, we focus on examples that have shown, to some extent, the successful application of pharmacophore-based virtual screening for new drug discovery. The overall features of these studies are summarized in **Table 1**.

3.1 Anticancer inhibitors

3.1.1 Tubulin inhibitors

Microtubules are cytoskeletal filaments consisting of α- and β-tubulin heterodimers. They are involved in crucial biological processes including cell division, cell mobility and cell-to-cell interactions, and have been identified as attractive targets for cancer therapy [33].

Chiang *et al.* [34] present an example of the successful application of pharmacophore-based virtual screening to identify novel compounds for inhibiting tubulin polymerization. A pharmacophore model was built on the basis of a training set of 21 indole compounds exhibiting inhibitory activity against the KB cell line within the concentration range of 1.2 nM – 6 μM [35], by applying the HypoGen module of Catalyst. By analyzing the chemical feature of the 21 compounds, four features were selected for pharmacophore model generation: hydrophobic group, hydrophobic aromatic group, HBA and HBD. To evaluate the effects of steric hindrance, an important factor for biological activities, the HypoRefine module was utilized to generate excluded volume features. By using these features and subsequently following constructive, subtractive and optimization phases, 10 top-ranked hypotheses were generated. These hypotheses were validated by using the CatScramble program implemented in Catalyst. Finally, the researchers obtained a refined pharmacophore model (Hypo 1), which was utilized to predict the activities of all the 21 training compounds.

The estimated activities predicted by Hypo 1 were close to the experimentally determined values, showing good predictability for biological activity, with high correlation coefficients of 0.96 and 0.89 for the training-set and test-set compounds, respectively. Once validated, the model was used as a 3D structural query for searching novel inhibitors from the chemical database of ChemDiv, Inc. (http://www.chemdiv.com) and collections of ~ 130,000 in-house compounds. The 1,000 best compounds predicted as highly active were subjected to further analysis. Finally, visual inspection of each molecule led to the selection of 142 compounds. Their anti-proliferative activities were then tested against the human oral squamous carcinoma KB cell line. It was seen that 4 compounds displayed 50% inhibitory concentration (IC_{50}) values of < 10 μM. The most potent compound (IC_{50} = 0.187 μM; **Figure 2**) inhibited the proliferation of various other cancer cell lines, including MCF-7, NCI-H640 and SF-268. Further biological characterization revealed that this compound (1) effectively inhibited tubulin polymerization and significant cell cycle arrest in the G2/M phase.

Kim *et al.* [36] designed and biologically evaluated novel tubulin inhibitors. They investigated the structural basis for the interaction of anti-mitotic agents with tubulin and proposed the binding models for a compound (MDL-27048) against the colchicine-binding site of tubulin [37,38]. The proposed model was not only consistent with the previous experimental data on the competition between colchicine

Table 1. Summary of successful application of hit generation by using pharmacophore-based virtual screening.

Disease	Target	Method	Activity (compound)	Ref.
Cancer	Microtubule	Catalyst PharmoMap™, PharmoScan™	IC_{50}: 0.187 μM (1), 15.7 μM (2)	[35,36]
	Aurora A kinase	Catalyst	IC_{50}: 3.8 μM (3), 9.4 μM (4)	[40]
	KDR (VEGFR-2)	Catalyst	100% inhibition, 50 μM (5)	[41,44]
	CHK1	Plurality (in-house program), FlexxPharm	IC_{50}: 450 nM (6)	[77]
	Aromatase	Catalyst/HipHop	IC_{50}: 274 nM (7)	[49]
Virus infection	HIV integrase	Catalyst/HipHop Gold	IC_{50}: 5 μM (8)	[50-53]
	HIV protease	Catalyst LigandScout	IC_{50}: 1.9 μM (9), 0.9 μM (10), 35 μM (11)	[54-55]
	CXCR4	MOE, Discovery Studio, PARAFIT08, Autodock3.0, FRED2.2.1, Gold3.0.1, HEX4.8	EC_{50}: 0.022 μg/ml (12)	[56]
	HCV polymerase	PharmoMap™, PharmoScan™ Unity, Flexx-Pharm	IC_{50}: 20 μM (13 – 15); cell viability: 1.26% (16)	[58-60]
	SARS-CoV protease	Catalyst, Dock4.0.2, Dock5.0, ComFA, ComSIA	IC_{50}: 3 μM (17)	[64-66]
Diabetes and obesity	11β-HSD1	Catalyst, Glide Catalyst/HipHop, Dock4.0 Catalyst	IC_{50}: 0.26 μM (18), 0.69 μM (19), 0.85 μM (20), 0.98 μM (21), 0.144 μM (22), 0.69 μM (23), 0.80 μM (24), 0.172 μM (25)	[69-70,73]
	CB1	Catalyst/HipHop Catalyst, MOE	K_i: 52.8 nM (26), 92 nM (27)	[74-75]
	DPP-IV	In-house program, GLIDE	Inhibition at 30 μM: 81.8% (28), 81.4% (29)	[76]
	PPARs	Catalyst/HipHop	PPAR-α IC_{50}: 1.5 μM (30), 1.0 μM (31), PPAR-γ IC_{50}: 44 μM (32), 13 μM (33)	[81]
	FFAR1	UNITY, Glide	EC_{50}: 7.6 μM (34), 3.6 μM (35), 8.2 μM (36)	[88]
Alzheimer	tau	Catalyst	IC_{50}: 12 μM (36), 9 μM (37)	[90]

11β-HSD1: 11β-hydroxysteroid dehydrogenase type 1; CHK1: checkpoint kinase-1; DPP-IV: dipeptidyl peptidase IV; FFAR1: free fatty acid receptor 1; HCV: hepatitis C virus; KDR: kinase insert domain-containing receptor tyrosine kinase; SARS-CoV: Severe acute respiratory syndrome coronavirus.

and MDL-27048 but also suggested the presence of an additional binding cavity on tubulin. On the basis of this finding of the MDL-27048 and tubulin complex, pharmacophore-based virtual screening was employed to identify new anti-mitotic agents from a chemical database. The pharmacophores were generated by using PharmoMap, an in-house software package developed by Equispharm as a pharmacophore generation tool. The pharmacophore models were employed as a search query to identify inhibitors targeting the colchicine-binding site from a 3D small molecule database, which comprises commercially available compounds of chalcone moieties (a total of 9,720 compounds). The structure-based virtual screening for the colchicine-binding pocket of tubulin was carried out by using the PharmoScan system, a structure-based virtual screening tool developed by Equispharm, Inc. (http://www.equispharm.com). A total of 24 compounds were finally selected for *in vitro* cell proliferation and tubulin polymerization assays. These exhibited activities with IC_{50} values in the range of 15.7 – 19.8 μM when tested in the tubulin polymerization assay. The most active compound (2; **Figure 2**) showed strong cell-cycle arrest in the G2/M phase and tubulin polymerization activity essential for anti-mitotic agents.

3.1.2 Kinase inhibitors

Aurora-A kinase, one of the most promising anticancer targets, has attracted both academia and pharmaceutical companies for the development of inhibitors [39]. Recently, Deng *et al.* [40] reported pharmacophore modeling and virtual screening of new Aurora-A kinase inhibitors. The analysis was employed to extract a common pharmacophore feature from known Aurora kinase inhibitors. A training set of 24 inhibitors from different literature resources was chosen for 3D pharmacophore model generation. Four molecular features, including HBA, HBD, general hydrophobicity and hydrophobic aromaticity, were selected to construct the pharmacophore model. The value for excluded volume was set to one to consider steric effects. The pharmacophore model was further validated by a test

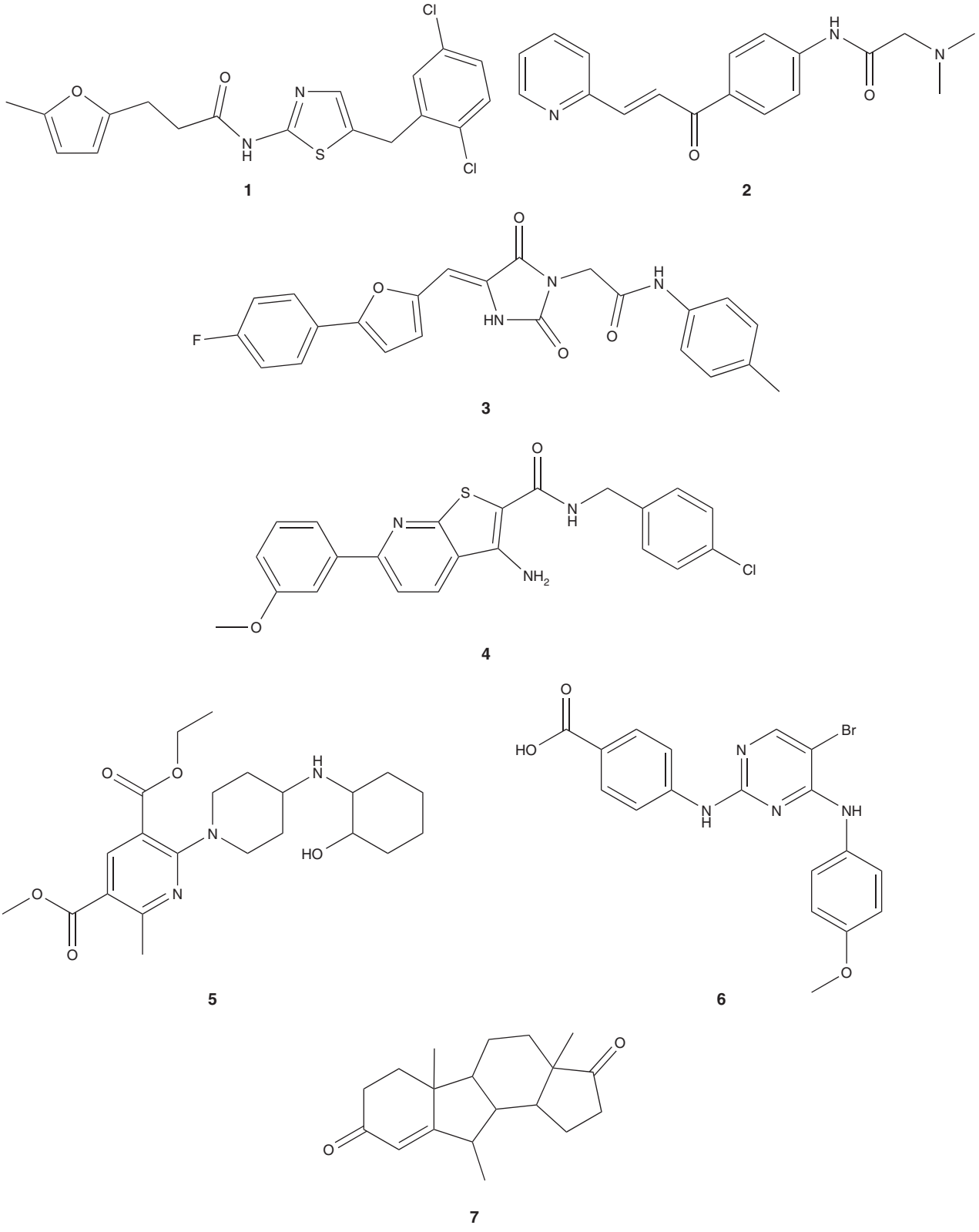


Figure 2. Chemical structures of active compounds identified by pharmacophore-based virtual screening for cancer inhibitor.

set. The results showed a fairly good correlation between the experimental and the predicted IC_{50} values, with a correlation coefficient of 0.904. Then, the model was used as a 3D query to screen the commercially available chemical library at Specs (http://www.specs.net) and the Chinese Natural Product Database. The resultant virtual screening hits were filtered by Lipinski's rules and docking studies to refine the retrieved hits. Finally, 39 compounds were purchased and screened *in vitro* against several human tumor cell lines in which Aurora-A is over-expressed, including A549, MCF7, HepG2 and PC-3. Two compounds, 3 and 4 (**Figure 2**), showed low micromolar inhibitory efficacy against the PC-3 and HepG2 cell lines.

Inhibitors of the kinase insert domain-containing receptor tyrosine kinase (KDR) exhibit potent antitumor activity and have emerged as promising novel cancer therapeutic agents [41]. Yu and coworkers [42] reported pharmacophore model generation, database searches and docking methodologies to identify a new structure for KDR inhibitors. The 3D pharmacophore models were generated from a set of 10 KDR inhibitors by using the HipHop algorithm [43] in Catalyst. The best pharmacophore model chosen contained four chemical features: one hydrophobic, one HBA, one HBD and one ring aromatic function. Then, the 3D structure of 4-amino-furo[2,3-*d*] pyrimidine, which was extracted from the crystal structure (PDB code 1YWN), was used to generate a shape query [44]. The Maybridge database (http://www.maybridge.com) was screened by using both the hypothesis and the shape information to discover new leads. The virtually screened compounds were then further examined for fit value, docking score, consensus score and binding conformation of the KDR–compound complex. Compound 5 (**Figure 2**) was shown to completely inhibit VEGF-stimulated KDR phosphorylation in HUVEC cells at 50 μM.

Lyne *et al.* [45] at AstraZeneca reported a successful 3D pharmacophore screening against checkpoint kinase-1 (Chk-1), an attractive target for cancer therapy [46]. A database of the AstraZeneca compound collection was filtered by molecular weight and rotatable bonds. Subsequently, a 3D pharmacophore was used for the screening of compounds against Chk-1. The hits from the pharmacophore searches were docked into the active site of Chk-1 by using the FleXX-Pharm program [47]. Each compound was then filtered by using a customized consensus-scoring scheme for Chk-1, followed by visual inspection of the docked compounds. Finally, 103 compounds were selected for further *in vitro* assay against Chk-1. A total of 36 compounds were found to inhibit the enzyme in a dose-dependent manner with IC_{50} values ranging from 100 nM to 68 μM. The most active compound (6) is represented in **Figure 2**.

3.1.3 *Aromatase inhibitors*

Aromatase, an enzyme involved in the conversion of androgen to estrogen, is an important target for the endocrine treatment of breast cancer [48]. Recently, Neves *et al.* [49] reported a pharmacophore-based approach for new aromatase inhibitors. Pharmacophore models were generated to accommodate putative binding requirements for the steroid aromatase inhibitors. The common chemical feature of C6-substituted steroid aromatase inhibitors, as deduced by the HipHop module in Catalyst, included two HBAs and three hydrophobic groups. In order to bind to the active site, the aromatase inhibitors must have both appropriate shape and appropriate volume. Thus, a pharmacophore model containing the combined pharmacophore and shape features was obtained. With this model, potential C6-substituted steroid aromatase inhibitors were screened by a 3D search query against the National Cancer Institute (NCI) database. Biological testing of some of these compounds showed low nanomolar IC_{50} values following the competitive inhibitory mode (7; **Figure 2**).

3.2 Antiviral inhibitors

3.2.1 *Inhibitors of HIV*

HIV, the causative agent of AIDS, expresses three structural polyproteins, Gag, Pol and Env, and a number of additional auxiliary proteins essential for viral replication. Among these, virally encoded enzymes integrase [50-53] and protease [54,55] have been subjected to extensive pharmacophore modeling for inhibitor screening. In addition, the glycoprotein has been subjected to pharmacophore modeling for successful identification of a receptor antagonist [56].

Dayam *et al.* [50] developed a pharmacophore model of quinolone 3-carboxylic acids, which are a novel class of integrase inhibitors, by using a training set of clinical candidates. From a database of 360,000 small-molecule compounds, 56 hit compounds were selected, followed by *in vitro* evaluation of the selected hits. After analysis of the structure–activity relationship (SAR) around the core of the compounds, a novel integrase inhibitor having diverse structural scaffolds was identified, with a IC_{50} value of 5 ± 2 μM (8; **Figure 3**).

A 3D hypothetical model for the binding of diketoacid analogues, which have been introduced into clinical studies, to integrase was built by means of Catalyst [51]. Ten compounds were finally selected from the CAP2002 database for integration assay, and two compounds, 9 and 10 (**Figure 3**), were identified as the most active inhibitors with IC_{50} values of 1.9 and 0.9 μM, respectively.

Another 3D pharmacophore model has been successfully generated for HIV-1 integrase by using 26 known inhibitors as a training set [53]. This model was validated by 14 other highly active compounds available from the literature. The model accurately predicted all the 14 previously known molecules as highly active, in perfect agreement with the actual biological data.

Disruption of the protein–protein interactions between protease and a cellular partner (LEDGF/p75), which has a critical role in HIV integration, has been reported by using

pharmacophore modeling [52]. A structure-based pharmacophore model for potential small-molecule inhibitors of the HIV-1 integrase and LEDGF/p75 interaction was developed by using the LigandScout software. Compound CHIBA-3002 was identified as an interesting hit for further optimization. After rational designing and synthesis of the derivatives, a new and more potent small molecule, CHIBA-3003 (11; **Figure 3**), was identified. The compound was shown to interfere with the HIV-1 integrase and LEDGF/p75 interaction at the IC_{50} value of 35 μM. By targeting the interface between the viral protein and the host factors, the novel inhibitors of integrase could provide an alternative to treat antiviral drug-resistant viruses against reverse transcriptase and protease.

HIV protease has also been actively pursued by pharmacophore-based virtual screening as a promising target in antiviral therapy [54,55]. To enhance the specificity and sensitivity of pharmacophore-based virtual screening, Pandit *et al.* [54] optimized the variables for the generation of small-molecule conformation, and for pharmacophore model construction of inhibitors. The key variables explored in their study included the selection of chemical features, involvement of excluded volumes, tolerance radius of excluded volumes, energy windows and maximum number of conformers. They tested the enhanced model by using HIV protease as the model target and verified that the model was able to identify 60 out of 75 known structurally diverse protease inhibitors correctly.

Successful application of pharmacophore-based screening was achieved by parallel screening and activity profiling of HIV protease inhibitors [55]. Four input data sets of approximately equal size comprising 89 known HIV protease inhibitors, 79 inhibitors of other proteases, 85 drug-like inactive compounds and presumably 80 inactive molecules taken from a virtual library of > 12,000 drug-like diverse compounds were employed. By using this pharmacophore-based screening technique, 59 known active structures were retrieved out of the 89 ligands with 66% predictability.

HIV entry into host cells involves the binding of the viral envelope glycoprotein (gp120) to both the host CD4 receptor and one of the CXCR4 or CCR5 chemokine coreceptors. Inhibitors of the receptor–ligand interaction were investigated by using the CXCR4 receptor as the target [56]. Various methods for structure- and ligand-based virtual screening were employed, including QSAR analyses, pharmacophore modeling and shape matching, to find new potential HIV entry inhibitors for the CXCR4 receptor. In this quest, pharmacophore modeling was performed by using the MOE and Discovery Studio software [57]. Five molecules in the hit list were selected and synthesized, and their *in vitro* EC_{50} values were in the range of 0.022 to > 4.1 μg/ml. Among them, a cyclam amine (12; **Figure 3**), a derivative of AMD3100, exerted anti-HIV activities with an EC_{50} value of 0.022 μg/ml, and is one of the most potent CXCR4 antagonists ever developed.

3.2.2 *Inhibitors of hepatitis C virus*

In the control of hepatitis C virus (HCV), HCV RNA-dependent RNA polymerase NS5B remains a challenging target for rational drug designing [58-60]. Not only the active sites of catalysis but also potential allosteric sites are being actively pursued for the identification of novel inhibitors.

Ryu *et al.* [58] generated a pharmacophore map by using PharmoMap (http://www.equispharm.com) for structure-based virtual screening and identified a potential allosteric binding pocket at the interface of the thumb and palm domain of NS5B. The constructed pharmacophore model was used as a search query for identifying inhibitors from a commercial 3D small-molecule database comprising 3.5 million compounds. Through the *in vitro* assay of NS5B activity with 119 compounds, 3 compounds (CVP09011, CVP09047 and CVP09081) with IC_{50} values of ~ 20 μM were identified (compounds 13 – 15; **Figure 4**).

By using 98 compounds known to be HCV polymerase inhibitors, a ligand-based virtual screening optimized procedure was employed to predict the potential inhibitors against genotype 1 HCV polymerase [59]. On the basis of the quantitative structure–activity patterns of the selected compounds, structural modifications were proposed to afford a better activity profile. Here, a QSAR model was developed by using five descriptors (Lipophilicity [ClogP], HOMO energy, Kier and Hall index order 2 [Ki2] and Kier and Hall information indices order 0 and 3 [KiInf0, KiInf3]) to predict the inhibitory potency and biological activities of the novel structures. Alternatively, aryl diketo-acid moiety-based inhibitors have been identified by using pharmacophore-guided virtual screening [61]. The UNITY-based pharmacophore search was employed as an efficient filtering tool. Following the docking-based virtual screening, novel anti-HCV diketoacid derivatives were generated. With this strategy, 40 compounds were selected as novel candidates from among 37,447 compounds in the Lead-Quest (http://www.tripos.com) chemical library. After cell-based biological screening of the selected hits, compound 16 (**Figure 4**) was identified as the most active compound with a perfect match to the hydrophobic hole in the aryl-binding site of HCV polymerase. In addition, an enhanced version of the flexibility induced through targeted evolutionary description (FITTED) docking program has been recently developed and applied to the virtual screening of potential HCV polymerase inhibitors [60].

3.2.3 *Inhibitors of influenza virus*

Influenza has long been recognized as the prime cause of acute respiratory disease with annual episodes and potential pandemic outbreaks. Recently, avian–human transmission of the H5N1-type virus and human–human transmission of a novel A/H1N1 virus have emerged as global concerns. For mitigating influenza infection, neuraminidase, one of the two major membrane glycoproteins on the virion, has been the promising target for antiviral drug development.

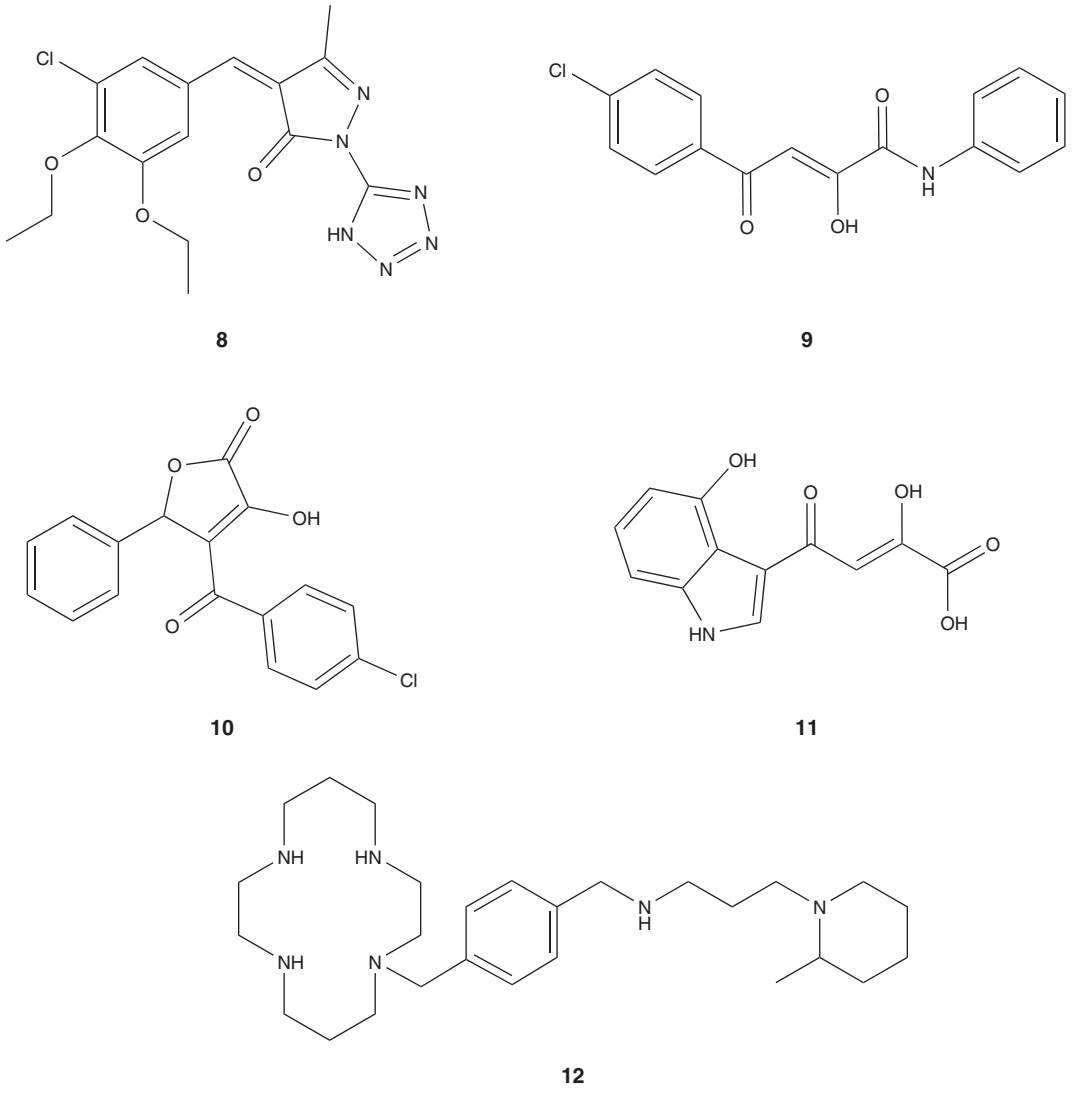


Figure 3. Chemical structures of active compounds identified by pharmacophore-based virtual screening for HIV inhibitor.

The recent emergence of resistant viruses to oseltamivir (Tamiflu), one of the two clinically available neuraminidase inhibitors, among H5N1-infected patients and the seasonal H1N1 viruses necessitate the development of next-generation neuraminidase inhibitors.

Highly selective pharmacophore models for inhibitors of viral neuraminidase were developed aided by Catalyst [62]. Here, chemical feature-based pharmacophore models were generated for the binding site of the enzyme on the basis of the X-ray crystallographic data on the neuraminidase complex with different inhibitors. The models were validated by virtual screening processes and could serve as a preliminary selection tool for candidate molecules based on their potential neuraminidase inhibitory activity.

Besides neuraminidase, influenza RNA polymerase remains a challenging target for antiviral drug designing. Interestingly, this polymerase carries intrinsic endonuclease activity as a novel target for antiviral activity [63]. In this study, the UNITY program was employed with the pharmacophore constraint obtained from ligand-based 3D QSAR studies. Among 693,042 compounds in the ChemDiv database, compounds with three oxygen atoms and three hydrophobic moieties, CD10, CD37 and CD57, exhibited the highest pIC_{50} values predicted by the 3D QSAR model, but await further biological validation.

3.2.4 *Inhibitors of severe acute respiratory syndrome coronavirus*

Severe acute respiratory syndrome coronavirus (SARS-CoV), first reported in Asia in 2003, is a newly emerging epidemic virus. Attempts have been made to control the virus by

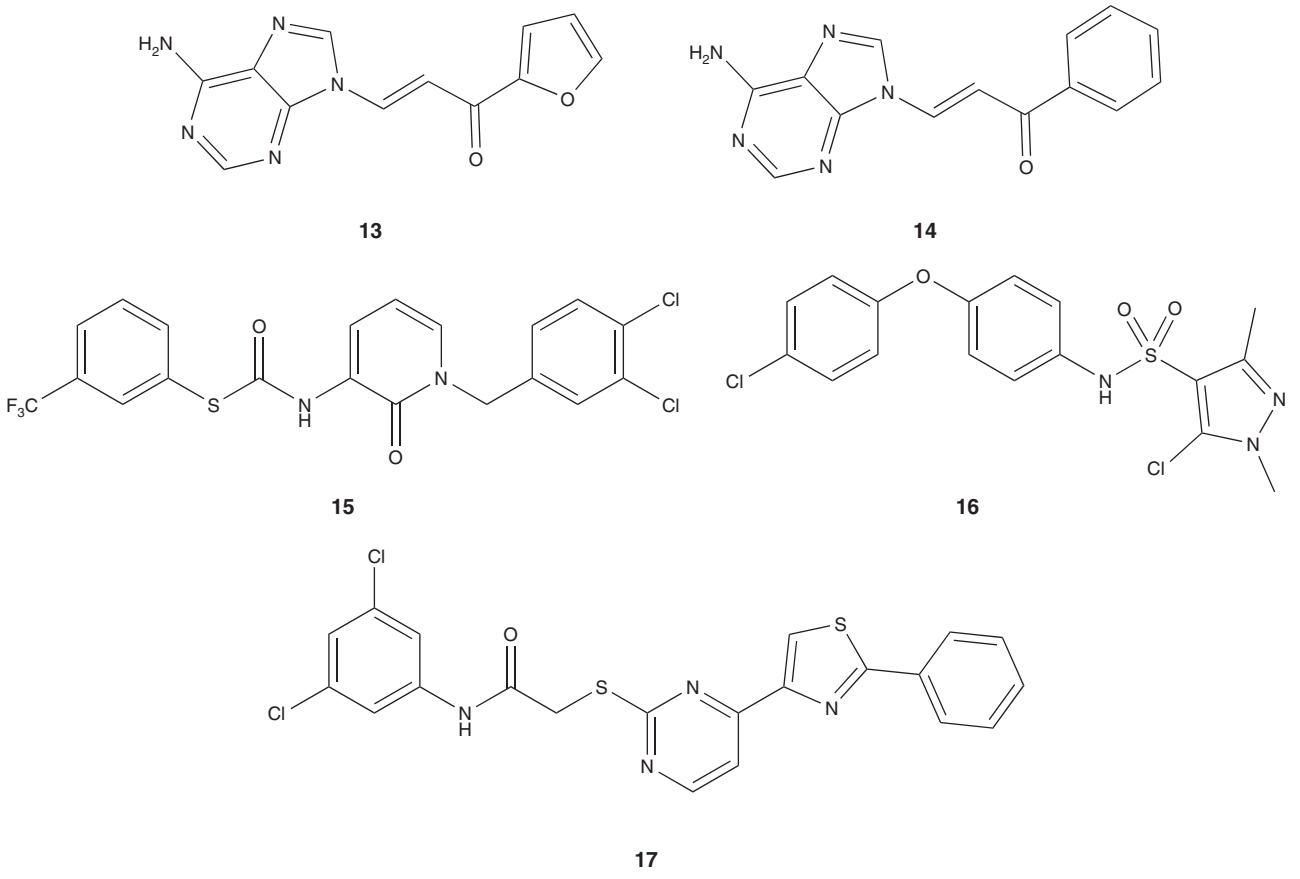


Figure 4. Chemical structures of active compounds identified by pharmacophore-based virtual screening for hepatitis C virus inhibitor.

targeting the viral protease, which plays an important role in viral replication [64-66].

Zhang *et al.* [64] developed a 3D pharmacophore model for the SARS-CoV protease to discover new anti-SARS-CoV inhibitors. They used the experimental structure of the SARS-CoV main protease complexed with its peptide inhibitor, CMK, along with the predicted structures of the main protease. The NCI 3D database, including 250,251 compounds, was screened by using the pharmacophore model to narrow the search down to 30 compounds. Among them, 6 were identical to previously known compounds with proven anti-SARS-CoV activity, suggesting the model's usefulness as a discovery tool for new anti-SARS-CoV inhibitors. In another study, a pharmacophore search was conducted on > 3.6 million compounds on the basis of the atomic coordinates of the complex obtained by the docking model of KZ7088, a derivative of a SARS-CoV main proteinase inhibitor [65].

Through the combination of structure-based virtual screening and 3D QSAR study, Tsai *et al.* [66] identified a novel family of potent SARS-CoV inhibitors. From the 59,363 compounds employed in their docking study, 21 were found to have biological activity and 3 of them shared common substructures. The core structure was then used as a query structure to search for analogues from the Maybridge, ChemBridge (http://www.chembridge.com) and SPECS_SC databases. Among the 28 structural analogues finally identified, inhibitor 1 showed activity with an IC_{50} value of 3 μM (17; **Figure 4**), demonstrating the method's usefulness in identifying novel inhibitors from a large chemical database.

3.3 Metabolic syndrome

3.3.1 11β-Hydroxysteroid dehydrogenase type 1 inhibitors

Enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts cortisone to cortisol. A physiological excess of glucocorticoids causes metabolic abnormalities such as obesity, impaired glucose tolerance, atherosclerosis, dyslipidemia and hyperglycemia [67,68]. Yang *et al.* [69] have described the discovery of highly active inhibitors of 11β-HSD1 by virtual screening using a ligand-based pharmacophore. Ligand-based pharmacophore modeling was employed to retrieve a pool of compounds with features

common to the previously known 11β-HSD1 inhibitors. The compounds with proven biological activity were used as the training set to build the pharmacophore models and identify common features of the inhibitors via the HipHop algorithm of Catalyst. The final pharmacophore model was selected by highlighting four hydrophobic features, an HBA and a ring structure of aromatic nature. A 3D database search among the 3,000 compounds prescreened by the DOCK 4.0 program was performed with the Best Flexible search mode followed by calculation of fit values. Finally, 82 compounds were evaluated by enzymatic assay, identifying compounds 18 and 19 as good inhibitors against human 11β-HSD1 (**Figure 5**).

Yang *et al.* [70] also employed the crystal structure of human 11β-HSD1 complexed with a synthetic inhibitor (PDB code 2IRW) to build pharmacophores [71]. After validation of the query pharmacophore model, virtual screening was carried out by using the Catalyst software. The hit compounds were ranked according to their best-fit values. Finally, the 1,000 compounds selected by virtual screening with a validated pharmacophore were subjected to docking study by using GLIDE [72]. Then, the compounds with the highest G scores were visually inspected and narrowed down to 56 hits. Among them, 9 compounds exhibited inhibition of human 11β-HSD1 with IC_{50} values ranging from 0.85 to 7.98 μM. The most potent compounds (20 and 21) are presented in **Figure 5**.

Schuster *et al.* [73] employed Catalyst to develop ligand-based pharmacophore models for 11β-HSD1 inhibitors. The virtual screening protocol selected 30 compounds, which were then subjected to biological analysis for the inhibition of human 11β-HSD1. At the concentration of 10 μM, 7 of 30 compounds inhibited >70% of the activity against 11β-HSD1. As shown in **Figure 5**, the IC_{50} values of all 7 compounds were <10 μM, with 4 being in the submicromolar range (compounds 22 – 25). The results suggest that inhibitor-based pharmacophore models in combination with suitable cell-based activity assays can be used for the identification of selective and potent inhibitors of 11β-HSD1.

3.3.2 *CB1 inhibitors*

Selective cannabinoid CB1 receptor antagonists have been proven clinically effective for treating obesity and related disorders. Recently, Wang *et al.* [74] have reported the identification of a novel class of azetidinone and CB1 antagonists by using pharmacophore-based virtual screening. The pharmacophore model for CB1 antagonists was constructed from known representative CB1 receptor antagonists. The model was then employed for screening a database of half million in-house compounds. The authors applied a stepwise filtering protocol based on the molecular weight, compound availability and modified rule of five to reduce the number of hits. The Bayesian modeling and clustering methods were adopted to select a final set of 420 compounds for *in vitro* inhibition assay. A total of 5 compounds exhibited >50% inhibition at 100 nM in a CB1 competitive binding assay. The IC_{50} values of the hit compounds were further evaluated to identify the most active compound at 52 nM against CB1 with more than fivefold selectivity against the CB2 receptor (26; **Figure 6**).

Foloppe *et al.* [75] also reported ligand-based virtual screening with a 3D pharmacophore leading to the discovery of novel drug-like ligands of human CB1. The authors used the previously reported data for potent and selective CB1 antagonists. Various pharmacophore models were generated by using Catalyst and then searched against commercially available chemical compounds. The query initially retrieved a pool of hits, which were subsequently filtered on a combination of computed physical properties related to drug likeness and the compatibility with CNS activity. Finally, 261 compounds were purchased from commercial vendors and assayed for binding to hCB1 by the displacement of radiolabeled rimonabant at a single concentration. The screening process yielded 42 compounds with >60% inhibition of rimonabant binding to hCB1. Of these, 30 were selected for the determination of the inhibitory constant (K_i) value against hCB1. The affinity to hCB1 ranged from 92 nM (27; **Figure 6**) to 10 μM, at which 12 compounds exhibited submicromolar affinity for hCB1.

3.3.3 *Dipeptidyl peptidase-IV inhibitors*

Dipeptidyl peptidase IV (DPP-IV) is known to be associated with type 2 diabetes. Ward *et al.* [76] employed a pharmacophore-based virtual screening approach by using an in-house pharmacophore searching method to identify DPP-IV inhibitors [77]. A database of 800,000 available compounds was filtered by physical properties, reducing the number to ~ 500,000 compounds. Enumeration of tautomers, the protonation state and the stereogenic center by using an in-house software and CORINA increased the number of structures to ~ 750,000 compounds in the single 3D conformer database. In addition, a multiconformer database was also built by using OMEGA [78]. The multiconformer database was then screened for the designed DPP-IV pharmacophores, and the hits from these pharmacophore searches were docked into the DPP-IV crystal structure [79] by using GLIDE. Finally, compounds of priority testing were listed, and 51 active compounds were identified from a list of 4,000 compounds tested. Compounds 28 and 29 (**Figure 6**) had activities ranging from 30 to 82% at the concentration of 30 μM in an enzyme inhibition assay.

3.3.4 *PPAR modulator*

Dysfunction of PPARs is closely associated with atherosclerosis, dyslipidemia, obesity, type 2 diabetes and other diseases caused by abnormal regulation of the glucose and lipid metabolism. Three subtypes, PPAR-α, -δ and -γ, are known to be involved in glucose and lipid homeostasis [80]. Markt *et al.* [81] reported a virtual screening approach based on pharmacophore modeling, 3D shape and electrostatic

Figure 5. Chemical structures of active compounds identified by pharmacophore-based virtual screening for 11β-hydroxysteroid dehydrogenase type 1 inhibitor.

similarity screening techniques to discover novel scaffolds for PPAR ligands. LigandScout was used to derive structures based on the pharmacophore models from crystallographic data of agonist–PPAR complexes available from the Brookhaven Protein Data Bank (PDB) [82]. Ligand-based pharmacophore models were generated from the known compound sets comprising structurally diverse PPAR agonists by using the HipHop algorithm [83] implemented in Catalyst. The performance of the pharmacophore models was evaluated by screening a known set of 357 PPAR ligands and a virtual database including 12,775 PPAR decoys. Then, the most selective pharmacophore models for PPAR-α, -δ and -γ, respectively, were determined and each PPAR agonist model was used to screen commercially available chemical databases. The hits that resulted from the pharmacophore modeling approach were then filtered with respect to physicochemical properties by using a Pipeline Pilot [84] script. The filtered compounds were screened by 3D shape and electrostatic similarity using ROCS [85] and EON [86] in the Openeye's software. Finally, PPAR activity was measured by using cell-based human PPAR ligand-binding domain (hPPAR-LBD) transactivation assay. Five compounds were able to activate one of the PPAR subtypes at 10-μM concentration. Compounds 30 and 31 (with IC_{50} values of 1.5 and 1.0 μM, respectively) were identified as PPAR-α ligands, whereas compounds

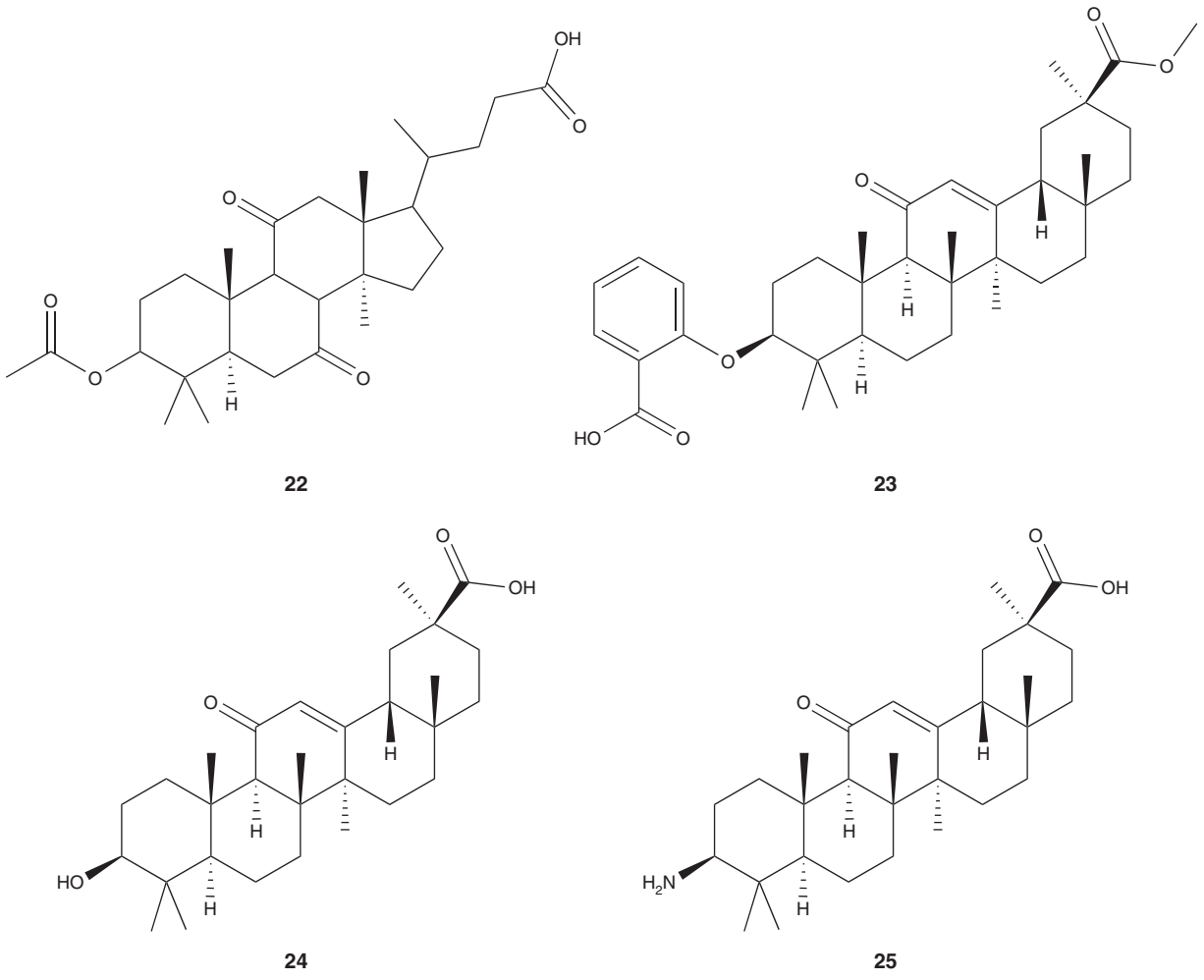


Figure 5. Chemical structures of active compounds identified by pharmacophore-based virtual screening for 11β-hydroxysteroid dehydrogenase type 1 inhibitor (continued).

32 (IC_{50} =) and 33 (with IC_{50} values of 44.0 and 13.0 μM, respectively) were able to bind to PPAR-γ (**Figure 6**).

3.3.5 *Free fatty acid receptor modulators*

Free fatty acid receptor 1 (FFAR1), previously known as GPR40, was initially identified as a G-protein coupled receptor and is a potential target for treating type 2 diabetes. This receptor is highly expressed in the beta cells of pancreatic islets, and its activation by long-chain free fatty acids is known to enhance glucose-stimulated insulin secretion [87]. Tikhonova *et al.* [88] successfully discovered novel FFAR1 agonists and antagonists through pharmacophore-based virtual screening by using the set of drug-like compounds retrieved from the ZINC database [89] of ~ 2.6 million commercially available compounds. As the first step of the virtual screening protocol, 2D similarity searches were performed on the basis of the structure of two potent FFAR1 agonists. Consecutively, a 3D pharmacophore search was performed in parallel with docking studies. A total of 70 compounds selected by virtual screening and a subsequent neighbor search were then tested for their ability to modulate FFAR1 activity, leading to identification of 15 compounds acting as either agonists or antagonists. The representative compounds are shown in **Figure** 7 (34 – 36).

These examples demonstrate the importance of pharmacophore-based virtual screening in the discovery of new molecular entities for diabetes and obesity management. The approach is very useful for lead identification, especially when combined with molecular docking, 3D shape similarity, electrostatic similarity and physicochemical property filtering techniques, for enriching novel scaffolds for antidiabetic and antiobesity activities.

3.4 Alzheimer inhibitors

Larbig *et al.* [90] have reported a ligand-based approach resulting in successful pharmacophore-based virtual screening of inhibitors of tau protein aggregation into helical filaments, which is thought to be one of the main causes of

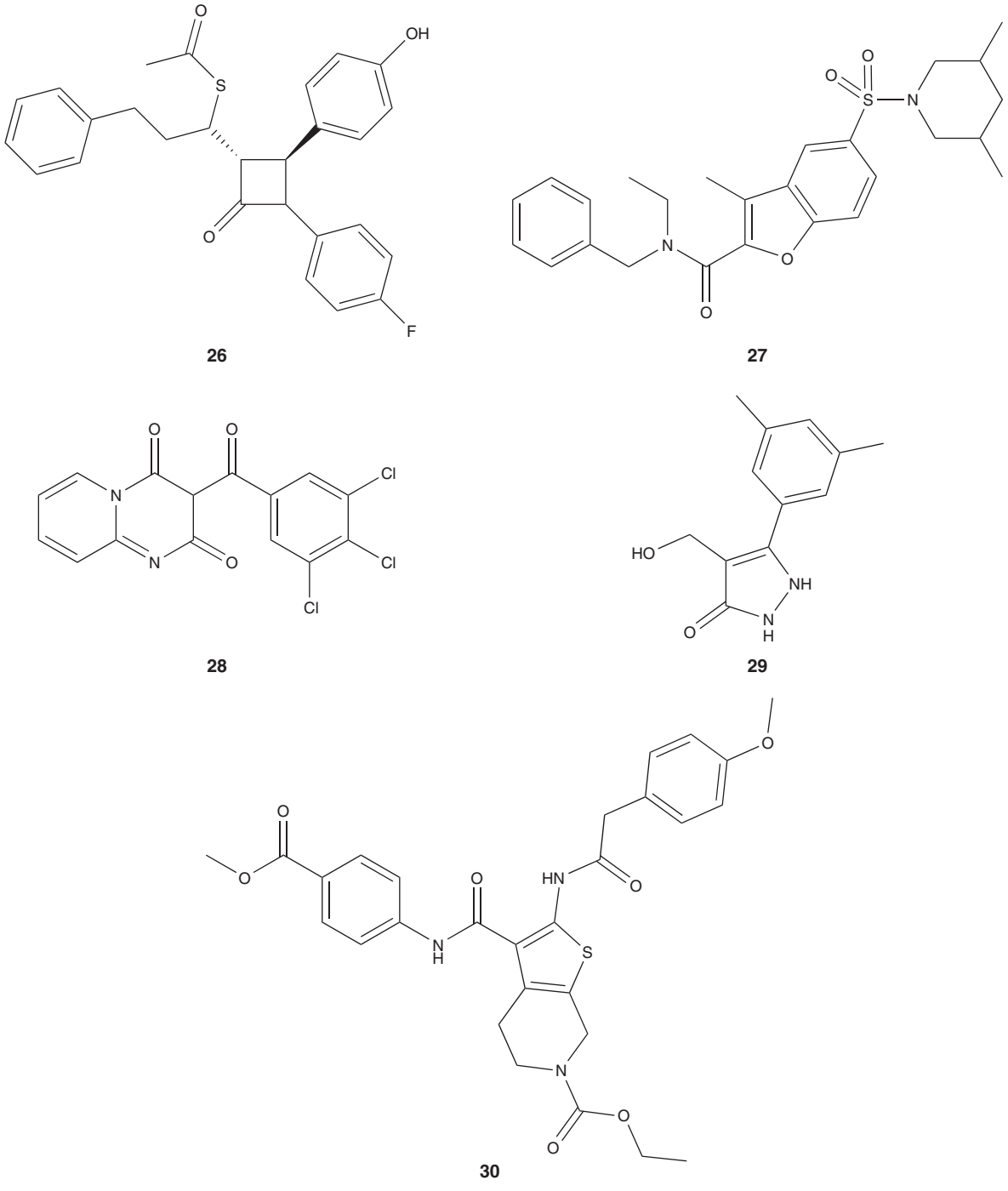
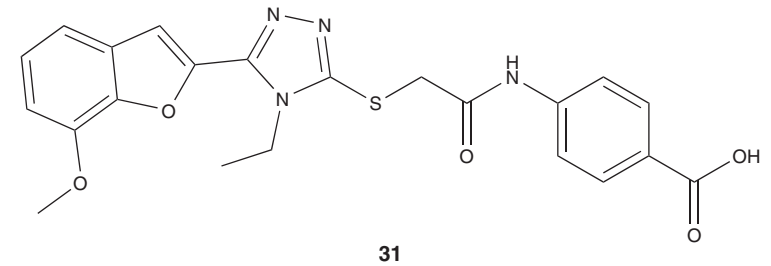


Figure 6. Chemical structures of active compounds identified by pharmacophore-based virtual screening for dipeptidyl peptidase IV inhibitor and PPARs modulator.

Alzheimer's disease. To identify novel leads against tau aggregation, they used Catalyst and generated several pharmacophore-based hypotheses. The training set of tau inhibitors was taken from hit compounds obtained from high-throughput screening [91]. The data set consisted of 21 selected compounds of different classes from 77 original hits. Catalyst was employed to generate several pharmacophore-based hypotheses. The best models were used for virtual screening of 59,676 molecules from the Maybridge Screening collection database. A total of 19 compounds were selected and assayed, and 2 lead structures with IC_{50} values of < 13 μM were identified (37 and 38; **Figure** 7).



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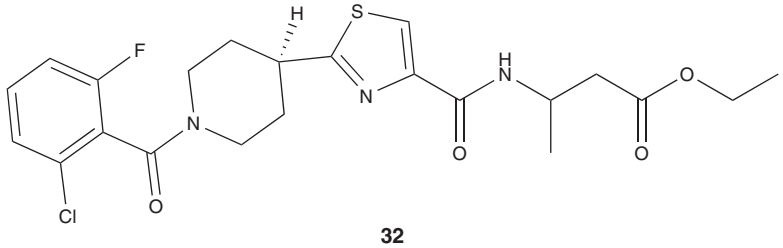

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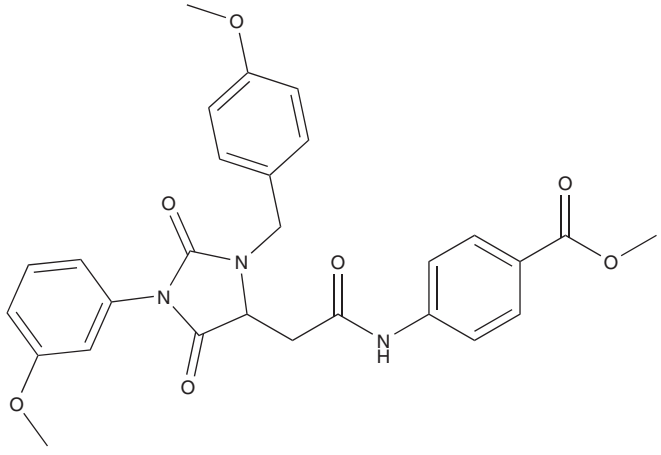

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Figure 6. Chemical structures of active compounds identified by pharmacophore-based virtual screening for dipeptidyl peptidase IV inhibitor and PPARs modulator (continued).

4. Expert opinion

In this review, we provide an overview of successful applications of pharmacophore-based virtual screening of compounds for therapeutic applications against cancer, metabolic diseases and viral infections. The numerous examples have shown that virtual screening is a useful tool for novel hit identification. Various virtual screening techniques have been developed during the last decade, and a consensus is emerging from the practical applications to date: i) virtual screening is an extremely fast and efficient method for the identification of biologically active compounds of a given target; and ii) successful hit identification by virtual screening depends, among others, on the quality of the search techniques combining the search methods, structural information of targets and chemical database. Despite improvements, the currently available pharmacophore-based virtual screening methods exhibit limited predictive power for a set of active compounds. To overcome this major hurdle, Wolfson and colleagues [92] have developed a highly efficient method for pharmacophore detection and virtual screening. They demonstrated that their pharmacophore detection algorithm is highly efficient, allowing rapid exploration of the chemical space by virtual screening of various compound databases. The performance of a new ligand-based method, PharmaGist, was well proven for pharmacophore detection, successfully evaluating a benchmark data set consisting of 74 drug-like ligands divided into 12 test cases [93]. However, several factors should be considered for further improvement. First, a database of compounds derived either from in-house or external vendor sources should be filtered by using validated drug-likeness filtering

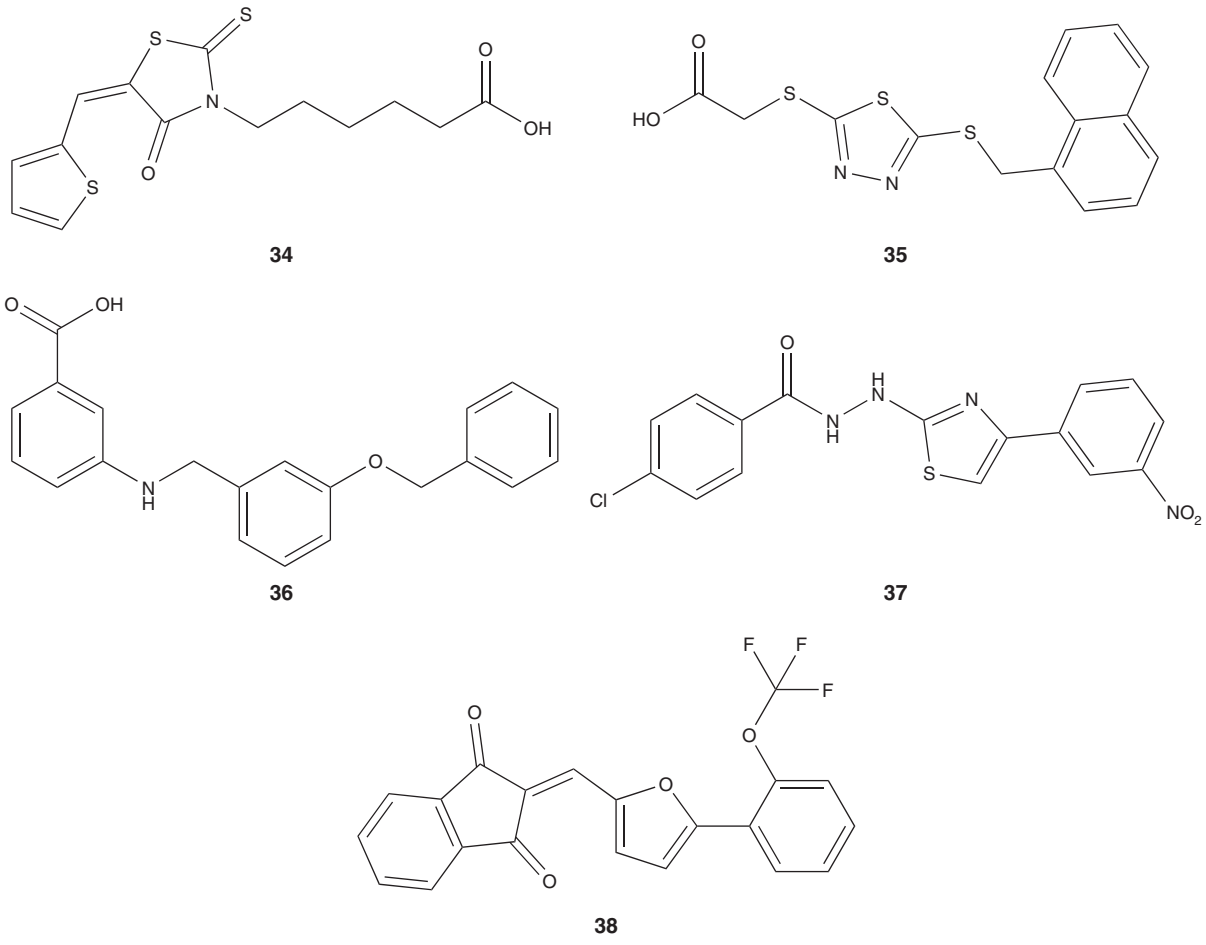

Figure 7. Chemical structures of active compounds identified by pharmacophore-based virtual screening for free fatty acid receptor 1 and tau inhibitor.

methods to eliminate nondrug-like compounds. To increase the efficiency of filtering, adequate and proper mapping of small molecules to a pharmacophore model is required, based on a precomputed ensemble of conformers for each compound. Therefore, the construction of a high-quality compound conformer database is one of the preliminary requirements for successful pharmacophore-based virtual screening. Second, in contrast to docking-based virtual screening, it is usually difficult to apply a reasonable scoring function for hit compound selection by pharmacophore-based virtual screening. This necessitates an alternative strategy of the preselection of compounds by various filtering techniques. As has been discussed in previous case studies, serious consideration should be given to the shape of previously known biologically active compounds or their excluded volume in terms of the space of the potential binding site of the target protein. Third, the efficiency of a given virtual screening method should be validated retrospectively by using a small number of known active compounds seeded into a set of false-positive (inactive) compounds. Fourth, for the better prioritization of candidate compounds, a method that simultaneously considers both pharmacophore- and docking-based virtual screening is recommended. Finally, along with the improved pharmacophore model techniques, an accurate and fast *in vitro* evaluation system for hit compounds should be used for identifying novel drug candidates. Despite such challenges ahead, virtual screening techniques will prove to be essential and dominant for the identification of new lead compounds with therapeutic applications.

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K-H Kim and ND Kim contributed equally to this work.

Declaration of interest

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