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Pharmacophore Modeling in Drug Discovery: Methodology and Current Status

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Abstract: A pharmacophore describes the framework of molecular features that are vital for the biological activity of a compound. Pharmacophore models are built by using the structural information about the active ligands or targets. The pharmacophore models developed are used to identify novel compounds that satisfy the pharmacophore requirements and thus expected to be biologically active. Drug discovery process is a challenging task that requires the contribution of multidisciplinary approaches. Pharmacophore modeling has been used in various stages of the drug discovery process. The major application areas are virtual screening, docking, drug target fishing, ligand profiling, and ADMET prediction. There are several pharmacophore modeling programs in use. The user must select the right program for the right purpose carefully. There are new developments in pharmacophore modeling with the involvement of the other computational methods. It has been integrated with molecular dynamics simulations. The latest computational approaches like machine learning have also played an important role in the advances achieved. Moreover, with the rapid advance in computing capacity, data storage, software and algorithms, more advances are anticipated. Pharmacophore modeling has contributed to a faster, cheaper, and more effective drug discovery process. With the integration of pharmacophore modeling with the other computational methods and advances in the latest algorithms, programs that have better perfomance are emerging. Thus, improvements in the quality of the phamacophore models generated have been achieved with this new developments.

Keywords: Computational approaches, computer-aided drug design, drug discovery, molecular modeling, pharmacophore.

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INTRODUCTION

Drug discovery and development is an expensive and complex process that takes more than 10 years (1). Drug design and discovery is a challenging task that needs the involvement of multidisciplinary approaches. Computer-aided drug design (CADD) methods are mainly employed in the early to mid-stage of the drug discovery process. CADD methods have contributed much to the drug discovery process with the rapid advance in computing capacity, data storage, software and algorithms (2–4). CADD has applications in target fishing, target validation, hit identification and selection of the lead and its optimization (5). Herein, pharmacophore modeling, which is among the CADD methods, is reviewed.

A pharmacophore is a molecular frame that describes the vital features responsible for the biological activity of a molecule (6). Pharmacophore models are generated to increase the understanding about the ligand–protein interactions. They can be empolyed in identifying new molecules that satisfy the pharmacophore requirements and thus expected to be active (7). Pharmacophore models can be built

by using the structural information about the active ligands that bind to the target if the target structure is not available. This is known as ligand-based pharmacophore modeling approach (8). In conditions where the structure of the target is available, pharmacophore models can be built by using the structural properties of the target. This is known as structure-based pharmacophore modeling approach (Figure 1) (7).

There are several pharmacophore modeling tools in use. HipHop, HypoGen, Pharmer, PHASE, GASP, PharmaGist, PharmMapper, MOE, LigandScout, and GALAHAD are examples of softwares used for pharmacophore model generation (5). With the use of such softwares, pharmacophore modeling has been employed at the various stages of the drug discovery process (9). Virtual screening, drug target fishing, ligand profiling, docking and ADMET (absorption, distribution, metabolism, excretion, toxicity) prediction are among its popular application areas (Figure 1) (10–12).

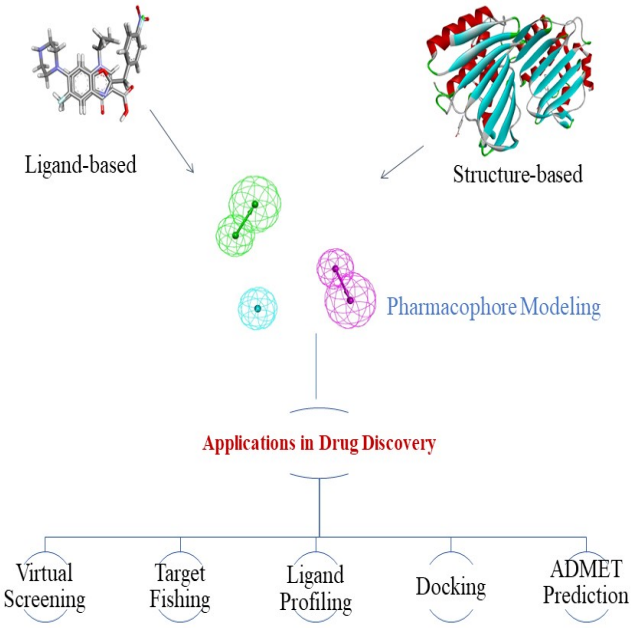

Figure 1: Overview of pharmacophore modeling and its applications.

The scope of the application of pharmacophore modeling in the drug discovery process has been increased by solving the challenges being faced. There are challenges in pharmacophore scoring functions used in virtual screening, modeling ligand flexibility, molecular alignment, and selection of training sets (13). In order to overcome these challenges the contribution of the other computational methods is crucial. Thus, the integration of pharmacophore modeling with the other computational methods is performed in a way that solves some of these limitations (14). For example, phamacophore modeling has been integrated with molecular dynamics simulations. With this integration better pharmacophore models have been built (15). Furthermore, with the contribution of the latest computational approaches such as machine learning the advances in pharmacophore modeling has got momentum (16).

In this study, the general principles of pharmacophore modeling and its major application areas in the drug discovery process are explained. Moreover, the challenges faced and their probable solutions through the advances in the computational methods are covered. This work aims to fill the information gap observed in pharmacophore modeling and to provide an updated informaion for the academia and the pharmaceutical industry.

PRINCIPLES OF PHARMACOPHORE MODELING

The pharmacophore concept was introduced by Paul Ehrlich in the early 1900s. Then, the term pharmacophore was coined and defined as molecular features that bears (phoros) the necessary properties for the biological activity of a drug (pharmacon) (17). In those year's pharmacophore was understood as chemical or functional groups on a molecule that are responsible for the biological activity. IUPAC (International Union of Pure and Applied Chemistry) defined pharmacophore as the sum of steric and electronic properties that are required for the interaction of a molecule with a target and thus provide the biological activity (13).

Pharmacophore is a pattern of features responsible for the biological activity of a compound. This shows that the concept of pharmacophore is more of about features than chemical groups. Each atom or group of a compound that shows features associated with molecular recognition can be converted into a pharmacophore pattern (18,19). Molecular pharmacophore patterns can be hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), positive features, negative features, aromatic rings, hydrophobic features and their combinations (20,21).

A pharmacophore model includes several patterns arranged in a particular 3D (three dimensional) pattern. Each pattern is depicted by a typical sphere containing radius that determines the deviation tolerance from the exact position. There are also various other displaying ways. These patterns can be displayed as a single pattern or their combinations (22).

There are two principal approaches of pharmacophore modeling that are used in the drug discovery process: Ligand-based pharmacophore modeling and structure-based pharmacophore modeling. In the ligand-based pharmacophore modeling approach, novel ligands are designed by using a set of active ligands available (23). This approach is employed if the target structure is not available. In a similar manner, the structure-based pharmacophore approach is employed when the structure of the target protein is available (24).

In the ligand-based pharmacophore modeling, first active ligands are identified by using the literature available or database search. The data set is split into a training set and test set. Then, feature analysis of the training set ligands is done. The common features are detected through the alignment of the active ligands. The next step is pharmacophore model generation and ranking of the generated models. Finally, pharmacophore model validation is performed and the best pharmacophore model is selected depending on the results obtained (23,25).

In the structure-based pharmacophore modeling, selection and preparation of target protein structure is the first step. The second step is binding site prediction. Then, complemental chemical features of the binding site amino acids and their layouts are identified by analyzing it carefully. After this, the pharmacophore features, which should be optimized by the adjusted tools in the programs employed, are generated. Finally, crucial pharmacophore features responsible for the activity are selected (7). LigandScout (26), MOE (27), Pocket v2 (28) and Snooker (29) are among the commonly used softwares for structure-based pharmacophore modeling. Similarly, there are various softwares and servers used in pharmacophore modeling. The commonly employed programs and servers are summarized in the alphabetical order (Table 1).

Table 1: Programs and servers used in pharmacophore modeling.

Program/Server	Brief Description
CATALYST-HipHop (30)	CATALYST is now part of the BIOVIA Discovery Studio. It consists of algorithms used in pharmacophore generation: HipHop and HypoGen. HipHop gives the alignment of active ligands against a specific target and finds the three dimensional arrangements of common features by overlapping various structures.
CATALYST-HypoGen (8)	It generates hypotheses that are able to estimate the activity of molecules quantitatively by using biological analysis data. Thus, it allows the correlation of the structural and activity data for pharmacophore modeling.
GALAHAD (31)	The program uses modified genetic algorithm and fixes certain shortcomings of the GASP program and thus increases its performance. It increases the computational speed by using prebuilt structures as a starting point.
GASP (32)	GASP is available in the SYBYL package. It uses genetic algorithm for the detection of pharmacophores. Unlike the other pharmacophore determinations, conformational search is carried out instantly in the GASP process and is an integral part of the program. A single low energy structure and random spinings are applied to examine conformational changes before superimposing on each input compound.
LigandScout (26)	Though it is possible to perform both structure-based and ligand-based phamacophore modeling with LigandScout, it is among the first programs specialized in structure-based pharmacophore modeling. Especially, if the structure of the target protein is present in its ligand bound state, LigandScout is widely used.
MOE (27)	MOE is able to perform ligand-based and structure-based

	pharmacophore modeling. Model building is performed by the pairwise alignment of the active ligands. It is recommended to decrease the magnitude of the training set by grouping similar molecules.
PharmaGist (33)	It is a freely accessible server used in ligand-based pharmacophore generation. This web server detects pharmacophores via multiple flexible alignments of the input molecules.
Pharmer (34)	It is a pharmacophore method that makes searching based on the width and complexity of the query instead of the molecular library screened. It is a very fast method and its source code is available under an open-source license.
PharmMapper (35)	It is a freely accessible web server used for the identification of potential targets for the input ligands. It calculates pharmacophores by using semi-rigid pharmacophore mapping.
PHASE (36)	It is provided by Schrödinger package. It is a convenient approach used in drug discovery with or without its receptor structure. It creates a hypothesis from one or more ligands, protein-ligand complexes and apo proteins. It has a special algorithm designed for use in optimization of lead compounds and virtual screening.

APPLICATIONS OF PHARMACOPHORE MODELING IN DRUG DISCOVERY

Pharmacophore modeling is employed in virtual screening, fishing drug targets, ligand profiling, docking, and ADMET prediction. New perspectives are also expected for various applications of pharmacophore modeling in the future due to the simplicity and versatility of the concept. In this way, besides the applications explained here, it may have applications in polypharmacology, drug repurposing and side effect prediction (24). In order to explain the scope of the application of pharmacophore modeling in drug discovery, publications in the last two decades are depicted here (Figure 2). These figures are the average of the number of documents published in Scopus, PubMed, and ScienceDirect. They are obtained by searching in these search engines using 'pharmacophore modeling' and 'drug discovery' as keywords. As illustrated by the publications generated, the use of pharmacophore modeling in drug discovery has been increasing (Figure 2).

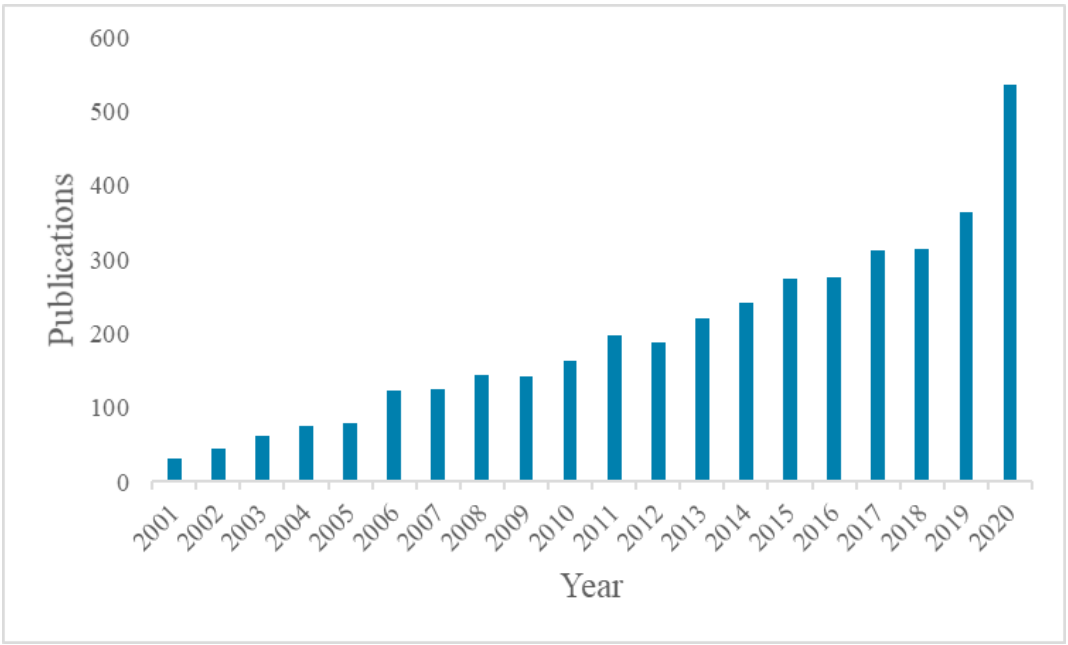

Figure 2: Publications in the application of pharmacophore modeling in drug discovery.

Applications in virtual screening

Pharmacophore modeling is frequently used in virtual screenings to identify compounds that trigger the intended biological activity. Therefore, researchers generate a pharmacophore model that codes the 3D structure of the desired interaction pattern correctly. There are various options to create a pharmacophore model according to the information about the query protein target. When several active ligands and their inactive derivatives

are available, separating the ligand data into training set and test set, for the validation of the pharmacophore models generated, is a common practice (37).

For example, pharmacophore modeling and virtual screening together with docking were used to identify novel *Mycobacterium tuberculosis* InhA (MtInhA) inhibitors. In this work, pharmacophore models were built using 36 known crystal structures of MtInhA. By the combination of ligand-based and structure-based data, four pharmacophore features were used to filter compounds that can meet the essential binding features of MtInhA. The compounds obtained from the pharmacophore-based virtual screening of the ZINC database was docked to compare the binding mode and score of the screened compounds. After thorough analysis of the *in silico* results, experimental testing was perfomed on six selected ligands. Three of these compounds were found to be potential InhA inhibitors (38).

In a recent work, pharmacophore modeling was used to repurpose drugs availabile in DrugBank for the fight against COVID-19. In this work, some potential candidates that can be used in the fight against the Coronavirus pandemic were screened (39).

Applications in drug target fishing

When mechanism of action of drug molecules isn't well known, CADD can be used to elucidate the mechanism. Chemoinformatics-based similarity search tools are used for the identification of similar ligands with known mechanisms of action (40). However, pharmacophore modeling can also be used for other purposes that are different from searching for molecules with a pharmacophore query. The compound under investigation can be the query and the purpose here is to determine the pharmacophore model that may be suitable for the compound. Such pharmacophore models can be developed manually or retrieved from the databases (41). Furthermore, this method can be used to find a target for a particular molecule whose activity is still unknown. For example, many plant metabolites have been investigated and several possible drug targets have been found for them (42).

Applications in ligand profiling

Pharmacophore modeling is utilized in ligand profiling to estimate the possible targets, their adverse effects and suggest new targets for drugs. Not only structure-based pharmacophore modeling but also ligand-based pharmacophore modeling is utilized in ligand profiling however structure-based pharmacophore modeling is preferable (43). Pharmacophore modeling can be an alternative to molecular docking for profiling of ligands (44).

In a study conducted by using pharmacophore modeling-based ligand profiling, targets are assigned correctly. In this study, through both structure-based and ligand-based pharmacophores, 16 metabolites from *Ruta graveolens* were screened against a dataset of 2208 pharmacophore models. The computational results were validated by experimental setups with a special focus on AChE (acetylcholinesterase), HRV (human rhinovirus) coat protein and CB2 (cannabinoid receptor type 2). The experimental results confirmed the binding profile obtained from the pharmacophore modeling (42).

Applications in docking

There are various means to combine pharmacophore-based and docking-based molecular modeling approaches. This may overcome some of the drawbacks of both approaches and may lead to generate better results. Pharmacophore models can be used as initial filters to reduce the number of molecules to be docked, during the docking process as pharmacophore guides and after docking as filters to select ligands and rank the poses (45). Pharmacophore models are used as filters to determine molecules that meet the basic structural and chemical functionality requirements of the query before molecular docking (46). Before the evaluation using docking, pharmacophore is utilized as a search query to filter the ligand database. Pharmacophore-constrained docking is also applicable in docking softwares available and permits certain types of ligand-protein interactions to exist in the docking pose (47). Similarly, pharmacophore models can be used in post-docking filtering to identify the correct binding mode of a compound (47,48). Therefore, pharmacophore models can be used in enriching the top ranking docking results (49).

For instance, pharmacophore and docking were used to filter Chk-1 (checkpoint kinase-1) inhibitors from a compound database. The pharmacophore model was used as initial filter in searching for small compounds that can interact with the adenine region of Chk-1 through a HBA and a HBD features. Then, docking was undertaken by incorporating these interactions in the pose identification. For each ligand, multiple poses were saved and rescoring was carried out. At the end of the study, compounds with potential binding affinity were identified (50). In another recent study, pharmacophore modeling and docking were used to discover new dual adenosine A1/A2A receptor antagonists (51).

Applications in ADMET

Low ADMET property is among the principal reasons for the failure of drug development efforts (52). Therefore, the necessity of determining ADMET properties in the early stages of the drug development process is generally accepted. Pharmacophore modeling methods are used in

estimating ADMET properties early to reduce the failures in the endeavor to develop novel drugs (53).

It is possible to use pharmacophore models in identifying the likely interactions between the drug and its metabolizer enzymes by comparing the similar chemical features of tested compounds and drugs whose ADMET profile is well known (54). For instance, a pharmacophore model that is able to estimate the binding of a drug-like molecule to some CYP (cytochrome P450) enzymes was generated by using the interactions of known drugs with CYP enzymes and the probable degradation by these enzymes was assesed (55). Similarly, ADMET pharmacophore model was generated for the 5'-diphospho-glucuronosyl transferase, which are enzymes related to drug excretion (56).

CHALLENGES IN PHARMACOPHORE MODELING

There are limitations in pharmacophore modeling that should be overcome. Therefore, there are new efforts to solve such problems and thus increase the quality of the applied modeling (57).

One of the application areas of pharmacophore modeling is virtual sceening by pharmacophore. However, there are no good scoring functions used in virtual screening by pharmacophore (58). Here, the extent of matching of the ligand to the pharmacophore query is usually expressed by the RMSD between the patterns of the query and atoms of the compound. However, this measurement does not consider the similarity with known inhibitors and thus cannot estimate the overall similarity with the receptor. Therefore, compounds that match the pharmacophore query may differ from the other known inhibitors and contain functional groups that cannot bind with the receptor binding site. This makes the molecules inactive though they are perfect matches (59).

Another challenging problem in pharmacophore-based virtual screening is higher 'false positive' rates, that is, the virtual hit ligands may not be biologically active (13). This limitation may result from lack of the required hypothesis, quality of the pharmacophore model and discrepancies from the real biological conditions. Using expertises, exhaustive validation, including important information about the target and integrating with the other computational methods need to be considered to overcome this drawback (60).

Modeling ligand flexibility is also an important challenge. To solve this, structure analysis based on predetermined structure databases or during the pharmacophore process can be used. The method based on predetermined structure databases has been found to have a better performance (61). However, there are deficiencies in virtual screening by pharmacophore that is performed using the method depending on predetermined structural databases. These databases consist of a few low energy structures per molecule. If the structure of an active ligand is missing, there is a possibility of not detecting it (62). This is especially true for various structures with rotatable bonds of small molecular functional groups like hydroxyl. It would be hard to differentiate the various rotations by RMSD value difference during structure generation. Generally, pharmacophore search tools can rotate such bonds during the matching process to find the right directional conformations of small flexible polar functional groups. The other limitation is the absence of a clear way to create a pharmacophore query (63).

Similarly, in structure-based pharmacophore modeling protein flexibility and ligand conformational flexibility are the major challenges. These limitations can be overcome by generating the pharmacophore model using docked complex built through flexible docking or by the generation and alignment of the models from protein-ligand molecular dynamics simulations simultaneously. In other words, combination of the structure-based approach with flexible docking and molecular dynamics simulations may alleviate these drawbacks (64). Furthermore, in structure-based approach generation of pharmacophore models is not straightforward. Especially, when various combination of features are likely, each pharmacophore model may lead to different set of compounds (65).

Molecular alignment is a difficult matter in pharmacophore modeling. Molecular alignments can be classified as point-based and feature-based approaches according to their basic nature. In the point-based algorithms, double atoms, fragments or chemical pattern points are overlapped using low square matching. The need for predetermined connection points is a major drawback of this approach. The feature-based algorithms use molecular domain determinants, often represented by Gaussian function sets, to create alignments. Development of new alignment methods continues (66).

The other challenging issue in the practical job is the right selection of the training set molecules. Although this issue is non-technical and straightforward, it may confuse users. The type of ligand molecule, size of the dataset and chemical variety have been shown to significantly affect the final pharmacophore model generated (13).

ADVANCES IN PHARMACOPHORE MODELING

The scope and depth of the utilization of pharmacophore modeling in the drug discovery process are increasing (Figure 2). As a result, in

order to keep up with the new developments in the area, contribution of the other computational methods is in need (45). Herein, the latest methods in the integration of the pharmacophore modeling with molecular dynamics (MD) simulations and the contribution of machine learning to its advance are presented. The necessity of the integration of it with the other compuational methods is apparent as it is demonstrated by its applications in the last three years (Table 2). Thus, more integrational approaches with the other complementary computational methods are anticipated (18). Moreover, the new approaches will help to overcome the difficulties encountered in pharmacophore modeling.

Integration with molecular dynamics simulations

Since ligands and receptors are dynamic bodies, it is clear that ligand-receptor complexes and thus the underlying interactions are also dynamic. Based on this concept, researchers began to integrate MD simulations in the generation of better pharmacophore models (67). In recent years most of the pharmacophore modeling implementations in the drug discovery process are in combination with MD simulations (Table 2). Thus, the integration of pharmacophore generation with MD simulations will have a profound effect on the improvement of this approach.

HSRP (hydration site restricted pharmacophore), SILCS (site identification by ligand competitive saturation) and dynophores are examples for methods that use conformations obtained from MD simulations for pharmacophore building (16). In the HSRP approach, the aim is to reduce the number of pharmacophore features by determining hydration points on the surface of the protein (68). The SILCS method uses the binding hot spots of probe compounds in MD simulations for the generation of pharmacophore models (69). On the other hand, the dynophore represents the fully automated integration of MD simulations with the pharmacophore generation (70).

Table 2: Recent applications of pharmacophore modeling in combination with other computational methods.

Aim of the Study	Major Findings	Other Computational Methods Used
Identification of 20S proteasome inhibitors (71)	Five promising compounds that might inhibit the β5 subunit of 20S proteasome were identified.	Virtual screening, molecular docking, and MD simulations
Identification of new *Mycobacterium tuberculosis* MurG inhibitors (72)	Ten potential inhibitors of MurG were identified.	Homology modeling, virtual screening, molecular docking, and MD simulations
Development of inhbitors against HER family proteins (73)	A lead compound with better properties than the reference ligand, afatinib, was found.	Molecular docking, ADMET property analysis, virtual screening and MD simulations
Identification of novel caspase-1 inhibitors (74)	Four compounds that can be leads for the development of new anti-inflamatory agents were identified.	Virtual screening, molecular docking, MD simulations and ADMET property analysis
Identification of novel compounds that inhibit TNFα and/or TNFR1 (75)	Fifteen promising leading compounds that can serve as novel TNFα and/or TNFR1 inhibitors were identified.	Molecular docking, virtual screening and ADMET property analysis
Identification of new therapeutic agents against resistant tuberculosis by targeting DNA Gyrase B (76)	Seven potential selective inhibitors of Gyrase B were detected.	MD simulations, virtual screening and molecular mechanics
Designing of new DprE1 inhibitors for tuberculosis (77)	A potential lead compound that can be used as DprE1 inhibitor was identified.	Molecular docking, free binding energy estimations, MD simulations, and ADMET property analysis
Elucidation of key interactions between SARS-CoV-2 main protease (Mpro) and its possible inhibitors (78)	Residual key for the interactions between SARS-CoV-2 Mpro and three drug candidates were revealed.	MD simulations
Exploration of novel drugs	Eight molecules that might	Homology modeling,

against COVID-19 by inhibiting its receptor binding domain (79)	hinder the attachment of the Spike protein of novel Coronavirus to the host receptor were predicted.	molecular docking, virtual screening and ADMET property analysis
Identification of new potential Coronavirus inhibitors (80)	Ten potential Coronavirus inhibitors were identified.	Virtual screening, molecular docking and MD simulations
Identification of new molecules for the regulation of glutamate signaling pathway (81)	Two potential dual negative allosteric modulator compounds against mGluRs in neurodegenerative diseases were identified.	Virtual screening, MD simulations and ADMET property analysis
Discovery of novel TNF-α inhibitors (82)	Sixteen molecules with better binding affinity than the previously known TNF-α inhibitors were identified.	Molecular docking, virtual screening, MD simulations, and free binding energy estimations
Identification of new GSTP1-1 inhibitors (83)	Four promising hGSTP1-1 enzyme inhibitors were screened.	Virtual screening and ADMET analysis

Involvement of machine learning

The improvements in computing capacity and the available data have contributed much to the drug discovery process. Machine learning, which is a subfield of artificial intelligence (AI), has been used in pharmacophore modeling over the last years. In the era of big data, machine learning methods have developed into more efficient approaches such as deep learning. Thus, several machine learning methods that use the concept of pharmacophores have been developed (16). In addition to this, machine learning has been used in improving the scoring functions (84).

HSPharm (hot spots guided receptor based pharmacophores), PharmIF (pharmacophore based interaction fingerprint) and DeepSite are examples for machine learning approaches trained with pharmacophores. The HSPharm trains random forest decision trees with pharmacophoric descriptors to decrease the number of pharmacophore features (85). The PharmIF trains support vector machine (SVM) with pharmacophoric fingerprints to rank docking poses of small molecules (86). The DeepSite trains convolutional neural network (CNN) with pharmacophoric descriptors to find out cavities and calculate binding affinities (87).

CONCLUSION

Pharmacophore is a pattern of features responsible for the biological activity of a molecule. There are various programs used in the generation of pharmacophore models. The pharmacophore models developed are used to identify new molecules that satisfy the pharmacophore requirements and thus expected to be biologically active.

Pharmacophore modeling has several applications at the various stages of the drug discovery process. Pharmacophore modeling is widely employed in virtual screenings to identify the molecules that trigger the desired biological activity. Pharmacophore models are also used as filters to identify molecules that meet the pharmacophore requirements prior, during and after docking. Furthermore, pharmacophore models are used in drug target fishing, ligand fishing, and ADMET property predictions.

It is possible to overcome the challenges encountered in pharmacophore modeling by using the other advanced computational methods. For instance, phamacophore modeling has been integrated with molecular dynamics simulations. This has the potential to alleviate problems faced in the modeling of the ligand flexibility. Lack of good scoring functions used in virtual screening by pharmacophore is another challenge observed. Machine learning can be used in improving such scoring functions as it has been employed in various computational approaches. Therefore, with the new advances in pharmacophore modeling, it is possible to develop pharmacophore models with better properties.

In short, pharmacophore modeling has played its own role in the drug discovery process. Improvements in computing capacity, increase in the available data, integration with the other computational methods and involvement of the latest algorithms have enhanced the quality of the pharmacophore models generated. As the quality of the pharmacophore model developed increases, its potential role in the drug discovery also increases. With this in mind, further improvements that will increase the quality of the pharmacophore models are still required.

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