



Utrecht
University

Virtual screening in multi-target drug design

Mirte Bredewout

5913586

28 February 2022

Internship Institute:

Utrecht University

Department of Pharmaceutical Sciences

Universiteitsweg 99, 3584CG

Utrecht

Supervisor/first examiner:

Dr. Ed E. Moret

Second examiner:

Dr. Dirk T.S. Rijkers

Abstract

Polypharmacology studies promiscuous drugs, which have the ability to interact with multiple targets. A few decades ago there was a paradigm shift from the traditional 'one drug-one target' approach to a 'one drug-multiple targets' approach. Currently, multi-target drugs are designed for complex diseases, and developed in case of drug resistance (desensitisation) and known drugs are repositioned, giving an existing drug a different therapeutic use. A wide variety of computational methods are available to identify new drugs via a ligand-based, structure-based or network-based approach. Here, we review virtual screening methods and their application in polypharmacology, including novel concepts developed for (rational) multi-target drug design. However, challenges remain including a lack of experimentally solved target structures, the need for more advanced computational methods, finding a combination of targets relevant for a disease, the selection of relevant protein conformations and the identification of initial hit compounds. The best approach to design a multi-target ligand is debatable and depends on the information that is available. Upcoming advancements in artificial intelligence (AI) and multi-omics will aid (network) polypharmacology. The ultimate goal is to process the high amounts of biomedical data with AI for personalized precision medicine.

Layman's summary

Traditional drug design used the lock-key analogy. In this model the key represents a drug and the lock represents a target, which is most often a protein. According to traditional drug design a specific key was needed to open or to block the lock. The pharmaceutical industry used to search for such a key using different drug design methods. However, most drugs that are designed can actually bind and unlock or block multiple locks, i.e. targets. Drugs that can bind multiple targets are also called promiscuous drugs or multi-target ligands. Nowadays it appears that it is often beneficial if a drug is able to bind to multiple targets and thus unlocks or blocks multiple locks. As such a drug has less side effects and less chance for losing activity due to resistance. Multi-target drugs are now designed on purpose. Hence, a masterkey is needed to fit multiple locks. Especially in complex diseases, such as cancer, which affects many cell components, a masterkey is needed.

How to design masterkeys? A masterkey can be designed by combining, fusing or merging known keys, that unlock or block a single lock. In addition, masterkeys can be designed from scratch. Via this approach targets (locks) have to be identified that are relevant for the disease. In the wet lab different keys can then be screened to find the ones that fit the locks and show a response. In contrast, virtual screening uses computer models to identify drugs that bind to a target. Using computers reduces cost and time, but has limitations as it is a computational model and does not represent the actual biological conditions. Consequently, after virtual screening the compounds that show the most promise are tested in the wet lab.

Different virtual screening methods exist and can be divided in ligand-based, target-based and network-based. For ligand-based methods information about the lock is often unavailable and features of known drugs are compared to other potential keys, to find a new drug. Whereas for structure-based methods the target structure is needed. Here, keys are tried to fit the lock using docking approaches. Docking has limitations like taking into account the flexibility of the key and lock and bad predictions of the effect of the key. In the network-based approach the system is considered, which includes other targets and drugs to identify new drug-target interactions. With improvements in artificial intelligence (AI) more and more data of targets and drugs can be processed. This data can be used to train a machine, which could eventually predict new drug-target interactions. Although, still a lot of limitations need to be overcome in multi-target drug design. For

instance, most of the current methods were used for traditional drug design and more adjustments are needed for their use in multi-target drug design. Moreover, more data of target structures is needed. A lot of data on compounds and their activity for a wide range of targets is unknown, but needed to train the machine in AI methods. Network-based approaches and multi-target drug design hold a lot of promise, also towards future personal medicine development.

Contents

Abstract	2
Layman's summary.....	2
Introduction.....	3
Chapter 1: Traditional drug design.....	5
1.1 Target discovery	6
1.2 Lead discovery	6
Chapter 2: Multi-target drug design.....	7
2.1 Multi-target drugs replace combination therapy?.....	7
2.2 Target finding	8
2.3 Designing a multi-target drug.....	8
Chapter 3: Virtual screening in multi-target drug design.....	9
3.1 Ligand-based virtual screening.....	12
3.2 Structure-based virtual screening	17
3.3 Network-based	24
Discussion	25
Best in silico approach to design a multi-target drug	26
Promiscuity and conformations of the ligand and target	26
Resistance and identification of novel modes of action	26
New use of multi-target drug.....	26
Towards precision medicine.....	27
Conclusion	27
References.....	27

Introduction

In 1907 Paul Ehrlich came up with the concept of a magic bullet, a drug that selectively targets one specific protein (Fig. 1).¹ This 'one drug, one target' approach has predominantly been used to rationally design new drugs since the 70s, including the development of captopril (Capoten®).^{2,3} Also, imatinib (Gleevec®) is a specific kinase inhibitor that acts on BCR-ABL, a deregulated fusion protein, which is produced by fusion of the genes encoding the breakpoint cluster region protein (BCR) and Abelson (ABL) tyrosine kinase.⁴

However, most drugs elicit their effect by interacting with multiple targets.⁵⁻⁷ Aspirin, a non-selective non-steroidal anti-inflammatory drug (NSAID), inhibits both cyclooxygenases COX-1 and COX-2.⁸

Inhibition of COX-1 has been associated with gastrointestinal bleeding. Therefore, selective COX-2 inhibitors, e.g. celecoxib, rofecoxib, valdecoxib, were developed, but rofecoxib and valdecoxib were later withdrawn from the market, due to life-threatening cardiovascular side effects associated with their long-term use.⁹ Suggesting that high selectivity towards a specific target might have fatal effects.

Another drug called clozapine, is a promiscuous drug, as it interacts with multiple targets, and has been used to treat resistant schizophrenia.¹⁰⁻¹² In contrast, highly selective anti-psychotic drugs failed to reach the market, as they were too selective.¹¹ Currently, the 'one drug-one target' paradigm has shifted to the design of compounds that act on multiple targets, except when there is a monogenetic cause, like for imatinib.¹³ Drugs that act on one target are prone to drug resistance (desensitisation) and reduced efficacy. Therefore, the concept of a magic bullet has now been updated with the idea of a magic shotgun, a drug that selectively targets multiple targets.¹⁰

In polypharmacology new drugs are designed that have their effect by modulating multiple targets, which may involve multiple diseases.¹⁴ Polypharmacology can be used to find therapeutics for complex diseases, such as cancer, Alzheimer and schizophrenia. A multi-target drug should have a higher efficacy, have a lower chance for resistance (desensitisation) and have less toxicity. Combination therapies use a combination of drugs that work synergistically (Fig. 1).¹⁵ For COVID-19 multi-target drugs and combination therapies have been investigated, including the Pfizers PF-07321332/ritonavir.¹⁶ Ritonavir blocks CYP450 3A4 to increase the half-time of PF-07321332, so the dose of the medicine can be lower.^{16,17} Compared to combination therapies, multi-target drugs are expected to avoid possible drug-drug interactions and to have a more positive synergistic effect.¹⁵ Polypharmacology can also be used to overcome drug resistance (desensitisation) and is used in drug repositioning. Sildenafil (Viagra), a PDE5 inhibitor, is nowadays used to treat erectile dysfunctions, but was originally designed to treat ischemic heart disease and hypertension.¹⁸ Multi-target agents were mostly discovered by serendipity, but more advanced methods enable rational design of multi-target drugs.¹¹

Before the 1980s phenotypic screening was performed using *in vivo* methods (or cells) to discover new drugs.¹⁹ This approach guarantees a high efficacy. However, for phenotypic screening a disease model is difficult to define, animals are needed, lead optimization is complicated and the throughput is low. In the 1980s, target-centric screening (*in vitro*) was introduced, for which no animals are needed and high throughput is possible.²⁰ Nevertheless, this method will not guarantee efficacy, as a molecular target alone does not represent the disease, because a target is part of a (protein) network.

The reductionist approach to develop a selective drug for a specific target was used with successful examples as imatinib and captopril.²¹ Now we are in a postreductionism era and the biological system is considered. As an example, kinase inhibitors are repositioned and multi-target drug design is chased to design new kinase inhibitors.⁴ Modern high-throughput screening (HTS), experimental screening of a library of compounds against biological targets, help to identify multi-target drugs.²² In addition, the design of multi-target compounds can be accelerated by *in silico* methods. Computer-aided drug design (CADD) uses computational methods to search for potential new drugs and to predict ligand-target interactions.²³ In 1997 the term virtual screening was introduced in the literature, which is an *in silico* method to screen compounds.²⁴ However, HTS is the 'gold-standard' to identify compounds with biological activity.²⁵ A recently published review discusses the computational side of polypharmacology.²⁶ Here, a ligand-based, target-based and network-based approach are presented.

In this review, we discuss computational screening methods used in multi-target drug design and discovery. First, we present the traditional ‘one drug-one target’ drug design approach. Second, we describe the multi-target drug design approach. Lastly, we focus on virtual screening methods and their use in multi-target drug design. Here, we critically analyse the current methods used in multi-target drug design and present the future challenges.

For this article literature reviews, that were published within the last 5 years, were used as a starting point. Followed by a more in-depth study of older and more recent published articles covering specific topics regarding multi-target drug design and virtual screening approaches.

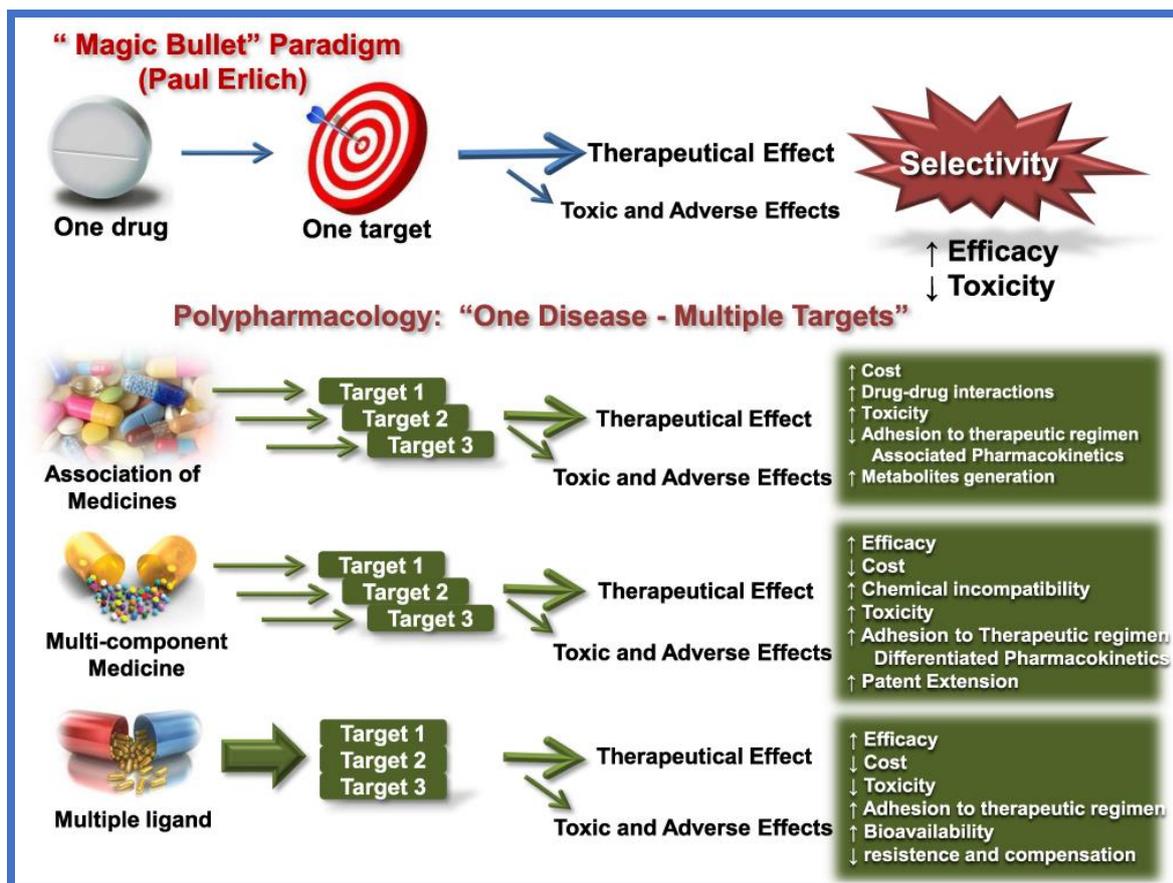


Figure 1. Paradigm shift: from single-target to multi-target drug design. Traditionally the ‘one drug-one target’ approach was used to design new drugs. Currently, polypharmacology is used in drug design and discovery. Here, combination therapies are developed and multi-target ligands are designed.²⁷

Chapter 1: Traditional drug design

The drug discovery and development route takes on average more than 10 years (Fig. 2).²² Stages of drug discovery and development typically consist of disease selection, target identification, lead discovery, preclinical studies, clinical trials and approval of the medicine.²⁸ However, the cost and approval time of the drug also depends on whether or not there exists an urgent medical need.²² Also, the cost to develop an orphan drug, which is designed for a rare or neglected disease, is considerably lower than for nonorphan drugs. New technologies help to accelerate the development of new drugs and pave the way for the development of personalized medicine. Finding a drug candidate from the lead discovery stage remains challenging. Leads often fail due to low efficacy and toxicity.

1.1 Target discovery

Target identification is considered the first step in the drug discovery process.²⁸ A biological target can for example be a protein, RNA and DNA. A good target should be disease relevant, druggable and safe to target. Target identification can be done at the start of the drug discovery process, followed by the screening of compounds during hit identification.²⁹ This approach is known as target-based screening (Fig. 2A). Whereas in phenotypic screening the target is identified after lead finding (Fig. 2B). During this approach compounds are identified that recover a disease phenotype. Therefore, phenotypic screening is a promising method to find new first-in-class drugs that act on a target, which could have initially been labelled as undruggable. Nevertheless, it remains a challenge to identify the target and clarify the mode of action of the identified hits. Different strategies can be followed to identify the target, using affinity-based or activity-based methods. New cell-based phenotypic assays, which make use of primary cells or stem cell derived human cells, enhance the number and types of cell-based disease models available and can be used for robotic screening.³⁰ Here, compounds that change the phenotype are identified as new active compounds. However, the disease relevance and screening throughput of the cell-based phenotypic assays still have to be improved. A combination of phenotypic and target based screening will be an optimal approach as they can complement each other.³¹

When in the drug discovery process the drug's target and mechanism of action should be identified is debatable.³² Some argue target identification is not needed, as there are drugs on the market with an unidentified target and an unknown mechanism of action. However, knowing the drug target is beneficial as next generation drugs can be designed against the target. These next generation drugs will have a higher efficacy and less side effects. Nevertheless, if there is an unmet need for a (rare) disease, target identification at a later stage in the drug discovery pipeline may be substantiated.

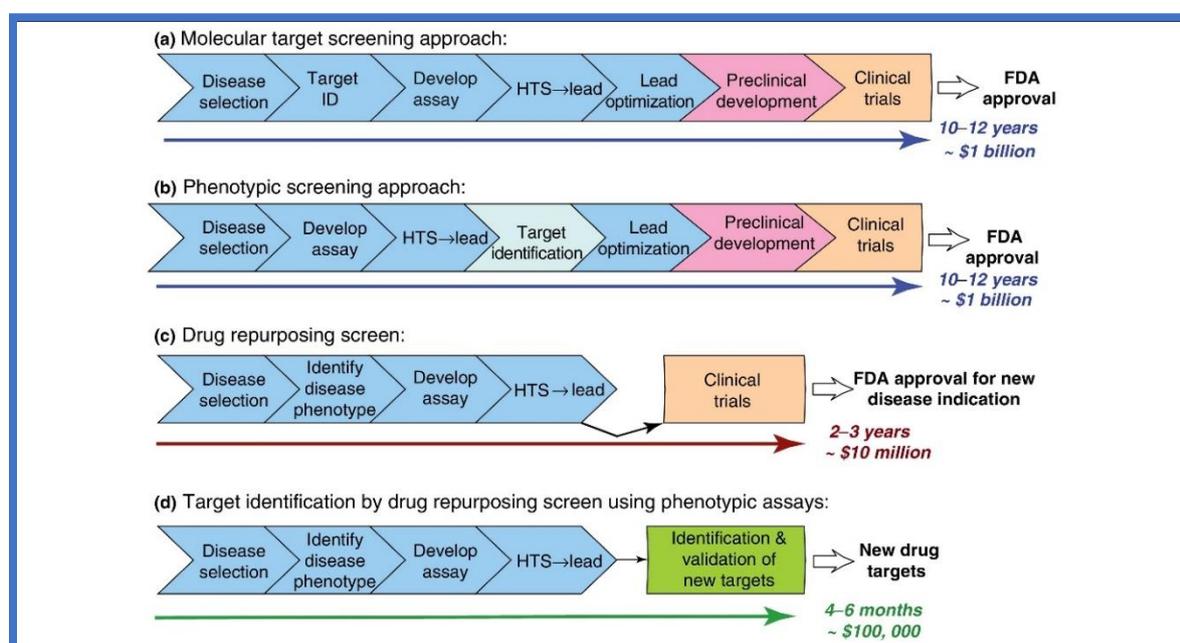


Figure 2: Target screening and phenotypic screening approaches in drug discovery and development. (A) The target screening approach starts with target identification. (B) In phenotypic screening the target is identified at a later stage in the drug discovery and development process. (C) Drug repurposing identifies known drugs for the treatment of a different disease. (D) Target identification of new targets in drug repurposing.³⁰

1.2 Lead discovery

Lead compounds can be discovered via various screening methods, e.g. HTS, phenotypic screening, knowledge based screening, DNA encoded library technology, virtual screening and fragment-based

screening.³³ The library used for screening can consist of natural compounds, synthetic compounds, computer generated compounds and biological agents.²² Natural compounds are structurally diverse and can be the starting point for a semisynthetic drug, a drug synthesized from a combination of natural and synthetic resources. In drug discovery natural drugs are less used as purification and characterization of the natural agents remains challenging.

In HTS thousands of small molecules (< 500 MW) are screened against a target.²² The small molecules are relatively chemically stable, easily synthesizable and characterized for their reactivity. Depending on the screening method, hits can be identified that elicit a biological response. Currently, ultra HTS can perform 100,000 assays a day in contrast to less than 10,000 assays a day in traditional HTS.

CADD reduces time and cost of drug discovery. CADD can be divided in ligand-based and structure-based.²³ The ligand-based approach is used when limited information on the target is available.²² This approach uses the physicochemical properties and biological activities of known compounds, which are used to design novel compounds. The structure-based approach uses the structure of the target to design compounds that fit in a binding site of the target. With CADD compounds can be designed that are active, easily synthesizable and have good ADMET (absorption, distribution, metabolism, excretion and toxicity) properties. During virtual screening also compounds that have never been synthesized can be screened. Also, with CADD penetration of the blood-brain barrier (BBB) can be predicted, as well as if the drug acts in the central nervous system. Furthermore, CADD is fast, reduces cost and less animals are needed for in vivo testing. A drawback of CADD is that it cannot predict the activity of the compound in the biological system, which consists of a whole network. Synergistic approaches use a combination of virtual screening and experimental methods. Virtual screening can be performed prior to HTS to reduce the number of compounds that need to be synthesized and tested.²⁴ This prefiltering will result in a list of top 30-500 compounds that can be tested via experimental approaches.

CADD can also be used for drug repositioning, interchangeable with drug repurposing, of existing drugs (Fig. 2C,D).^{22,34} New uses are identified and developed for the existing drugs, which become again first-in-class drugs.¹⁹ The use of existing drugs reduces development risks, as the drugs properties are already known. Thereby offering an advantage over the search for a new drug via the traditional *de novo* drug discovery process. Lead compounds often need to be optimized to minimize their toxicity and optimize the activity and pharmacokinetics. Furthermore, for oral availability the Lipinski's rule of Five should be considered. Considering that the structure size will probably increase in the lead optimization phase and become more hydrophobic. For fragment-based lead discovery a rule of three has been suggested.

Chapter 2: Multi-target drug design

2.1 Multi-target drugs replace combination therapy?

For certain diseases it is required to administer a cocktail of drugs that act on different targets.³⁵ A common approach is the use of combination therapy, where two or more selective drugs are administered. Co-administration of drugs can result in a higher efficacy and lower toxicities, due to lower dosages used. This approach has been used in the treatment of complex diseases, such as cancer. Another benefit of a drug combination is the synergistic effect from the use of different drugs, where synergy means that the overall therapeutic effect of the combination is greater than the sum of effects caused by individual components.³⁶ However, the number of different drugs, finding the appropriate dose regime and potential drug-drug interactions are a downside of combination therapies. For those reasons, the design of a single drug that can selectively bind multiple targets would be advantageous (Fig. 3). Main reasons for the development of multi-target

drugs are cases of resistance (desensitisation), drug repositioning and complex diseases.³⁷ A multi-target drug could act as an agonist on one target and an antagonist on another target. Designing a multi-target drug that acts on proteins from the same family is easier than targets across the human proteome.³⁸ Currently, multi-target drugs exist in the treatment against complex diseases, such as schizophrenia, depression and cancer.⁵

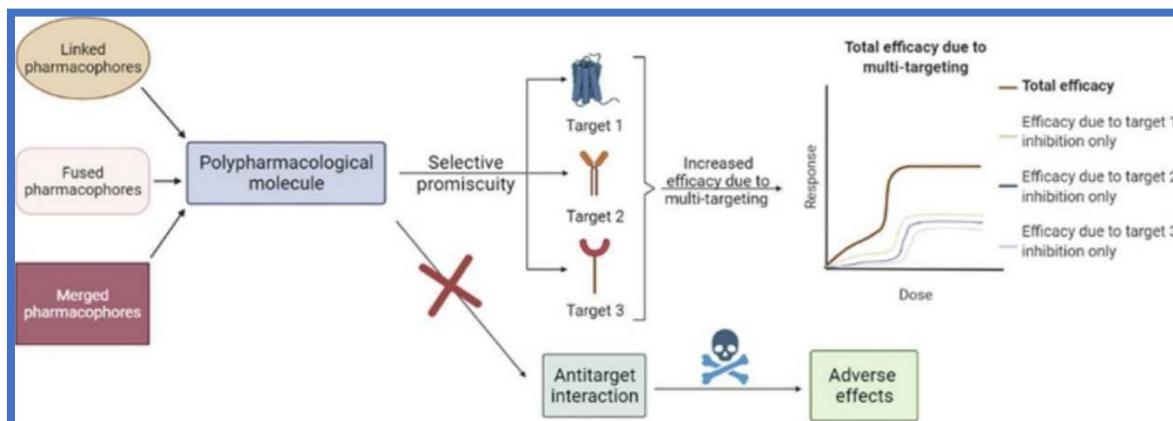


Figure 3. Different approaches in multi-target drug design in polypharmacology. Pharmacophores of known selective compounds can be combined to obtain a selective multi-target drug. This multi-target drug increases the efficacy compared to a drug targeting a single-target.¹⁴

2.2 Target finding

Selecting the drug targets in multi-target drug design remains a challenge.⁵ The targets should be important in the disease and targeted with some selectivity (Fig. 3). Moreover, it is beneficial if a drug that acts on both targets has additive or synergistic effects. When the targets belong to the same pathway there could be an additive effect. However, synergy can be obtained from targets belonging to complementary pathways. In the case of additive or synergistic effects lower doses of a drug are required for an effect than drugs acting on a single target. Subsequently, toxicity should be less as the dose is lower and off-target interactions should be minimized from the start. In addition, it is easier to design a ligand for similar targets, which are assumed to have a similar binding to compounds.³⁹ Thus, targets across different protein classes with similar binding sites could be targeted simultaneously.

Traditionally potential targets were identified using protein isolation and mass spectrometry.⁴⁰ These experimental methods require a lot of time and are expensive. Therefore, *in silico* approaches are used to identify the potential targets of a compound, this is known as target fishing or reverse screening. With this method drug-target interactions (DTIs) can be identified, possible adverse effects and can be used in polypharmacology.

2.3 Designing a multi-target drug

Multi-target drugs were initially identified by serendipity, but are now rationally designed.¹¹ Different approaches exist for the rational design of multi-target drugs.³⁸ Multi-target drugs can be designed from known selective drugs, starting from their molecular properties and pharmacophores. These compounds can be divided according to their pharmacophore into three groups, namely linked pharmacophores, fused pharmacophores and merged pharmacophores (Fig. 3).⁴¹ Linked pharmacophores are two molecules that are conjugated via a linking group.⁴² However, these compounds are often very large and could prove too large to find their way to the targets they are supposed to bind. Then again, a linker can improve the solubility of the compound. This approach is also used to link a drug to an antibody, which helps the drug to reach the drug target. Cefiderocol is

an example of a compound designed by linking pharmacophores (Fig. 4A). Fused pharmacophores are two molecules that are joint without a linking group. The resulting molecule could still have a high molecular weight and high lipophilicity, which could limit the solubility of the compound. Rivastigmine and rasagiline were fused to create ladostigil, which acts on acetylcholinesterase and monoamine oxidases (Fig. 4B). Linked or fused pharmacophores may be cleavable depending on the type of linker and bonds. Merged pharmacophores often have a lower molecular weight as a part of the two molecules overlaps to create a new molecule. Ziprasidone is an antipsychotic drug, which was developed and optimized by merging the pharmacophores of dopamine and 5-HT₂R (Fig. 4C). Finally, the position of linking, fusing or merging is important as the molecule needs to be able to interact with multiple targets.

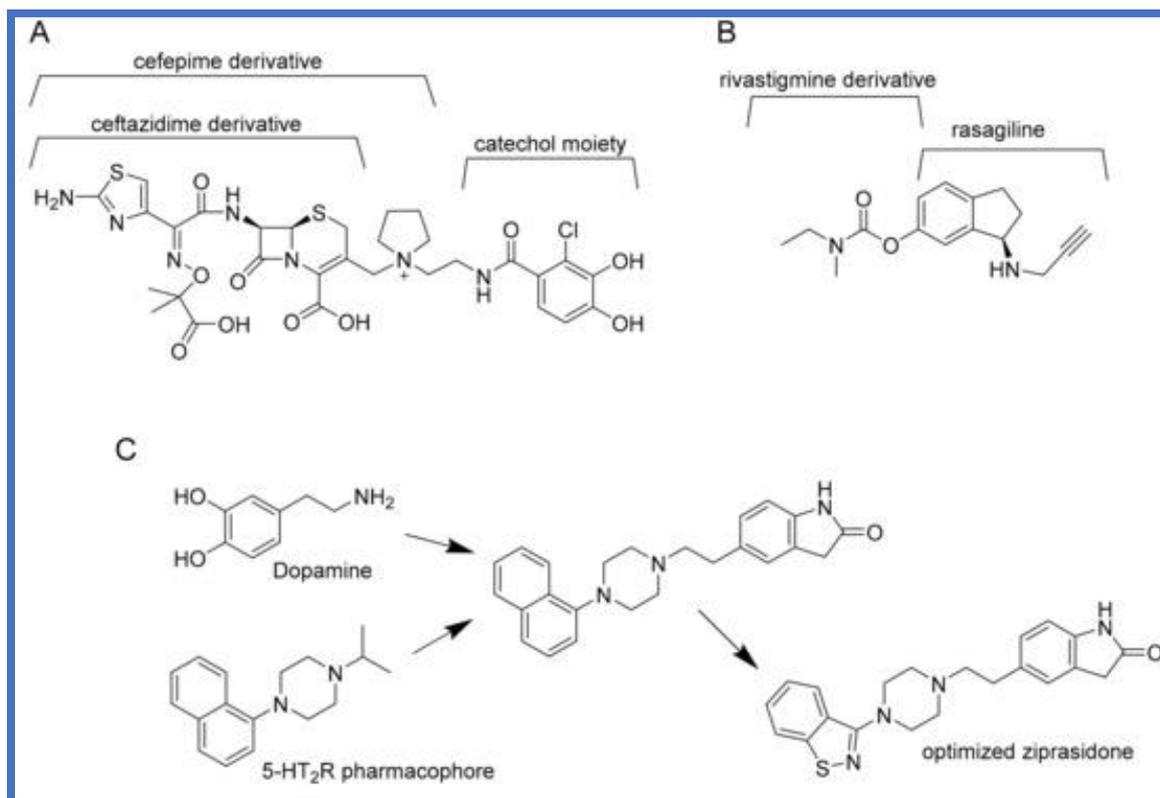


Figure 4. Pharmacophores multi-target drugs designed from existing pharmacophores. (A) Example of linked pharmacophores in cefiderocol. Ceftazidime derivative and catechol moiety with a cefepime derivative as linker. (B) Example of fused pharmacophores in ladostigil. Rasagiline is fused with rivastigmine. (C) Example of merged pharmacophores (dopamine and 5-HT₂R pharmacophore) and optimization of the molecule to ziprasidone.⁴²

Another approach for the design of multi-target drugs is to start from dirty drugs, which are non-selective drugs that can bind multiple targets.⁴¹ So, lead compounds could be promiscuous ligands. These could be optimized into a more selective multiple-target drug. For instance, olanzapine, an analog of clozapine, is a second-generation promiscuous drug against schizophrenia.⁴³ Olanzapine has less severe side effects compared to clozapine.

In addition, multi-target ligands can be rationally designed using *de novo* multi-target drug design.⁴⁴ Here, ligands can be built from scratch using fragment linking or growing. Moreover, multi-target ligands can be found using (virtual) screening approaches.⁵

Chapter 3: Virtual screening in multi-target drug design

Virtual screening methods can be divided in ligand-based and structure-based VS (LBVS and SBVS, respectively).⁴⁵ Network-based approaches are promising, as they incorporate multi-omics methods

and systems biology.²⁶ Here, the basis of the disease is studied by considering protein networks and the effects of a drug on a network. Also, a combination of LBVS and SBVS can be applied using sequential methods, parallel methods or hybrid methods (Fig. 5).⁴⁶ In sequential methods ligands are often prefiltered using LBVS methods and SBVS is performed in the end. Parallel methods use both LBVS and SBVS independently and the best-ranked compounds are used for biological testing. LBVS and SBVS can be combined in a standalone method, known as a hybrid method. Interaction-based methods are an example of hybrid methods.

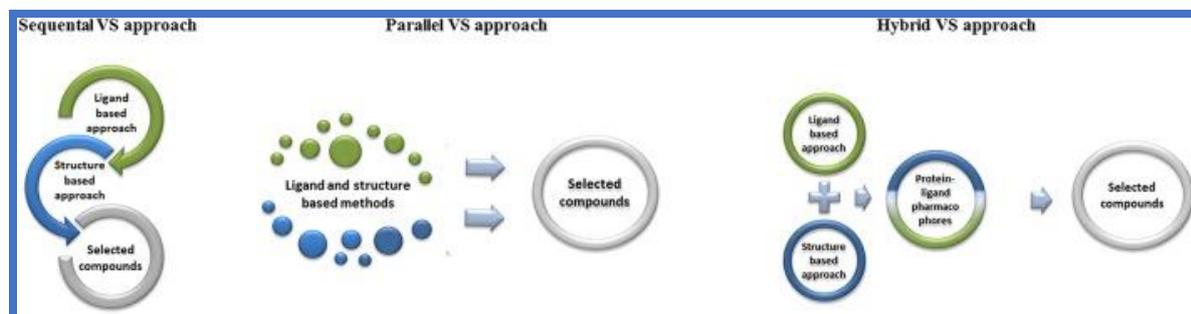


Figure 5. LBVS and SBVS approaches can be combined, resulting in sequential methods, parallel methods or hybrid methods. Sequential methods start with LBVS methods to prefilter the compounds and followed by SBVS. LBVS and SBVS are performed independently in parallel methods and best-ranked compounds are selected. The hybrid approach integrates both LBVS and SBVS methods.⁴⁷

LBVS methods make use of the 2D or 3D chemical structure or molecular descriptors of known active compounds to find new ligands.²⁶ The information of known ligands is used to search for similar structures and investigate the quantitative structure-activity relationship. Approaches for LBVS include 2D (fingerprint) and 3D similarity search methods (pharmacophore), 2D or 3D QSAR (quantitative-structure activity relationship) modelling and other, like shape-similarity (Fig. 6). SBVS makes use of the knowledge of the target binding site for receptor-based pharmacophore screening and docking. During the latter approach compounds or fragments are docked into the binding site, followed by scoring and ranking of the compounds. Postprocessing can be done, for example making use of rescoring using a different scoring function. Network-based methods use algorithms to predict DTIs. Also, target profiles of drugs can be analysed using multi-omics data.

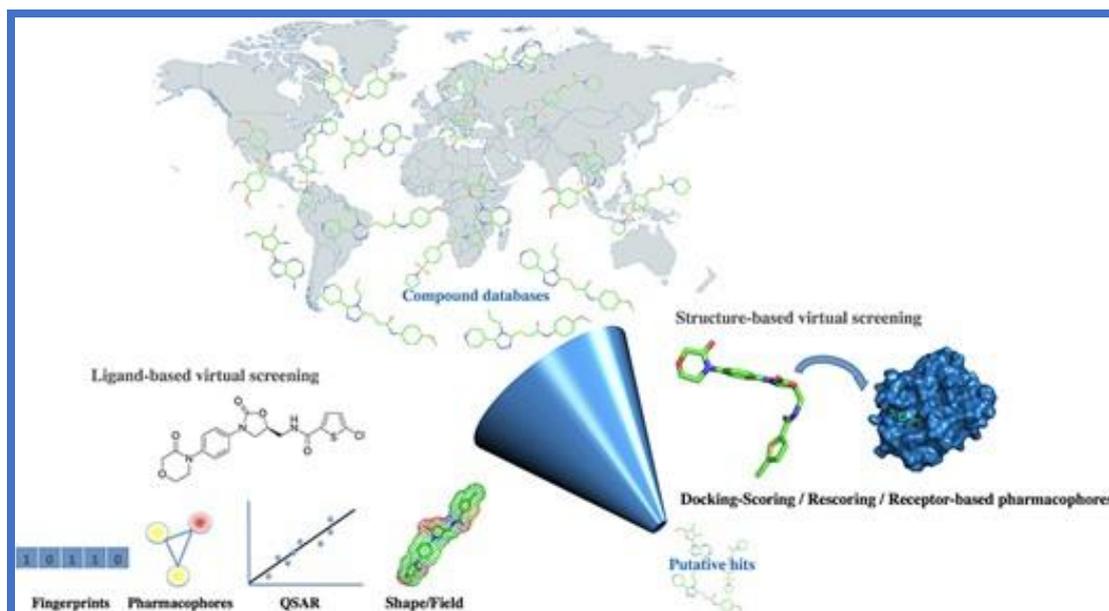


Figure 6. General overview of LBVS and SBVS methods. LBVS methods include similarity searches, ligand-based pharmacophores and QSAR. SBVS uses docking and structure-based pharmacophores.²⁴

For multi-target drug design the traditional computational methods can be modified to be used in polypharmacology (Fig. 7).²⁴ To predict targets of a multi-target ligand target fishing or in silico profiling is performed. This can be done with chemical similarity using bioactivity information of small compounds stored in databases. Here, targets with ligands similar to the query compound will be identified. Tools that can be used include a 2D or 3D similarity search, machine learning and docking. Target fishing makes use of reverse or inverse docking methods, these methods will later be explained in more detail.

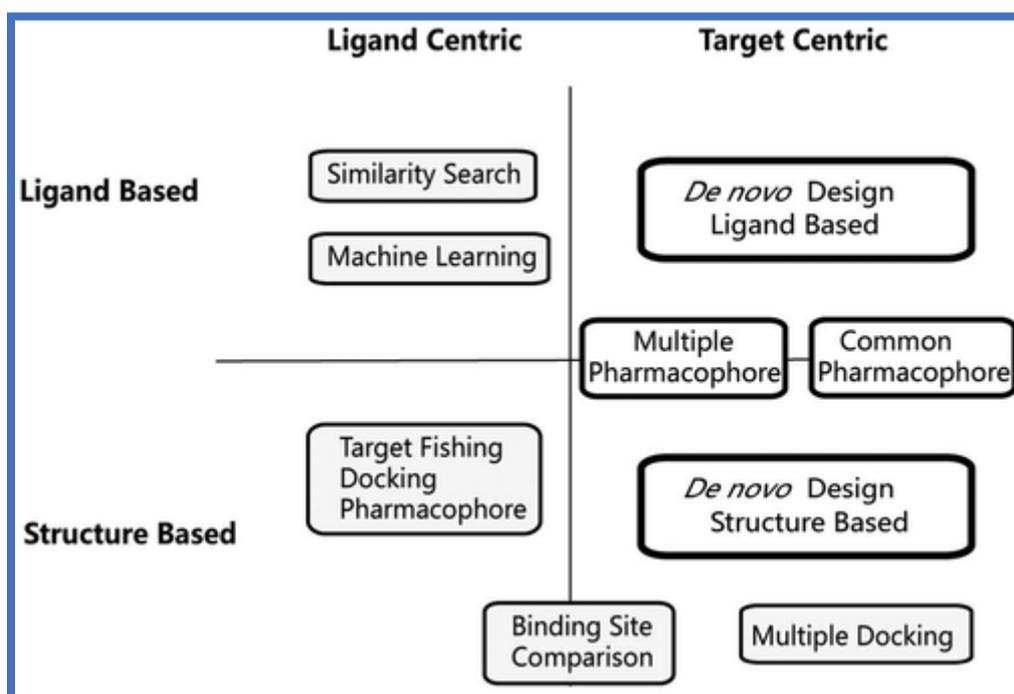


Figure 7. Computational methods used in multi-target drug design. Similarity searches and machine learning aim to identify novel multi-target drugs. Target fishing and reverse docking are used to identify the targets of a compound. In addition, multi-target drugs can be designed using a *de novo* based method. Furthermore, pharmacophores of known selective compounds can be used to design a multi-target drug.⁴⁸

3.1 Ligand-based virtual screening

3.1.1 Similarity searching

A 2D similarity method can use molecular fingerprints of known ligands to find new compounds with a similar fingerprint in a library.²⁴ There exist different similarity types and distance metrics. As an example, the Tanimoto coefficient takes on values between 0 and 1, which represent the similarity to the input query. In addition, other methods can evaluate the shape similarity of compounds.

In traditional drug discovery SAR/QSAR (quantitative-structure activity relationship) models predict the activity of a compound against individual targets.²⁶ QSAR uses the information about the activity of the ligand towards the target to obtain a relationship between compound features and the biological activity.²⁴ To obtain a relationship both active and inactive compounds are used. With 3D-QSAR methods steric and electrostatic energies of 3D conformations of analogs were calculated and correlated with their bioactivity.⁴⁷ Currently, 3D-QSAR methods can also consider multiple conformations of a compound, which gives a better representation of the dynamic nature of ligands, in contrast to the use of only one static conformation of a ligand.

For the design of multi-target drugs SAR/QSAR models are needed for all relevant targets, to predict the activity of a multi-target compound.²⁶ The method used in single-target drug design can be adjusted to be used for multi-target drug design, if there is missing data.⁵ Here, families of compounds can be used instead of single compounds with known activity. Also, from the QSAR model new analogs of a multi-target lead can be designed to improve the activity and predict toxicity.⁴⁷ QSAR models are an *in silico* method that can distinguish between antagonists and agonists.

Multi-target QSAR (mt-QSAR) models can be used to virtually screen compounds based on more than one target.⁴⁹ Furthermore, for multi-target drug design the metacore (MC) concept was introduced (Fig. 8).⁵⁰ MCs can be extracted from the structural cores of analog series (ASs) and thus represent a set of ASs. For the development of multi-target drugs ASs can be selected that contain analogs with multi-target activity. Furthermore, the MCs will then contain cores that are linked to different target families. The relevance of MCs can be evaluated and used to design novel multi-target drugs.

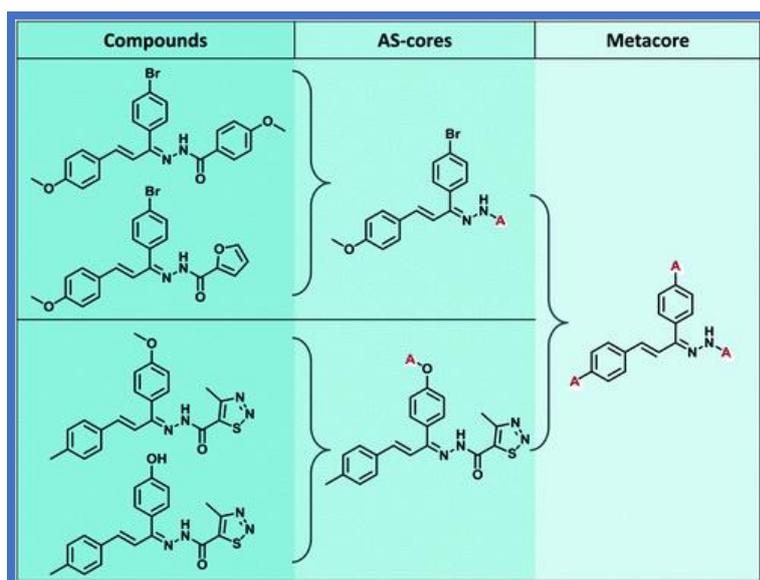


Figure 8. Metacores (MCs) are obtained from analog series (ASs). Here, the AS-cores were generated from two compounds. AS-cores can then be further reduced to a MC.⁵⁰

In contrast to QSAR and chemogenomics, which only use information on ligand similarity, proteochemometric (PCM) modelling also incorporates information of the similarity of targets (Fig. 9).⁵¹ PCM can predict the affinity of ligands for highly similar targets by using extrapolation. In addition, PCM can be used in polypharmacology and can aid the discovery of ligands for orphan receptors.

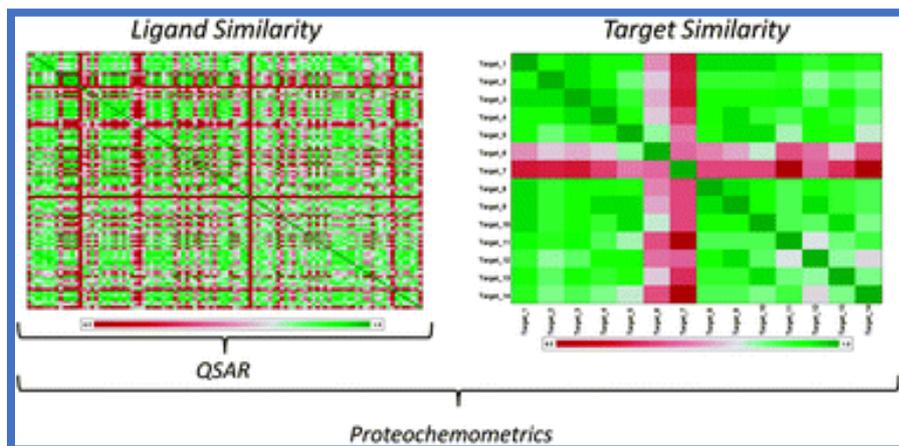


Figure 9. Proteochemometrics combines ligand similarity with target similarity. In contrast, QSAR only uses ligand similarity. Here, heatmaps represent the similarity of different ligands or targets.⁵¹

3.1.2 Ligand-based pharmacophores

A pharmacophore consists of a framework with chemical features, such as hydrogen bond donors, hydrogen bond acceptors, charged groups or lipophilic groups, that are involved in the interaction between the ligand and macromolecular target.^{24,52} For each feature the spatial tolerance and weight can be altered by adjusting the size and importance.⁵² In addition, for hydrogen bonds and aromatic interactions a direction can be added to the feature. Also, an exclusion volume constraint can be used, which could impose a barrier for the binding of a certain ligand. Together the features are responsible for the biological activity of a compound towards the target.²⁴ Pharmacophore modelling is usually performed in 3D.

Virtual screening using pharmacophore models started in the late 80s and early 90s.⁵²

Pharmacophore models can be designed via a ligand-based or structure-based approach, which one is used depends on the information that is available of the target and ligands. There could be data available of the active ligands, the ligand in complex with its target or a structure of the target (apo). To elucidate the 3D pharmacophore different methods can be used, which can be divided into feature-based, substructure pattern-based or molecular field-based methods. Feature-based methods use geometric descriptors or molecular interactions to identify features. Substructure pattern-based methods define substructures, such as hydroxyl groups are defined as hydrogen bond acceptors or donors. Lastly, molecular field-based methods calculate the interaction energy using different chemical probes that sample the ligand or target. The interaction energy maps can be converted to chemical features. Combining 3D pharmacophores and molecular dynamics (MD) would help to sketch physiologically relevant interactions. Furthermore, machine learning and AI could make use of the 3D pharmacophore model.

The ligand-based approach is chosen when there is no structural information available on the macromolecular target. In the ligand-based approach first the chemical features of the ligands, that are important for binding to a target are identified by using a set of known active compounds. Alignment of different conformations of the active compounds helps to identify the shared features (Fig. 10). When the compounds share a feature, it can become a pharmacophoric feature. From the

features a model is developed. Furthermore, to identify if a compound shares the pharmacophore a prefiltering step can be used. Here, the interfeature distances of a molecule are compared with feature combinations of the pharmacophore model, to check if a specific combination of features is present. Next, the compound is aligned by considering the placement of the pharmacophoric features. Also, the importance of the features can be related to the biological activities of known active compounds. Multiple low energy conformations for each molecule are used as the active conformations of molecules are often unknown. In addition, structural dissimilar molecules may have a different binding mode, which makes alignment difficult. Separate pharmacophores may be needed. A library of compounds is screened against the model and compounds that match the model are identified.

Pharmacophore-based screening can be performed with a 3D pharmacophore obtained from ligand-based or structure-based pharmacophore modelling. The models are screened against a library of molecules. These libraries are updated to contain the different conformers of the molecules, to account for the flexibility of the molecules. During the actual screening pharmacophoric features from the query pharmacophore and library compound are compared. From the library molecules that meet the requirements of the query pharmacophore are collected. For the comparison of the pharmacophoric features a fingerprint-based or 3D alignment-based approach can be used. Fingerprint-based methods check for the presence of certain features and convert it to fingerprint-like descriptors to compare with the query pharmacophore. Alignment-based methods align the pharmacophoric features of a compound to a set of the pharmacophoric features of the query pharmacophore. When they are able to align there is a hit. As this approach is time consuming and expensive a pre-filtering step can be performed in which feature presence or distant checks can be done. Pharmacophore-based virtual screening can be followed by docking or molecular dynamic simulations to obtain further structural information. In contrast to in vitro HTS with virtual screening the hit rate is often higher and reduces the amount of molecules that need to be tested with experimental testing.

Validation of the 3D pharmacophore models can be done if there is experimental data on binding ligands. Therefore, known active, inactive and decoy ligands are selected. The active molecules need to share the same binding mode with the target, as 3D pharmacophores are made for a specific binding pose. Also, the inactive molecules could be inactive due to different reasons, such as being unable to reach the target in cell-based assays or being insoluble. Rather decoys are used than inactive molecules, as these have similar physicochemical properties as active molecules and said to be inactive. For validation the set of ligands can be used to evaluate the 3D pharmacophore and if needed optimize further. Here, screening is performed and the number of correct identified actives and inactive molecules is checked. Metrics, like the yield of actives (YA), can be used for evaluation. YA represents the amount of actives found in the total hit list. Retrospective validation of the 3D pharmacophores can be described by plotting receiver operating characteristics (ROC) curves with active and inactive molecules identified in the hit list over time. This is an indication of the sensitivity and specificity of the 3D pharmacophore. Classic pharmacophore modelling will be improved with MD and machine learning (ML), which allow for flexibility and generating of 3D pharmacophores from data, respectively. For instance, dynophores (dynamic pharmacophores) incorporate MD in a 3D pharmacophore. Combining pharmacophores is often used in multi-target drug design.⁴²

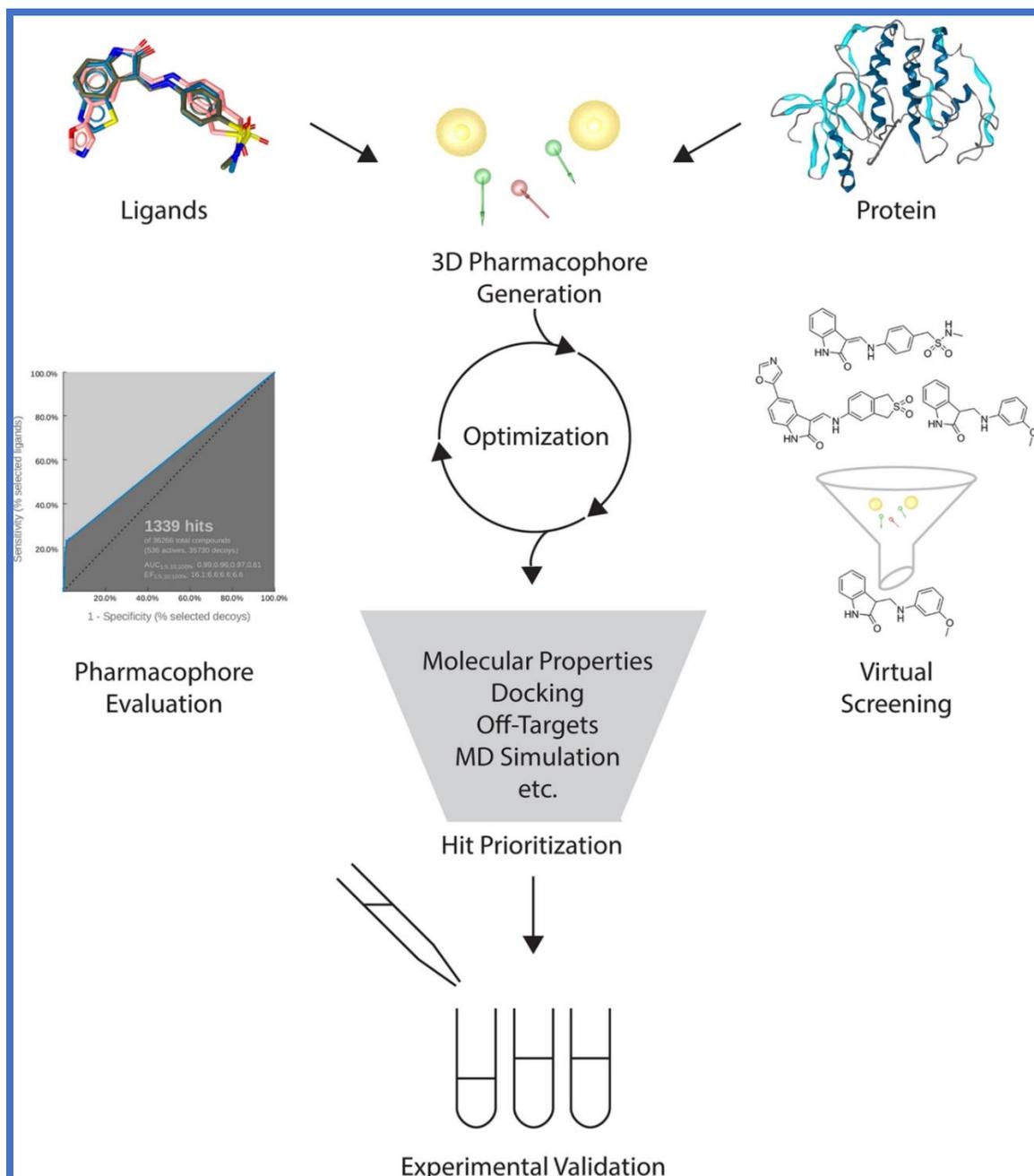


Figure 10. Virtual screening using 3D pharmacophores generated by either LBVS and SBVS. Common features of known ligands or essential features in the binding site are selected to create a pharmacophore. This pharmacophore is evaluated using a ROC plot and is optimized. Next, the model is used for virtual screening to obtain putative hits and the model can be used in combination with e.g. MD. Eventually, hits are tested using experimental methods.⁵²

3.1.3 Machine Learning

Machine Learning (ML) is a part of AI.⁵³ ML has been used in (early) drug discovery pipeline, because it can process an enormous amount of data.⁵⁴ It has been used to predict biological activities, DTIs and toxicity using ML methods, like Support Vector Machines, Naive Bayes, Random Forest and Deep Neural Networks. ML uses computer algorithms to learn from raw data and is later used to perform a certain task.⁵³ Algorithms and a large amount of data are given to the machine to train the network. However, the specific model or formula to solve the problem at hand is not given to the machine. Supervised and unsupervised learning are two learning methods used in ML. Supervised learning is applied to be able to make predictions from data. Here, the machine is trained with known input and

output data. Later the machine will be able to predict the output from new input data. Unsupervised learning is used to cluster data by identifying hidden patterns. This method only uses known input data to train the network.

In drug discovery the ML methodology that is most often used for the prediction of new drugs consists of 5 steps (Fig. 11).⁵⁴ First, data is collected on small molecules and peptides, which are represented as SMILES or FASTA. The molecules in the data set must have characteristics of a potential drug, such as high specificity, low toxicity and good absorption. In addition, these molecules need to be relatively easy to handle in the production process. Second, mathematical descriptors are generated. Sequences are converted into matrices and the different compounds can be labelled. The dataset now consists of mathematical descriptors and can be processed by the ML model. For training and testing the dataset is divided in a training set and testing set. Training sets contain a larger amount of data than testing sets. Third, the amount of variables within the training set is reduced, as a part of the data is redundant, due to collinearities. Different methods exist to obtain a subset of variables from the training set. Fourth, the ML model will be trained. Before the actual training the appropriate algorithms with parameters have to be chosen. Next, the training data is used in subsequent runs, but iterations have to be limited to prevent obtaining a biased model. Cross-validation (CV) can be used to check if the model is unbiased and generalizable. From the original data a new training set and new validation set are used in each CV run. Thereafter, a model is selected, which has the highest performance on relevant parameters. Finally, the selected model is validated using the test set from the original set. Once the results are significant the model can be used for the prediction of new drugs.

Deep learning (DL) is a subset of ML and works with artificial neural networks to detect features from enormous amounts of data.⁵⁴ Input features are given to the input layer of a neural network. Next, nonlinear transformations are performed in multiple hidden layers. Finally, the output is generated in an output layer, often backpropagation of errors is used to limit the difference in expected and obtained output. Deep neural networks can be used for *de novo* drug design and bioactivity prediction.

Recently, a generative neural network was modified for the use in *de novo* multi-target drug design.⁵⁵ Here, data sets of known multi-target ligands, single-target ligands and inactive compounds were extracted from publicly available experimental screening data. A generative model (REINVENT), used for traditional *de novo* design, was fine-tuned using transfer learning. Generative models are trained using datasets with the SMILES data of known compounds and fine-tuned to design compounds with desired properties. Fine-tuning via transfer learning includes the use of additional learning phases with smaller datasets of compounds that have the desired characteristics. The model learns common features and is able to generate new compounds with these properties. In this study, they acquired a model that could tell multi-target ligands apart from single-target ligands and inactive compounds. Also, the model could design new multi-target ligands, suggesting the application of the model in *de novo* multi-target drug design.

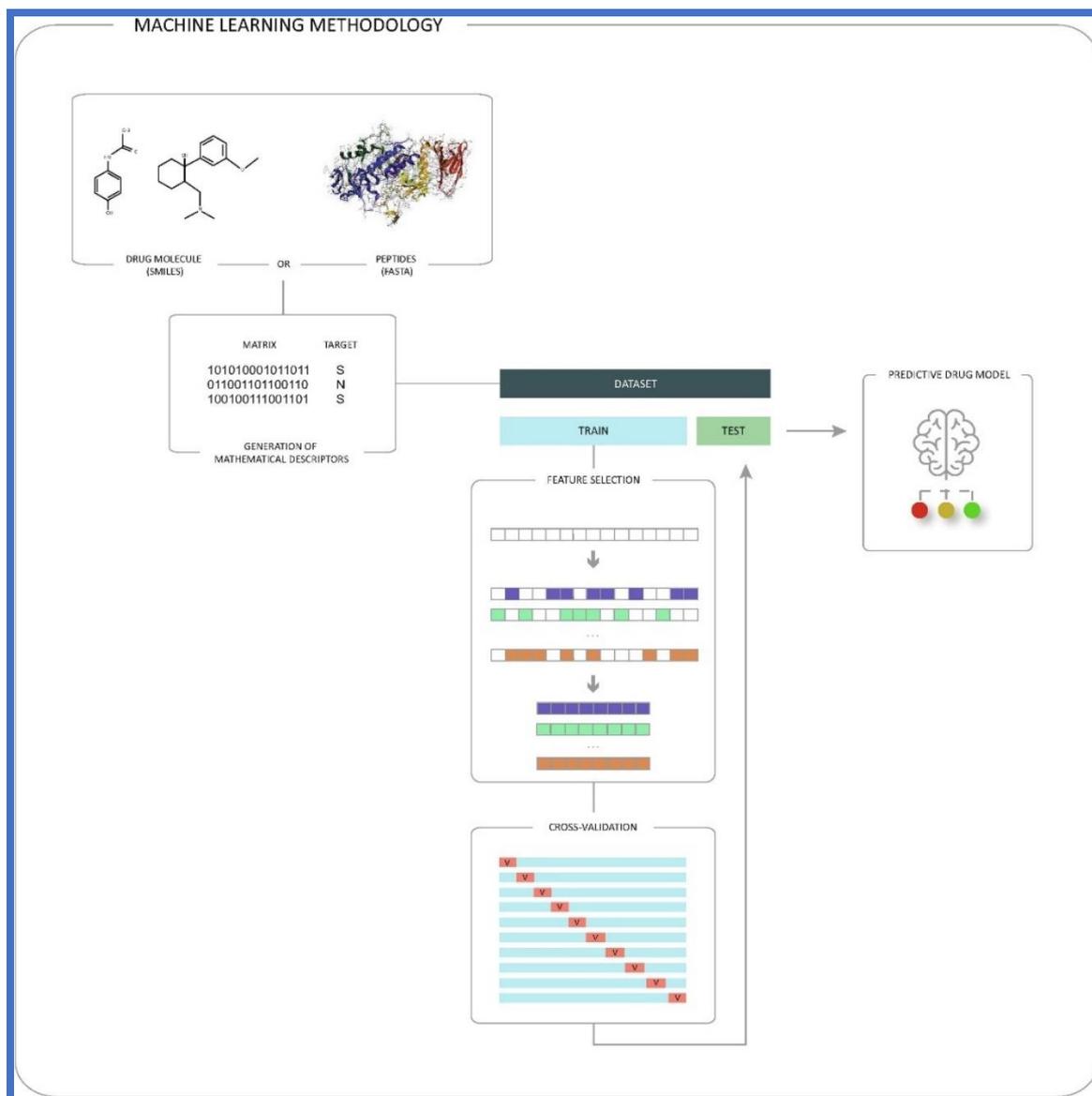


Figure 11. Machine Learning (ML) in drug discovery. Data of small molecules and peptides is collected and represented as SMILES or FASTA. Next, mathematical descriptors are generated using matrices and labelling. This dataset can be processed by the ML model. The dataset is divided in a training set and testing set. Only a subset of relevant features is used from the training set. The model is trained with the training data using multiple runs. Cross-validation can be used to check if the model is unbiased. Finally, a model is selected and validated using the test set. When the model is significant it can be used as a predictive drug model.⁵⁴

3.2 Structure-based virtual screening

SBVS makes use of structural information that is available of the target, which are mostly proteins.²⁴ In general, the 3D structure of the target is solved by crystallography or another biophysical method, and can even be a homology model (Fig. 12). In contrast to LBVS no information is needed about the ligands. However, a priori knowledge about the target binding to known (endogenous) ligands will help to choose and optimize the best approach for finding or designing (new) ligands. As more structural information comes available, the SBVS approaches becomes more appealing for *de novo* ligand design. For instance, fragment-based *de novo* ligand design is used to develop new ligands. Furthermore, MD can be applied during SBVS for various reasons, like the influence of the solvent, the conformation of the ligand and target, and to investigate potential allosteric binding. Lastly, as mentioned earlier LVBS methods can be combined with SVBS methods.

For multi-target drug design there are more limitations for SBVS compared to traditional drug design.⁵⁶ As the multi-target drug has to bind different targets, molecules that will fit these targets have to be found, limiting the ligand possibilities. In addition, the structure of many targets is not determined experimentally, so homology models have to be used. For the disease at hand it is likely that no structure is available for all targets, forcing the use of homology models. In the future, more structural models of on-targets and off-targets will help to find ligands that are selective toward the on-targets. For now we will outline two SBVS approaches, namely structure-based pharmacophore models and molecular docking approaches, and discuss their use in multi-target drug design. Followed by an example of SBVS used in dual-target drug design.

Furthermore, especially in polypharmacology selection of the relevant protein conformation is a challenge.⁵⁷ Artifacts in the binding site can result in potential biases and result in the selection of certain compounds over others during screening. Using an ensemble of protein conformations or allowing receptor flexibility during docking will improve the quality in single target VS. For multi-target drug design using an ensemble of protein conformations will give a higher chance to find common binding site features and compounds with multi-target activity. In addition, for similarity of the binding site of the proteins also consider the presence of water molecules needed for ligand-target interactions or unfavorable water molecules. Also, when there are more ligand-target complex structures available, ligand-based and structure-based approaches can be performed in parallel to identify similar protein conformations that can be used for docking. A ligand-based approach could be to identify a set of protein conformations that can bind similar (co-crystallized) ligands. With a structure-based approach similar features in the binding site can be identified between structures. Furthermore, with MD simulations and ML approaches the conformational landscape of proteins can be sampled. Selection of protein conformations in multi-target drug design will help to design and predict multi-target ligands better.

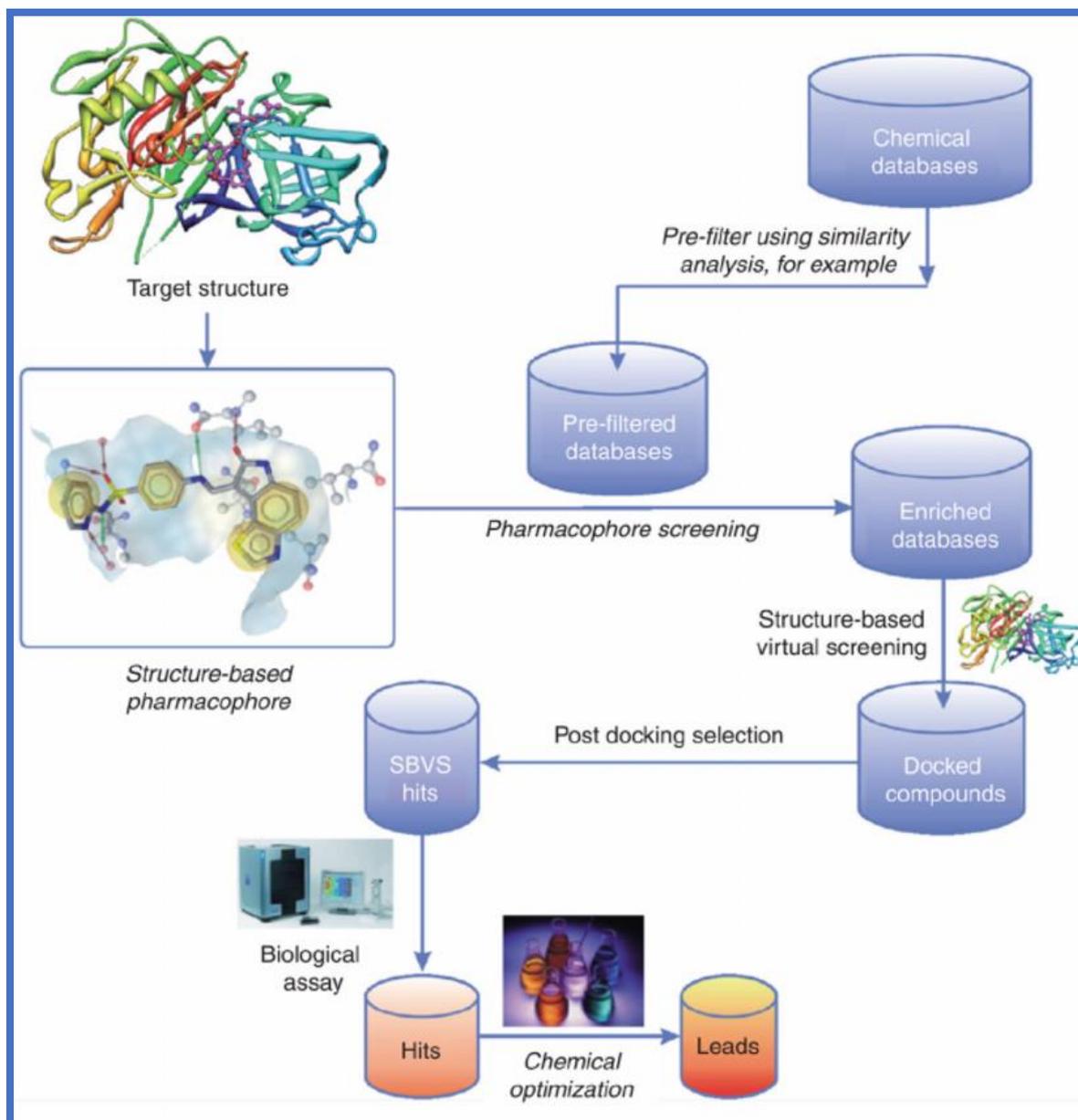


Figure 12. Schematic of the SBVS workflow. First, compounds can be pre-filtered from a library of compounds using for example compound similarity searches. The target structure is obtained from crystallographic data or a homology model is used. A structure-based pharmacophore model can be created, which can be used to screen compounds. This can be followed by SBVS using docking. The compounds are ranked and SBVS hits are selected. Biological assays can be performed to identify hits, which can be optimized into lead compounds.⁵⁸

3.2.1 Structure-based pharmacophores

When the structure of a ligand-target complex or of only the target (apo) is available a structure-based pharmacophore model can be developed.⁵² The structure of a ligand-target complex can be obtained from crystallization or by docking. When there are no known ligands for a certain target the apo structure can be used as a *de novo* approach to create a 3D pharmacophore. This approach can also be used if the ligand is known and a structure of the ligand-target complex is available. Advantageously, this will create a pharmacophore model for the binding site that is not biased by the presence of the known ligand. Therefore, a different region of the chemical space can be sampled.

Feature-based methods can be used for both the complex structure as the apo structure. For the complex the ligand-target interactions are identified and result in the pharmacophoric features. In

contrast, for the apo structure fragments are docked into the binding site using a docking program. Fragments which are energetically suitably docked are used to create a 3D pharmacophore hypothesis. Also, molecular field-based methods can be used to obtain pharmacophoric features by using molecular interaction fields (MIFs) (Fig. 13). Beforehand a binding site is defined and a grid is placed and different probes sample the interactions that occur between functional groups of the ligand and target. Energies between the probe and target are calculated and make it possible to identify sites of favorable interactions. Together the interactions describe the MIFs, and maps are generated that show how the interaction energy alters between the probe and the target. Local minima of the MIFs are converted to pharmacophoric features per probe.

For an apo structure too many possible features are generated and should be reduced before virtual screening. With too many features it will be very hard to obtain any hits, but there should be enough to obtain specificity.

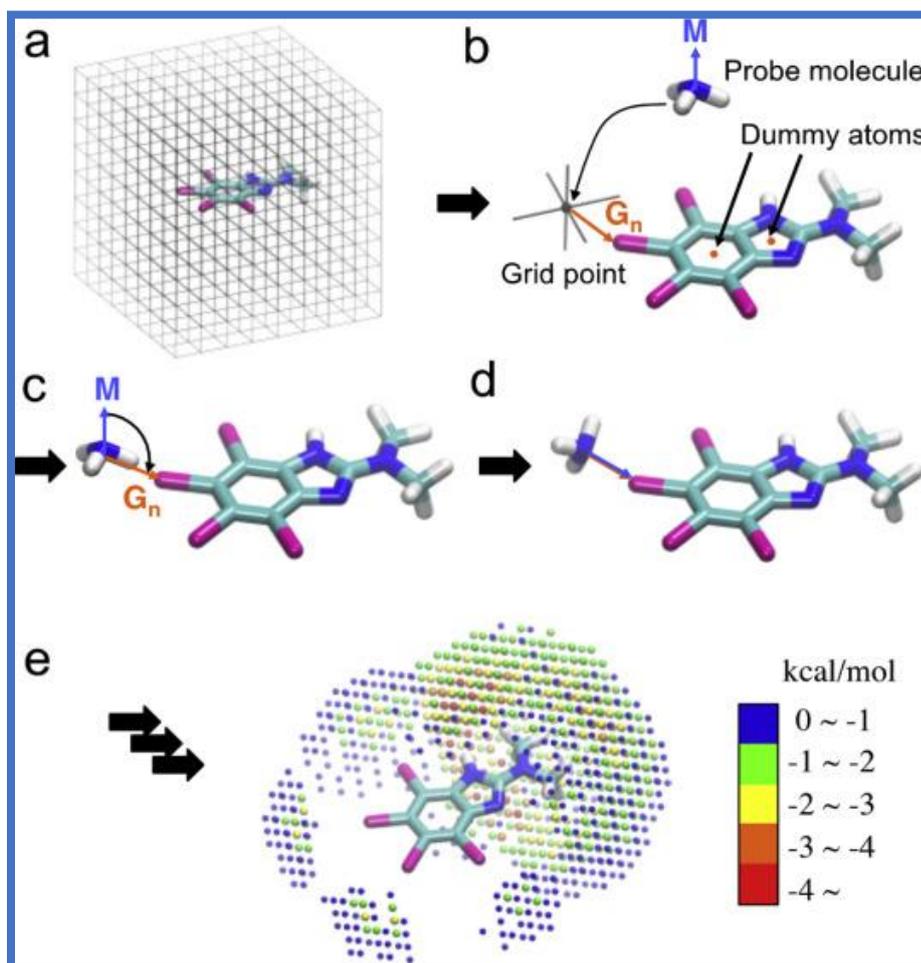


Figure 13. Calculation of MIF of a compound using a grid. (A) A grid is placed over the ligand. (B-D) A probe molecule is used to calculate the interaction energy at different grid points. (E) An example of a MIF.⁵⁹

3.2.2 Molecular docking

Molecular docking is performed to predict the interactions between a compound and a target.⁶⁰ Often first the orientation of the compound in the binding site of the target is predicted, which is followed by scoring to assess their complementarity.

Virtual libraries contain a set of compounds that are docked against a target.⁶¹ A crystal structure of the target was always needed to perform docking, but recent advancements have made it possible to

use homology models (possibly optimized with MD).²⁵ Virtual compound libraries can be classified into general, focused and targeted.⁶¹ General libraries contain compounds for a large number of different targets. Focused libraries are more specific towards a family of related targets. Most specific is the targeted library, which contains a set of compounds meant for a single target. Library size can vary, but often consists of a high number of compounds. During docking the compounds or fragments from the library are docked and parameters are defined. Unfortunately, sampling of the conformations of the ligand and target is often limited.²⁵ Also, different constraints can be used, such as distant restraints, which can keep the compound in the binding site.²⁴ Scoring functions are used to rank the compounds. Often one scoring function is used, which can for example be force field based, empirical or knowledge based. However, scoring functions often do not resemble the experimental binding affinities.²⁵ Post-docking refinement or rescoring can help to obtain better hits, as these methods use more rigorous binding free energy calculations or use machine learning scoring functions.²⁴ Other types of rescoring approaches involve various types of molecular interaction fingerprints as the different ligands of a given target often share key molecular interaction patterns. MD can be applied to sample more of the conformational space of ligands, targets and ligand-target complexes and improve docking results.²⁵ In addition, a validation can be done by re-docking the original ligands in the binding site.⁶²

In structure-based docking visual inspection is needed to evaluate the binding mode due to inherent inaccuracies.⁶³ Often binding modes are inspected on criteria as shape, hydrogen bonds and hydrophobic contacts. However, human inspection has drawbacks, such as their experience and the number of poses they can correctly examine. In addition, docking methods have limitations like not taking into account protein flexibility, omitting part of the entropy and the use of inaccurate scoring functions. A study challenged experts to separate native and non-native poses by visual inspection. The results suggested visual inspection bias exists, but remains limited when discussing results with colleagues.

In addition, to the insight in ligand-target interactions, molecular docking can be used for drug repositioning, reverse screening, prediction of adverse drug reactions and in multi-target drug design.²⁵ Reverse docking is used to predict the targets of a ligand, a method that allows for target fishing and profiling (Fig. 14).^{25,64,65} In this approach the library consists of target structures and targets are ranked with scoring functions.²⁵ Results of standard docking scoring functions are normalized to prevent biases or depending on the use of target. In RD the target flexibility is also limited.⁵⁹ Combining ML methods with RD is also being explored to improve docking results.²⁵ Some limitations of RD with ML are the need for more advanced computational approaches, the amount of data needed for training of the model and unavailability of some target structures. As more data on target structures comes available ML will be used more in the future.

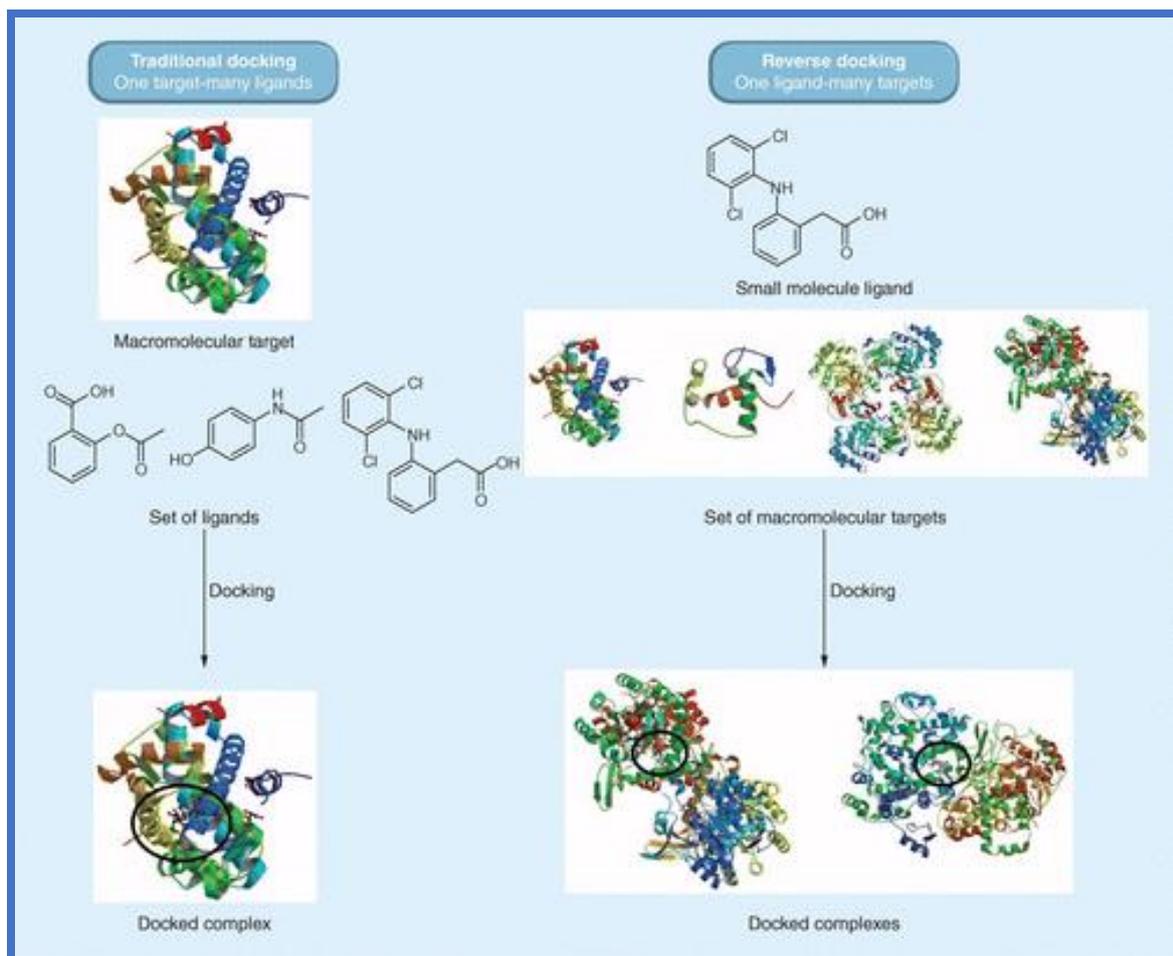


Figure 14. Traditional docking and reverse docking. During traditional docking many ligands are screened against one target. In contrast, reverse docking uses one ligand, which is docked against multiple targets.⁶⁵

In polypharmacology molecular docking can be performed for *de novo* multi-target design, to find new drugs that bind selectively to a subset of targets relevant for a certain disease.⁴⁴ This can be fragment-based *de novo* multitarget drug design via an iterative fragment-growing strategy.⁶⁶ Fragments are more promiscuous and combining them is a strategy to develop a multi-target ligand. Although the use of docking is especially challenging based on selection of protein conformations and structural similarity of the binding sites of targets.²⁵ Therefore, docking is often combined with other in silico methods, for example pharmacophore modelling. A combination of in silico methods for the design of multi-target ligands needs to be decided on based on the available resources and biological data.

Covalent docking in combination with quantum mechanics (QM) is the 'gold-standard' to design covalently binding drugs.⁵² Lower doses are needed for covalent-binding ligands as they remain at their target, which makes these ligands popular. However, QM calculations have a high computational cost and require much time. For these reasons pharmacophore models are often used in virtual screening of large libraries.

Example of dual-target ligands designed using SBVS

So far the pharmaceutical industry believes multi-target drugs to be a challenge to design, as the lead optimization step is complex.¹⁴ Recently, dual-target ligands against G-protein coupled receptors (GPCRs) were designed using SBVS.⁶⁷ This study was the first to report the use of SBVS to design ligands with activity against two distinct GPCRs. These dual-target ligands showed antagonistic

activity against the A_{2A} adenosine receptor and agonistic activity against the D₂ dopamine receptor. Both receptors are relevant for Parkinson's disease, a complex disease, for which traditional drug design is challenging. For A_{2A}AR crystal structures were available and for D₂R a homology model was used. The binding sites of the two targets were compared, and seemed to differ a lot from structural alignment (Ballesteros-Weinstein residue numbering) and ChEMBL data (Fig. 15A). After inspection targeting of the orthosteric and secondary pockets seemed to be the best approach (Fig. 15B).

A virtual library was designed using N-methyl-2-aminoindane as core scaffold, which was positioned in the orthosteric site of D₂R and secondary binding pocket of A_{2A}AR (Fig. 15C). Building blocks were linked to the core scaffold to generate compounds for the compound library. The optimal length of the linkers needed to be found, so both the orthosteric and secondary binding pockets could be accessed. Building blocks were obtained from known single-target A_{2A}AR ligands from ChEMBL and commercial building blocks of the ZINC15 database. Structure-based docking was performed using the compounds from the library. First, docking was performed against a crystal structure of A_{2A}AR using thousands of different conformations and orientations of each compound in the binding site. The top-ranked compounds were visually inspected and ten were picked for synthesis based on interactions with key residues.

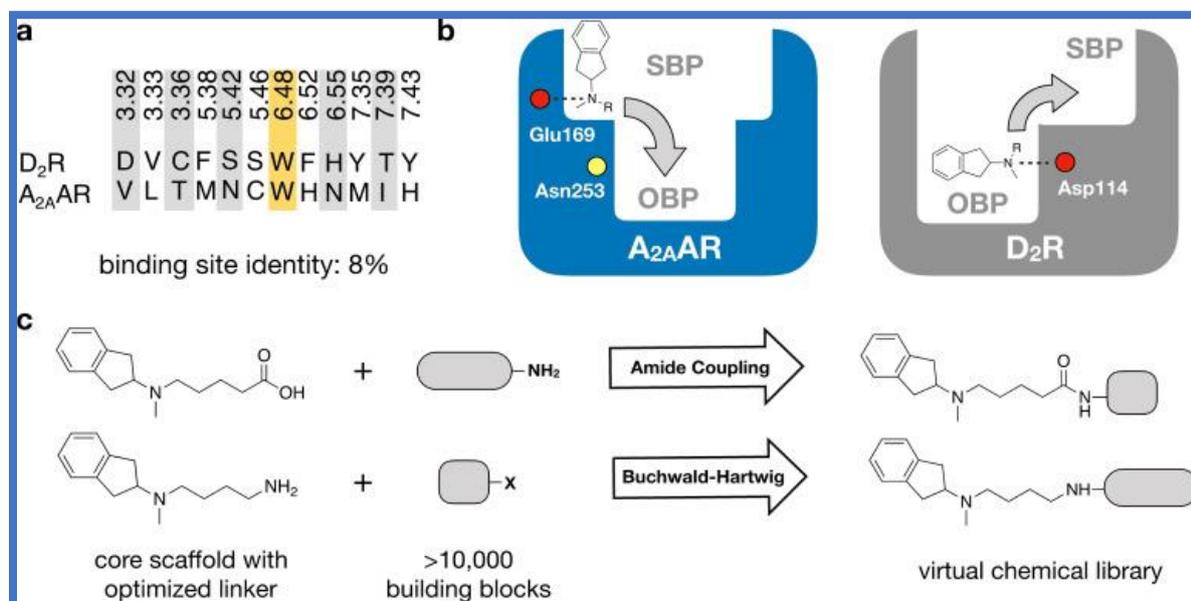


Figure 15. Design of a virtual chemical library. (A) The binding sites of A_{2A}AR and D₂R were compared using structural alignment (Ballesteros-Weinstein residue numbering) and ChEMBL data. (B) The orthosteric and secondary binding pockets of A_{2A}AR and D₂R were inspected and N-methyl-2-aminoindane was used as core scaffold. (C) A virtual library was designed with N-methyl-2-aminoindane as core scaffold and an additional linker. Different building blocks were linked to the core scaffold.⁶⁷

The binding of the ten synthesized compounds was assessed using a competition binding assay. One compound was selected as the most promising starting point for hit-to-lead optimization, with the best affinity for the A_{2A}AR and reasonably high affinity for D₂R. To improve affinity and optimize functional potency analogs were designed and tested to obtain structure–activity relationships. Selectivity of analogs was evaluated using closely related targets. One analog was selected, for which BBB permeation and in vivo testing was performed. The compound could cross the BBB and had an antiparkinsonian effect. In the future, such dual-targeting compounds could potentially be used by Parkinson patients and SBVS can be used to design multi-target drugs for other complex diseases.

3.3 Network-based

Network pharmacology allows the repositioning of FDA-approved drugs for the treatment of other diseases.²³ Polypharmacology holds the principle that targets which are molecularly similar are assumed to have a similar binding to compounds. Proteins with similar binding pockets across the human proteome hold potential to be targeted simultaneously.³⁹ Identification of similar binding sites helps to discover new possibilities to design multi-target ligands in the system of (protein) interactions underlying a disease. The system describes the interactions from omics (proteomics and genomics) data, which also describes dynamic interactions.⁶⁸ So, in systems biology the DTIs in biological networks can be studied with the help of computational resources. For network-based methods in drug target discovery it is important to consider the isoform level, especially the identification of the major isoform, which can be analysed with gene expression profiles and an isoform coexpression network.⁶⁹ Insight in the major isoform and drug targeting of different isoforms can be obtained for drug discovery. In addition, the design of novel multi-target therapeutic strategies can be identified. Eventually, drug-drug networks, drug-target networks and disease-specific protein-protein networks can be made.

New DTIs can be identified with the network-based approach, as was done for GPCRs (Fig. 16).⁷⁰ First, an atlas of the human GPCRs was made. Second, the network of DTIs was reconstructed. Here, they made a local DTI network using only drugs marked as 'approved' in the DrugBank. In addition, a global DTI network was made using drugs marked as 'approved' and 'investigational' in the DrugBank, purchasable GPCR ligands and multi-target GPCR ligands. Third, predictive models were made by using different network-based methods and chemical substructures. Fourth, computationally potential DTIs were predicted from a compound library. Fifth, from the compounds a selection was made of ligands that bound to one or multiple GPCRs that were relevant. Lastly, experimental assays were done, which validated the activity of the new ligands.

The network-based approach has several advantages compared to methods like docking and machine learning.⁷⁰ First of all, for network-based methods crystal structures of the GPCRs are not needed. Second, only knowledge of the active DTIs is needed, in contrast to machine learning, where both the active and inactive DTIs is required. Which is hard as there is a lack of high-quality data on inactive DTIs. Although the network-based approach is promising there are still improvements to be made. Orphan GPCRs, GPCRs without known endogenous ligands, cannot be incorporated into the network. Therefore, identifying their endogenous ligands would enable the incorporation of the orphan receptors. Furthermore, methods that are able to predict potential DTIs for orphan receptors are being developed, aiding the development of new drugs against orphan GPCRs.

Systemic chemogenomics/QSAR can be used in phenotypic virtual screening.⁷¹ When the whole system is encoded the connection between the first ligand-target interaction and the final phenotype can be unravelled. Systemic chemogenomics/QSAR can be used to select ligands that induce the desired phenotype. Also, proteochemometric modelling can be used for drug repositioning.²³

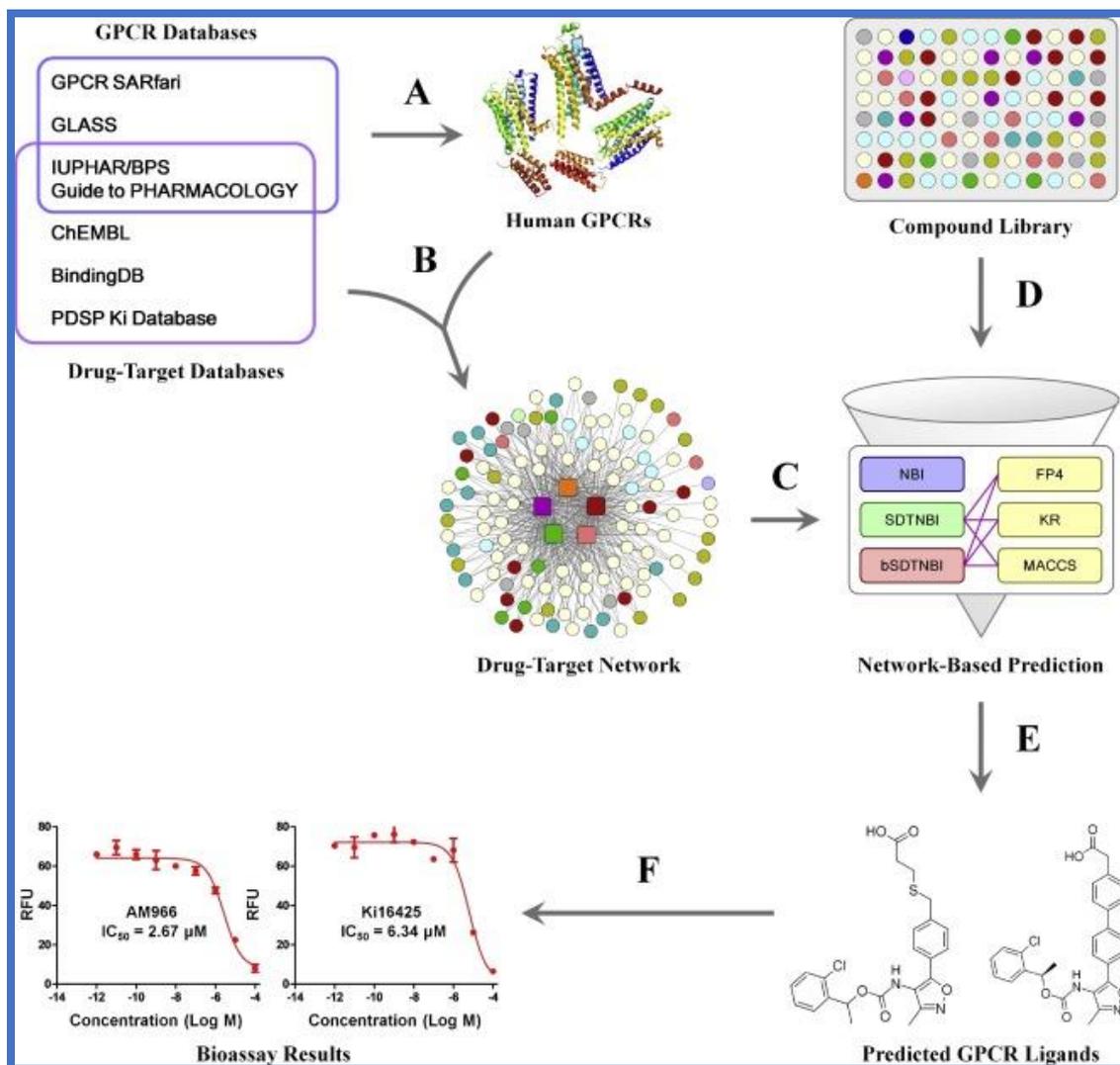


Figure 16. Network pharmacology used to identify new DTIs for GPCRs. (A) Information on human GPCRs was collected from different GPCR databases. (B) Drug-target network was reconstructed from drug-target databases and human GPCRs. (C) Network-based predictive models were made using network-based methods. (D) DTIs were predicted using a compound library. (E) Relevant GPCR ligands were selected from the compounds. (F) The ligands were validated with bioassays.⁷⁰

Discussion

Traditional drug design rationally designed selective drugs for a specific target.¹³ However, most drugs are actually promiscuous.¹⁴ For complex diseases a multi-target drug is needed for better efficacy. Polypharmacology studies can be used to reposition drugs, overcome resistance, predict possible adverse effects and design multi-target drugs, e.g. for complex diseases. Virtual screening methods have been used and improved to be used in multi-target drug design.²⁶ Here, we presented ligand-based, structure-based and network-based approaches used to screen for multi-target drugs. Traditional virtual screening methods have been modified for the use in multi-target screening, but still need to be improved. New methods and algorithms are needed and more data, such as structural data of targets and activity data of compounds for multiple targets. Novel concepts have been introduced for multi-target drug design. In ligand-based mt-QSAR and the introduction of MCs seem promising. Structure-based the use of reverse docking and *de novo* multi-target drug design. Network-based considers the system and uses omics data and AI to find DTIs. A lot of progress is being made to alter the use of methods for the use of multi-target drug design and concepts are

being introduced. However, more alterations are needed for the applicability in multi-target drug design

Best in silico approach to design a multi-target drug

The best approach to use virtual screening in the multi-target design process differs. As the available information about ligands and targets is different per disease. Maybe in the future there will be one approach for the design of new drugs, the network approach shows great promise. Here, high performance computers need to handle all the data and there should be enough (experimental) data. Therefore, current limitations need to be overcome. More and more progress is made to obtain structural data targets and identify orphan drugs and targets. In the end still experimental screening is needed, with HTS as the gold-standard. AI can be used in ligand-based and structure-based *de novo* multi-target drug design.⁷² Incomplete data of compound activities (negative and positive data, i.e., ligands bind on or off target) makes the design of multi-target drugs challenging. In addition, available data is often primarily for target classes as GPCRs and kinases. In the future we need more data on compounds and their activity for different targets, which means that also negative data needs to be published. When designing a network using incomplete data, this will result in a bias and will limit the applicability of the model. Use of DL in multi-target drug design holds much promise, as it could predict synergistic effects. However, the lack of data for training is limitation.

Promiscuity and conformations of the ligand and target

Computational methods are available to study the promiscuity of both drugs and targets.⁷³ Promiscuity of both the ligands and targets needs to be considered. While promiscuous ligands have been studied extensively the promiscuity of the binding site has been studied to a lesser extent. Drugability of the binding site needs to be evaluated. Dopamine and cocaine both bind to the dopamine transporter (DAT) using a different binding mode, with different interactions, thus have a different pharmacophore.^{74,75} Recently, a new allosteric binding site of cocaine of DAT was identified.⁷⁶ Therefore, a pharmacophore model cannot describe the interactions of cocaine and dopamine, due to the different conformations of the DAT receptor during binding. In addition, flexibility of the binding site is of great importance. The use of MD in docking is needed to account for flexibility. Also, the binding of a drug to different protein classes or proteins from the same family need to be considered, which is linked to sampling of the entire chemical and biological space.

Resistance and identification of novel modes of action

Resistance (desensitisation) against newly discovered drugs should be circumvented and the probability of resistance should be minimized from the start.⁷⁷ Next to the magic bullet and magic shotgun, the term magic bomb was introduced for a new mode of action against parasitic diseases.⁷⁸ Here, a prodrug is activated inside the parasite by reductive bioactivation, relying on electron transfer, which is followed by the formation of reactive radicals. These drugs are promiscuous as they target multiple proteins and try to prevent the emergence of resistance. Highlighting the emergence of new potential uses of multi-target approaches.

New use of multi-target drug

Synthetic lethality is the cell death due to multiple genetic events, e.g. in cancer, whereas a single genetic event will not result in cell death.⁷⁹ Multi-target drugs can be designed that act on specific pathways involved in synthetic lethality. Polypharmacology studies both the on-target and off-target effects, indicating it can also be used to predict adverse reactions. In addition, a multi-target drug can act on different targets and may act agonistic and antagonistic. For the design of a multi-target drug more information is needed in advance to predict if it will be an agonist or an antagonist. The multi-target drug has to remain long enough in the blood to be able to reach and act on multiple targets.

Furthermore, in each case we should investigate if weak partial inhibition is better than the full inhibition of a target. Additionally, the drug should have approximately the same affinity for the relevant targets. Finally, a multi-target drug should show synergy by modulating multiple targets.

Towards precision medicine

The term precision medicine has been coined, which takes into account the genes, environment and lifestyle of each person to create new treatments.⁵⁴ This approach will eventually replace the ‘one-size-fits-all’ approach. As only for the perfect patient the predefined dose of a drug is correct, each patient ideally needs a different dose. This also holds for the usage of combination drugs or multi-target drugs. For the development of personal medicine new methodologies need to be developed. In addition, a lot of omics data is needed to find specific treatments. Furthermore, cell-based disease models (phenotypic) may aid the development of personal medicine.

Conclusion

In summary, multi-target drug design remains challenging, but recent and upcoming advancements show great promise. Methods are being updated and novel concepts are introduced to aid the design of multi-target drugs. In this review we highlighted the traditional drug design approach and the shift towards polypharmacology. Especially focussing on the virtual screening methods in drug design and their applicability in multi-target drug design. We introduced limitations and promising approaches in multi-target drug design. Future approaches will aid network-based drug design using omics data and AI methods.

References

- (1) Strebhardt, K.; Ullrich, A. Paul Ehrlich’s Magic Bullet Concept: 100 Years of Progress. *Nat. Rev. Cancer* **2008**, *8* (6), 473–480.
- (2) Medina-Franco, J. L.; Giulianotti, M. A.; Welmaker, G. S.; Houghten, R. A. Shifting from the Single- to the Multitarget Paradigm in Drug Discovery. *Drug Discov. Today* **2013**, *18* (0), 501.
- (3) Acharya, K. R.; Sturrock, E. D.; Riordan, J. F.; Ehlers, M. R. W. Ace Revisited: A New Target for Structure-Based Drug Design. *Nat. Rev. Drug Discov.* **2003**, *2* (11), 891–902.
- (4) Cohen, P.; Cross, D.; Jänne, P. A. Kinase Drug Discovery 20 Years after Imatinib: Progress and Future Directions. *Nat. Rev. Drug Discov.* **2021**, *20* (7), 551–569.
- (5) Ramsay, R. R.; Popovic-Nikolic, M. R.; Nikolic, K.; Uliassi, E.; Bolognesi, M. L. A Perspective on Multi-Target Drug Discovery and Design for Complex Diseases. *Clin. Transl. Med.* **2018**, *7* (1), 3.
- (6) Saginc, G.; Voellmy, F.; Linding, R. Cancer Systems Biology: Harnessing off-Target Effects. *Nat. Chem. Biol.* **2017**, *13* (12), 1204–1205.
- (7) Klaeger, S.; Heinzlmeir, S.; Wilhelm, M.; Polzer, H.; Vick, B.; Koenig, P. A.; Reinecke, M.; Ruprecht, B.; Petzoldt, S.; Meng, C.; Zecha, J.; Reiter, K.; Qiao, H.; Helm, D.; Koch, H.; Schoof, M.; Canevari, G.; Casale, E.; Re Depaolini, S.; Feuchtinger, A.; Wu, Z.; Schmidt, T.; Rueckert, L.; Becker, W.; Huenges, J.; Garz, A. K.; Gohlke, B. O.; Zolg, D. P.; Kayser, G.; Voeder, T.; Preissner, R.; Hahne, H.; Tönisson, N.; Kramer, K.; Götze, K.; Bassermann, F.; Schlegl, J.; Ehrlich, H. C.; Aiche, S.; Walch, A.; Greif, P. A.; Schneider, S.; Felder, E. R.; Ruland, J.; Médard, G.; Jeremias, I.; Spiekermann, K.; Kuster, B. The Target Landscape of Clinical Kinase Drugs. *Sci.* **2017**, *358* (6367), eaan4368.
- (8) Radi, Z. A.; Khan, N. K. Effects of Cyclooxygenase Inhibition on the Gastrointestinal Tract. *Exp. Toxicol. Pathol.* **2006**, *58* (2–3), 163–173.

- (9) Schönthal, A. H.; Chen, T. C.; Hofman, F. M.; Louie, S. G.; Petasis, N. A. Celecoxib Analogs That Lack COX-2 Inhibitory Function: Preclinical Development of Novel Anticancer Drugs. *Expert Opin. Investig. Drugs* **2008**, *17* (2), 197–208.
- (10) Roth, B. L.; Sheffer, D. J.; Kroeze, W. K. Magic Shotguns versus Magic Bullets: Selectively Non-Selective Drugs for Mood Disorders and Schizophrenia. *Nat. Rev. Drug Discov.* **2004**, *3* (4), 353–359.
- (11) Hopkins, A. L.; Mason, J. S.; Overington, J. P. Can We Rationally Design Promiscuous Drugs? *Curr. Opin. Struct. Biol.* **2006**, *16* (1), 127–136.
- (12) Hu, Y.; Bajorath, J. Entering the ‘Big Data’ Era in Medicinal Chemistry: Molecular Promiscuity Analysis Revisited. *Futur. Sci. OA* **2017**, *3* (2), FSO179.
- (13) Hopkins, A. L. Network Pharmacology: The next Paradigm in Drug Discovery. *Nat. Chem. Biol.* **2008**, *4* (11), 682–690.
- (14) Kabir, A.; Muth, A. Polypharmacology: The Science of Multi-Targeting Molecules. *Pharmacol. Res.* **2022**, *176*, 106055.
- (15) Long, M. J.; Liu, X.; Aye, Y. Genie in a Bottle: Controlled Release Helps Tame Natural Polypharmacology? *Curr. Opin. Chem. Biol.* **2019**, *51*, 56.
- (16) Kontoghiorghes, G. J.; Fetta, S.; Kontoghiorghes, C. N. The Need for a Multi-Level Drug Targeting Strategy to Curb the COVID-19 Pandemic. *Front. Biosci. (Landmark Ed.)* **2021**, *26* (12), 1723–1736.
- (17) Rock, B. M.; Hengel, S. M.; Rock, D. A.; Wienkers, L. C.; Kunze, K. L. Characterization of Ritonavir-Mediated Inactivation of Cytochrome P450 3A4. *Mol. Pharmacol.* **2014**, *86* (6), 665–674.
- (18) Ghofrani, H. A.; Osterloh, I. H.; Grimminger, F. Sildenafil: From Angina to Erectile Dysfunction to Pulmonary Hypertension and Beyond. *Nat. Rev. Drug Discov.* **2006**, *5* (8), 702.
- (19) Swinney, D. C. Phenotypic vs. Target-Based Drug Discovery for First-in-Class Medicines. *Clin. Pharmacol. Ther.* **2013**, *93* (4), 299–301.
- (20) Pereira, D. A.; Williams, J. A. Origin and Evolution of High Throughput Screening. *Br. J. Pharmacol.* **2007**, *152* (1), 61.
- (21) Earm, K.; Earm, Y. E. Integrative Approach in the Era of Failing Drug Discovery and Development. *Integr. Med. Res.* **2014**, *3* (4), 211–216.
- (22) Berdigaliyev, N.; Aljofan, M. An Overview of Drug Discovery and Development. *Future Med. Chem.* **2020**, *12* (10), 939–947.
- (23) Schaduangrat, N.; Lampa, S.; Simeon, S.; Gleeson, M. P.; Spjuth, O.; Nantasenamat, C. Towards Reproducible Computational Drug Discovery. *J. Cheminform.* **2020**, *12* (1), 1–30.
- (24) Singh, N.; Chaput, L.; Villoutreix, B. O. Virtual Screening Web Servers: Designing Chemical Probes and Drug Candidates in the Cyberspace. *Brief. Bioinform.* **2021**, *22* (2), 1790–1818.
- (25) Pinzi, L.; Rastelli, G. Molecular Docking: Shifting Paradigms in Drug Discovery. *Int. J. Mol. Sci.* **2019**, *20* (18), 4331.
- (26) Chaudhari, R.; Fong, L. W.; Tan, Z.; Huang, B.; Zhang, S. An Up-to-Date Overview of Computational Polypharmacology in Modern Drug Discovery. *Expert Opin. Drug Discov.* **2020**, *15* (9), 1044.

- (27) Makhoba, X. H.; Viegas, C.; Mosa, R. A.; Viegas, F. P. D.; Poee, O. J. Potential Impact of the Multi-Target Drug Approach in the Treatment of Some Complex Diseases. *Drug Des. Devel. Ther.* **2020**, *14*, 3235–3249.
- (28) Hughes, J. P.; Rees, S. S.; Kalindjian, S. B.; Philpott, K. L. Principles of Early Drug Discovery. *Br. J. Pharmacol.* **2011**, *162* (6), 1239–1249.
- (29) Ha, J.; Park, H.; Park, J.; Park, S. B. Recent Advances in Identifying Protein Targets in Drug Discovery. *Cell Chem. Biol.* **2021**, *28* (3), 394–423.
- (30) Zheng, W.; Thorne, N.; McKew, J. C. Phenotypic Screens as a Renewed Approach for Drug Discovery. *Drug Discov. Today* **2013**, *18* (21–22), 1067–1073.
- (31) Eder, J.; Sedrani, R.; Wiesmann, C. The Discovery of First-in-Class Drugs: Origins and Evolution. *Nat. Rev. Drug Discov.* **2014**, *13*, 577–587.
- (32) Davis, R. L. Mechanism of Action and Target Identification: A Matter of Timing in Drug Discovery - ScienceDirect. *iScience* **2020**, *23* (9), 101487.
- (33) Leveridge, M.; Chung, C. W.; Gross, J. W.; Phelps, C. B.; Green, D. Integration of Lead Discovery Tactics and the Evolution of the Lead Discovery Toolbox. *SLAS Discov.* **2018**, *23* (9), 881–897.
- (34) Langedijk, J.; Mantel-Teeuwisse, A. K.; Slijkerman, D. S.; Schutjens, M. H. D. B. Drug Repositioning and Repurposing: Terminology and Definitions in Literature. *Drug Discov. Today* **2015**, *20* (8), 1027–1034.
- (35) Cheng, F.; István, I.; Kovácskovács, A.; László, A.-L.; Lászlóbarabási, L. Network-Based Prediction of Drug Combinations. *Nat. Commun.* **2019**, *10*, 1197.
- (36) Chen, D.; Liu, X.; Yang, Y.; Yang, H.; Lu, P. Systematic Synergy Modeling: Understanding Drug Synergy from a Systems Biology Perspective. *BMC Syst. Biol.* **2015**, *9*, 56.
- (37) Talevi, A. Multi-Target Pharmacology: Possibilities and Limitations of the “Skeleton Key Approach” from a Medicinal Chemist Perspective. *Front. Pharmacol.* **2015**, *6*, 205.
- (38) Zhou, J.; Jiang, X.; He, S.; Jiang, H.; Feng, F.; Liu, W.; Qu, W.; Sun, H. Rational Design of Multitarget-Directed Ligands: Strategies and Emerging Paradigms. *J. Med. Chem.* **2019**, *62* (20), 8881–8914.
- (39) Duran-Frigola, M.; Siragusa, L.; Ruppín, E.; Barril, X.; Cruciani, G.; Aloy, P. Detecting Similar Binding Pockets to Enable Systems Polypharmacology. *PLOS Comput. Biol.* **2017**, *13* (6), e1005522.
- (40) Galati, S.; Di Stefano, M.; Martinelli, E.; Poli, G.; Tuccinardi, T. Recent Advances in In Silico Target Fishing. *Molecules* **2021**, *26* (17), 5124.
- (41) Proschak, E.; Stark, H.; Merk, D. Polypharmacology by Design: A Medicinal Chemist’s Perspective on Multitargeting Compounds. *J. Med. Chem.* **2019**, *62* (2), 420–444.
- (42) Stelitano, G.; Sammartino, J. C.; Chiarelli, L. R. Multitargeting Compounds: A Promising Strategy to Overcome Multi-Drug Resistant Tuberculosis. *Molecules* **2020**, *25* (5), 1239.
- (43) Citrome, L.; McEvoy, J. P.; Todtenkopf, M. S.; McDonnell, D.; Weiden, P. J. A Commentary on the Efficacy of Olanzapine for the Treatment of Schizophrenia: The Past, Present, and Future. *Neuropsychiatr. Dis. Treat.* **2019**, *15*, 2569.
- (44) Yuan, Y.; Pei, J.; Lai, L. LigBuilder V3: A Multi-Target de Novo Drug Design Approach. *Front. Chem.* **2020**, *8*, 142.

- (45) Stumpfe, D.; Bajorath, J. Current Trends, Overlooked Issues, and Unmet Challenges in Virtual Screening. *J. Chem. Inf. Model.* **2020**, *60* (9), 4112–4115.
- (46) Vázquez, J.; López, M.; Gibert, E.; Herrero, E.; Javier Luque, F. Merging Ligand-Based and Structure-Based Methods in Drug Discovery: An Overview of Combined Virtual Screening Approaches. *Molecules* **2020**, *25* (20), 4723.
- (47) Nikolic, K.; Mavridis, L.; Djikic, T.; Vucicevic, J.; Agbaba, D.; Yelekci, K.; Mitchell, J. B. O. Drug Design for CNS Diseases: Polypharmacological Profiling of Compounds Using Cheminformatic, 3D-QSAR and Virtual Screening Methodologies. *Front. Neurosci.* **2016**, *10*, 265.
- (48) Zhang, W.; Pei, J.; Lai, L. Computational Multitarget Drug Design. *J. Chem. Inf. Model.* **2017**, *57* (3), 403–412.
- (49) Kleandrova, V. V.; Scotti, L.; Bezerra Mendonça Junior, F. J.; Muratov, E.; Scotti, M. T.; Speck-Planche, A. QSAR Modeling for Multi-Target Drug Discovery: Designing Simultaneous Inhibitors of Proteins in Diverse Pathogenic Parasites. *Front. Chem.* **2021**, *9*, 58.
- (50) Stumpfe, D.; Hoch, A.; Bajorath, J. Introducing the Metacore Concept for Multi-Target Ligand Design. *RSC Med. Chem.* **2021**, *12* (4), 628–635.
- (51) Van Westen, G. J. P.; Wegner, J. K.; Ijzerman, A. P.; Van Vlijmen, H. W. T.; Bender, A. Proteochemometric Modeling as a Tool to Design Selective Compounds and for Extrapolating to Novel Targets. *Medchemcomm* **2011**, *2* (1), 16–30.
- (52) Schaller, D.; Šribar, D.; Noonan, T.; Deng, L.; Nguyen, T. N.; Pach, S.; Machalz, D.; Bermudez, M.; Wolber, G. Next Generation 3D Pharmacophore Modeling. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* **2020**, *10* (4), e1468.
- (53) Vamathevan, J.; Clark, D.; Czodrowski, P.; Dunham, I.; Ferran, E.; Lee, G.; Li, B.; Madabhushi, A.; Shah, P.; Spitzer, M.; Zhao, S. Applications of Machine Learning in Drug Discovery and Development. *Nat. Rev. Drug Discov.* **2019**, *18*, 463–477.
- (54) Carracedo-Reboredo, P.; Liñares-Blanco, J.; Rodríguez-Fernándezbc, N.; Cedrón, F.; Novoa, F. J.; Carballal, A.; Maojod, V.; Pazosabe, A.; Fernandez-Lozanoabe, C. A Review on Machine Learning Approaches and Trends in Drug Discovery. *Comput. Struct. Biotechnol. J.* **2021**, *19*, 4538–4558.
- (55) Blaschke, T.; Bajorath, J. Fine-Tuning of a Generative Neural Network for Designing Multi-Target Compounds. *J. Comput. Aided. Mol. Des.* **2021**, 1–9.
- (56) Weiss, D. R.; Karpiak, J.; Huang, X. P.; Sassano, M. F.; Lyu, J.; Roth, B. L.; Shoichet, B. K. Selectivity Challenges in Docking Screens for GPCR Targets and Antitargets. *J. Med. Chem.* **2018**, *61* (15), 6830–6845.
- (57) Pinzi, L.; Caporuscio, F.; Rastelli, G. Selection of Protein Conformations for Structure-Based Polypharmacology Studies. *Drug Discov. Today* **2018**, *23* (11), 1889–1896.
- (58) Liao, C.; Sitzmann, M.; Pugliese, A.; Nicklaus, M. C. Software and Resources for Computational Medicinal Chemistry. <http://dx.doi.org/10.4155/fmc.11.63> **2011**, *3* (8), 1057–1085.
- (59) Hayakawa, D.; Sawada, N.; Watanabe, Y.; Gouda, H. A Molecular Interaction Field Describing Nonconventional Intermolecular Interactions and Its Application to Protein–Ligand Interaction Prediction. *J. Mol. Graph. Model.* **2020**, *96*, 107515.
- (60) Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath, J. Docking and Scoring in Virtual Screening for Drug Discovery: Methods and Applications. *Nat. Rev. Drug Discov.* **2004**, *3* (11), 935–949.

- (61) Kalyanamoothy, S.; Chen, Y. P. P. Structure-Based Drug Design to Augment Hit Discovery. *Drug Discov. Today* **2011**, *16* (17–18), 831–839.
- (62) Cleves, A. E.; Jain, A. N. Knowledge-Guided Docking: Accurate Prospective Prediction of Bound Configurations of Novel Ligands Using Surflex-Dock. *J. Comput. Aided. Mol. Des.* **2015**, *29* (6), 485–509.
- (63) Fischer, A.; Smieško, M.; Sellner, M.; Lill, M. A. Decision Making in Structure-Based Drug Discovery: Visual Inspection of Docking Results. *J. Med. Chem.* **2021**, *64* (5), 2489–2500.
- (64) Lavecchia, A.; Cerchia, C. In Silico Methods to Address Polypharmacology: Current Status, Applications and Future Perspectives. *Drug Discov. Today* **2016**, *21* (2), 288–298.
- (65) Kharkar, P. S.; Warriar, S.; Gaud, R. S. Reverse Docking: A Powerful Tool for Drug Repositioning and Drug Rescue. *Future Med. Chem.* **2014**, *6* (3), 333–342.
- (66) Shang, E.; Yuan, Y.; Chen, X.; Liu, Y.; Pei, J.; Lai, L. De Novo Design of Multitarget Ligands with an Iterative Fragment-Growing Strategy. *J. Chem. Inf. Model.* **2014**, *54* (4), 1235–1241.
- (67) Kampen, S.; Duy Vo, D.; Zhang, X.; Panel, N.; Yang, Y.; Jaiteh, M.; Matricon, P.; Svenningsson, P.; Brea, J.; Loza, M. I.; Kihlberg, J.; Carlsson, J. Structure-Guided Design of G-Protein-Coupled Receptor Polypharmacology. *Angew. Chem. Int. Ed. Engl.* **2021**, *60* (33), 18030.
- (68) Vitali, F.; Mulas, F.; Marini, P.; Bellazzi, R. Network-Based Target Ranking for Polypharmacological Therapies. *J. Biomed. Inform.* **2013**, *46* (5), 876–881.
- (69) Ma, J.; Wang, J.; Ghoraie, L. S.; Men, X.; Liu, L.; Dai, P. Network-Based Method for Drug Target Discovery at the Isoform Level. *Sci. Rep.* **2019**, *9*, 13868.
- (70) Wu, Z.; Lu, W.; Yu, W.; Wang, T.; Li, W.; Liu, G.; Zhang, H.; Pang, X.; Huang, J.; Liu, M.; Cheng, F.; Tang, Y. Quantitative and Systems Pharmacology 2. In Silico Polypharmacology of G Protein-Coupled Receptor Ligands via Network-Based Approaches. *Pharmacol. Res.* **2018**, *129*, 400–413.
- (71) Cruz-Monteagudo, M.; Schürer, S.; Tejera, E.; Pérez-Castillo, Y.; Medina-Franco, J. L.; Sánchez-Rodríguez, A.; Borges, F. Systemic QSAR and Phenotypic Virtual Screening: Chasing Butterflies in Drug Discovery. *Drug Discov. Today* **2017**, *22* (7), 1007.
- (72) Yang, X.; Wang, Y.; Byrne, R.; Schneider, G.; Yang, S. Concepts of Artificial Intelligence for Computer-Assisted Drug Discovery. *Chem. Rev.* **2019**, *119* (18), 10520–10594.
- (73) Cerisier, N.; Petitjean, M.; Regad, L.; Bayard, Q.; Réau, M.; Badel, A.; Camproux, A. C. High Impact: The Role of Promiscuous Binding Sites in Polypharmacology. *Molecules* **2019**, *24* (14), 2529.
- (74) Beuming, T.; Kniazeff, J.; Bergmann, M. L.; Shi, L.; Gracia, L.; Raniszewska, K.; Newman, A. H.; Javitch, J. A.; Weinstein, H.; Gether, U.; Loland, C. J. The Binding Sites for Cocaine and Dopamine in the Dopamine Transporter Overlap. *Nat. Neurosci.* **2008**, *11* (7), 789.
- (75) Wang, K. H.; Penmatsa, A.; Gouaux, E. Neurotransmitter and Psychostimulant Recognition by the Dopamine Transporter. *Nat.* **2015**, *521*, 322–327.
- (76) Xu, L.; Chen, L. Y. Identification of a New Allosteric Binding Site for Cocaine in Dopamine Transporter. *J. Chem. Inf. Model.* **2020**, *60* (8), 3958–3968.
- (77) Matthew, A. N.; Leidner, F.; Lockbaum, G. J.; Henes, M.; Zephyr, J.; Hou, S.; Rao, D. N.; Timm, J.; Rusere, L. N.; Ragland, D. A.; Paulsen, J. L.; Prachanronarong, K.; Soumana, D. I.; Nalivaika, E. A.; Kurt Yilmaz, N.; Ali, A.; Schiffer, C. A. Drug Design Strategies to Avoid Resistance in

Direct-Acting Antivirals and Beyond. *Chem. Rev.* **2021**, *121* (6), 3238–3270.

- (78) Wittlin, S.; Mäser, P. From Magic Bullet to Magic Bomb: Reductive Bioactivation of Antiparasitic Agents. *ACS Infect. Dis.* **2021**, *7* (10), 2777–2786.
- (79) Topatana, W.; Juengpanich, S.; Li, S.; Cao, J.; Hu, J.; Lee, J.; Suliyanto, K.; Ma, D.; Zhang, B.; Chen, M.; Cai, X. Advances in Synthetic Lethality for Cancer Therapy: Cellular Mechanism and Clinical Translation. *J. Hematol. Oncol.* **2020**, *13*, 118.