REVIEW ARTICLE

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De novo Drug Design: An overview

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***De novo* drug design is an iterative process in which the three-dimensional structure of the receptor is used to design newer molecules. It involves structure determination of the lead target complexes and the design of lead modifications using molecular modeling tools. It can also be used to design new chemical classes of compounds that present similar substituents to the target using a template or scaffold, which is chemically distinct from previously characterized leads.**

If a three-dimensional structure of the receptor or the 3D-pharmacophore is known then new lead compounds can be explored by two ways. In one way, a known molecule is allowed to interact with the receptor. This approach commonly referred to as structure-based drug design is usually implemented by searching a database. In another way, entirely new molecules are designed from scratch, an approach commonly referred to as *de novo* drug design.

In *de novo* drug design or *de novo* ligand design, the three-dimensional structure of the receptor or the 3D-pharmacophore is used to design new molecules. There are two basic types of *de novo* design algorithms. The first type of method has been described as 'outside in' method[1]. Here the binding site is first analyzed to determine where specific functional groups might bind tightly. These groups are connected together to give molecular skeletons, which are then converted into 'real' molecules. In the 'inside out' approach, molecules are grown within the binding site, under the control of an appropriate search algorithm with each suggestion being evaluated using and energy function. These two approaches are represented in fig. 1. *De novo* drug design methods can be classified as follows[2-7].

ACTIVE SITE ANALYSIS METHODS

Methods for analysis of the active site do not construct ligands; rather they analyze the properties of the active site, and usually determine favorable binding locations for individual atoms or small fragments. Fig. 2 shows an example of how a typical fragment placement method works. In this example a collection of benzene rings have been placed in a lipophilic pocket of a receptor active site, a collection of formaldehyde molecules have been placed near a hydrogen bond donor site, and several hydroxyl groups have been placed near a hydrogen bond acceptor site. Methods in this category includes GRID[8,9], GREEN[10,11], HSITE[12,13], Multiple Copy Simultaneous Search[14,15] (MCSS), Monte Carlo or simulated annealing based methods[16-18] and HINT[19].

Advantages and disadvantages:

A small number of well-placed fragments, e.g., lipophilic and hydrogen-bonding can provide significant binding energy. As the diversity of reasonable fragments increases, so will the chance of suggesting a synthetically tractable molecule. The one limitation of this approach is that they do not directly propose ligands for testing. A great deal of addition work must be done to convert the fragment locations into a complete ligand.

GRID:

Goodford and his colleagues have developed GRID, which computes the interaction of small organic fragments with an enzyme[8,9]. The method places probes at regularly spaced grid points within the active site and determines the regions with the most favorable scores. Each probe

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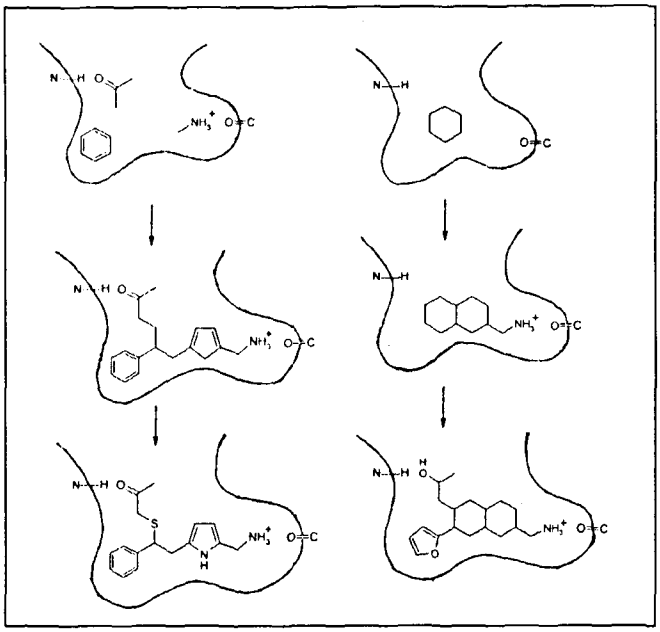

Fig. 1: Approaches to *de novo* design, a) outside in approach and b) inside out method

represents a simple functional group, such as water or methyl, and can be calculated in a few minutes on a typical workstation. Grid has been applied successfully to the design of thymidine synthase inhibitors[20,21].

HINT[19]:

Hydrophobic interactions help to evaluate and visualize the binding interactions between enzyme and ligand. It uses a set of empirical parameters to estimate logP or to produce a hydrophobic field that can be added to CoMFA or other 3D-QSAR treatment. It also allows an estimate of all atom-atom pair-wise interactions between ligand and receptor from which the ligand-binding energy may be estimated. Finally, with the ancillary programs LOCK and KEY it allows the user to map the hydrophobic and polar nature of the active site, as well as the interactions between enzyme and ligand.

WHOLE MOLECULE METHODS

The whole molecule techniques fit ligands into a receptor active site, using either shape complementarity alone, or coupled with electrostatic fitting. Whole molecule methods include DOCK[22,23], perhaps the first computer program in the field of structure based ligand design. DOCK uses a shape fitting approach, searching many possible ways to fit ligands into the receptor active site. Optionally, electrostatics also may be added to the scoring function. It is designed to search through databases containing thousands of molecules. Related techniques use combinations of Monte Carlo and simulated-annealing search approaches, such as AUTODOCK[24,25], or distance geometry[26] approaches to flexibly fit the ligand.

Advantages and disadvantages:

Known or synthesizable compounds are generally studied by these methods. Any hit produced may readily be tested for activity. They may be used to screen large databases of small fragments. These methods have the ability to perform an in-depth analysis of all reasonable binding modes for individual compounds. A disadvantage of these methods is that they are time consuming. Another

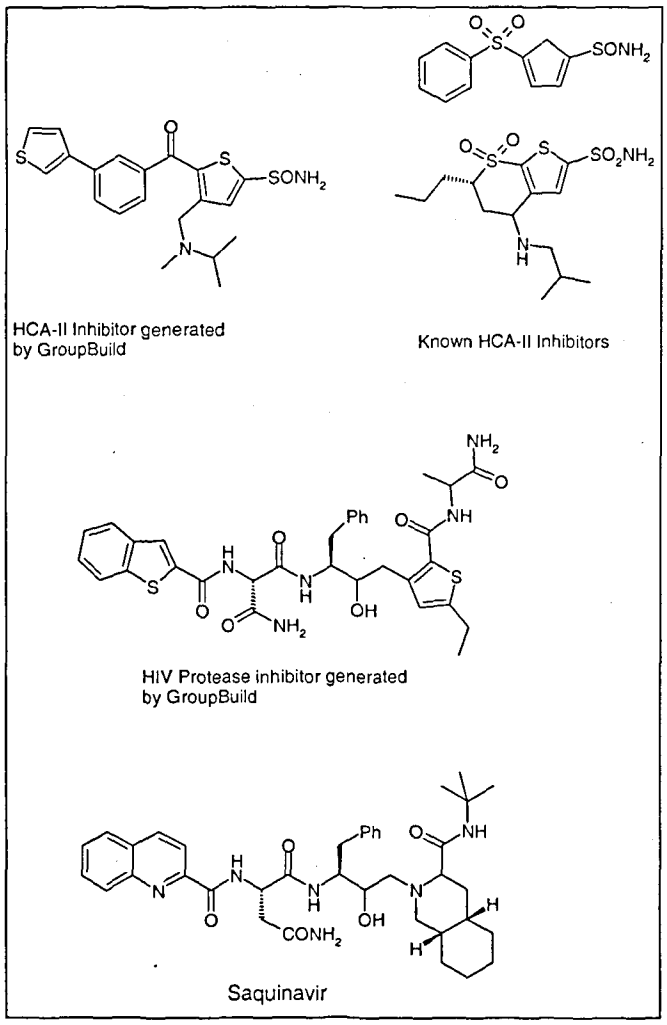

Fig. 2: Inhibitors designed by GroupBuild

disadvantage is that of rigid body, whole molecule fitting is likely to miss many good candidates.

AUTODOCK[24,25]:

AUTODOCK is a conformational search engine, taking a small number of ligands and performing a thorough search of the many conformations that those ligands may adopt. Side chains within the active site also can be allowed to flex.

Distance geometry approaches[26]:

In this method, the active site is first defined by overlapping spheres of variable radii, in a manner similar to DOCK. With use of distance geometry, the ligand is then generated directly in the binding site in a random orientation and conformation. The fit between the ligand and spheres is then optimized, also using a DG method. The ligand is completely flexible during this process; when the minimization is complete, the entire volume of the ligand is inside the collection of active site spheres. The spheres are then removed and the ligand is further refined with a standard force field.

CONNECTION METHODS, SITE-POINT CONNECTION METHODS

A site point is a point in space at which a suitable ligand atom can make favorable interactions with one or more enzyme atoms. For example, in the vicinity of a phenylalanine side chain, there will be several favorable hydrophobic sites. Site points with appropriate ligand atoms nearby are said to be satisfied. Site point connection methods attempt to place small fragments in the active site so that one or more site points are satisfied, and fragments are thereby placed in favorable regions. Site-point connection methods include CLIX[27] and LUDI[28,29].

Advantages and disadvantages

These methods are fast. Another advantage of these methods is their versatility. It is not necessary to match every site point to achieve good binding. A disadvantage is their dependence on proper site- point placement. Ligands, designed to superimpose on poorly selected site points will most likely be poor ligands. If site points need to be matched perfectly, most fragments will miss those site points and be rejected. Another disadvantage is the lack of flexibility of the individual fragments.

CLIX[27]:

It is a hybrid approach that may be viewed as either a site-point or a whole molecule docking method. It uses the output from GRID calculations, carried out with a variety of probes, to characterize the receptor site in terms of an ensemble of favorable binding positions for different groups or fragments. This information is then used to query a chemical database for candidate molecules having good coincidence for individual fragments with members of the ensemble. The receptor is rigid. Binding energy is estimated using the energy information in the GRID interaction energy maps.

LUDI[28,29]:

It is similar to CLIX, is primarily a method for fitting molecular fragments to site points within an active site. LUDI accepts the output from GRID in the same manner as CLIX. LUDI also has the capability to calculate site points suitable for lipophilic interactions or hydrogen bonds. For the enzyme DHFR, placements of key functional groups in the well-known inhibitor methotrexate were reproduced by LUDI.

FRAGMENT CONNECTION METHODS

In the Fragment connection methods, isolated fragment, which have been selected in a variety of ways, are connected. This is done in one step using a single scaffold or linker. This approach relies on the concept that a small number of well-placed fragments, each making favorable interactions with the enzyme is capable of providing a significant overall binding energy. Methods in this category include CAVEAT[30,31], HOOK[32], SPLICE[33], NEWLEAD[34] and PRO_LIGAND[35,36].

Advantages and disadvantages:

Information about favorable fragments locations may be obtained from any source. If one already has a set of candidate fragments placed in the active site, these methods allow one to quickly stitch together those binding elements. In this way pharmacophore hypothesis may be tested. Compounds suggested from these methods can be quite rigid thereby lowering the overall entropy of the system. Many choices of scaffolds are available, ranging from rigid polycyclics to completely flexible hydrocarbon chains. These methods have many disadvantages. They have slow search times for those methods that perform flexible 3D searching or that use very large multi conformational databases. Any scaffold may be rejected if some portion of it overlaps the receptor. Molecules suggested by these methods are generally complicated and thus impractical for the medicinal chemists.

CAVEAT[30,31]:

It is designed to identify scaffolds that can link together any number of isolated ligand fragments. Bonds are treated as vectors, and the method works by comparing the relation between those vectors in the isolated ligand fragments with those of each molecule in the database. It is fast and easy to use.

HOOK[32]:

This method uses molecular skeletons from a database to connect multiple isolated functional groups. Each skeleton has two or more 'hooks' which are specific bonds designated as connection points. The skeletons can be selected from various sources, such as Cambridge structural database, or may be generated *de novo*. Skeletons are treated as rigid, so if a skeleton is actually flexible, it is treated as a set of distinct, rigid conformations. The degree of overlap between the isolated fragments and the skeleton may be controlled by the user. In addition, linkages can occur in several other ways. Functional groups can be linked with unused hooks directly through bond fusion, or an extra methylene group may be used as a spacer to connect the functional group and the hook. After all possible connections have been made between the skeleton and the isolated fragments, the resulting molecule is scored using a simple model.

NEWLEAD[34]:

This automatically generates candidate structures by connecting two isolated ligand fragments with spacers assembled from small chemical entities (atoms, chains, or ring moieties). The building blocks for the connecting linker may be single atoms, library spacers, or fused-ring spacers. The library spacers are used to directly connect two pharmacophoric pieces. The single atom spacers and fused ring spacers are connected to one of the pieces, and the atoms of the spacer are then used for connection to another pharmacophoric piece with a library spacer. For the test cases, known ligands were dissected, key pharmacophoric elements were kept, and the rest of the atoms discarded.

PRO_LIGAND[35,36]:

It employs a design base that contains information about the desired structural features of the ligands. This information may be derived from a model or structure of the receptor, or from a pharmacophore model. PRO_LIGAND can grow a ligand in a continuous, linear fashion, or it can be used to bridge between fragments.

SEQUENTIAL BUILDUP METHODS

The sequential build up techniques based on the philosophy that ligands can be constructed piece by piece. The construction need not be linear, each piece can be added anywhere on the existing ligand. Atom-by-atom approaches include LEGEND[37,38], GenStar[39] & GROWMOL[40]. Fragment-by-fragment approaches include GROW[41], GroupBuild[42] and SPORUT[43,44].

Advantages and disadvantages:

Ligands suggested by these methods are smaller and more efficient. Because each piece is added sequentially, it is possible to perform more detailed conformational analyses, leading to fewer misses. There are disadvantages as well. The most important is the problem of crossing 'dead zones' – open spaces of the active site where few enzyme contacts are possible. Another problem is that they are prone to 'combinatorial explosion'. The last one is the synthetic accessibility.

GrowMol[40]:

It builds ligands one atom or small functional groups at a time in linear fashion. At each step, location, atom or functional group, and torsional angle are randomly chosen. Scoring is based on 'chemical complemetarity' to the receptor, so atoms and groups making good van der Waals contacts or hydrogen bonds are scored highly. Newly grown atoms and groups may be connected to previously generated portions of the same ligand, leading to polycyclic and fused aromatic systems. After this, various post processing steps are followed. Ligands, which are not making a sufficient number of hydrogen bonds and hydrophobic contacts with the enzyme are eliminated. Then, each molecule is energy minimized within the active site, and the strain energy of the bound conformation is used to eliminate compounds that are binding in high energy conformations. Next, the potency of each remaining compound is estimated, using a regression equation derived from the experimental data available for that particular receptor. This equation simply counts the number of hydrophobic contacts and hydrogen bonds between the ligand and the enzyme. Finally the remaining compounds are clustered into families.

GROW[41]:

It is one of earliest de novo design programs, which uses a buildup procedure to determine the best peptide ligand or substrate for a given enzyme. This is designed to avoid the difficult problem of connecting isolated fragments

by using buildup procedures linearly connecting each fragment to the preceding one.

GroupBuild[42]:

It suggests chemically reasonable structures that efficiently fill the active sites of enzymes. These structures are composed entirely of simple functional groups (also known as building blocks or fragments) that the program chooses from a small predefined library. The method was designed to propose molecules in which every fragment provides the greatest degree of steric and electrostatic contact with the enzyme while existing in a low energy conformation. Representative examples are shown in fig. 2.

SPROUT[43,44]:

This is a general purpose program intended to be useful for a range of applications, including ligand design as well as the design of catalysts and agents for asymmetric synthesis. It divides the structure generation process into two phases: primary and secondary structure generation. Primary structure generation produces a 3D molecular graph consistent with the shape of the receptor site and matches target sites (i.e., hydrogen bonding regions). 3D graphs are composed from combinations of templates, which represent common building blocks and may be joined in various ways. A unique collection of templates is called a skeleton. Skeletons, which are composed of hydrocarbon fragments, are scored based on steric contact with the enzyme, the number of rotatable bonds, the strain energy, and so forth. Secondary structure generation is the process of converting the graph into a 'real' structure with appropriate bonds, atom types, etc. Secondary structure generation phase uses information about the active site, such as electrostatics and hydrophobicity.

RANDOM CONNECTION AND DISCONNECTION METHODS

This set of methods contains some features of the sequential buildup procedures, but also includes methods for altering the bond connectivity of the ligand(s) as they are being constructed. This category includes genetic algorithm methods[45], CONCEPTS[46], CONCERTS[47], DLD[48] and MCDNLG[49].

Advantages and disadvantages:

Ability of these methods to explore 'drug space', superior to other methods and, in general, they will generate a more diverse set of suggestions. These methods are capable of generating a huge variety of compounds. This means that within a given set of building blocks and ways to connect them, the problem of finding productive combinations is higher than with other methods.

Genetic Algorithms[45]:

Molecular structures are generated that match an enzyme active site or a pharmacophore model. Either random molecules or known ligands may be used as starting points. The method can be initiated from ethane as the seed molecule, or from a series of fragments randomly selected from a library of common building blocks, including benzene, cyclohexane, naphthalene, and the like. Alternatively, a known starting point may be used, which can be frozen in place or allowed to move: it may also be partially frozen by using additional constraints that penalize any changes to the atomic positions or atom types of the fragment. Several mutation operators are available. Mutations that tend to form hydrogen bond are slightly preferred. After the new molecules are formed, they are 'cleaned up' with molecular mechanics.

CONCERTS[47]:

It uses small organic fragments as the basic building blocks. The method is slow. Ligands generated by this method are not necessarily synthetically accessible, this method definitely falls into the category of idea generators.

MCDNLG[49]:

Monte Carlo *de novo* ligand generator (MCDNLG) method starts with a random collection of atoms packed tightly into the active site of the receptor and slowly anneals it into a chemically stable molecule. Each atom is represented by its element type, hybridization, hydrogen bonding possibilities, and so on. Changes to the ligand are made randomly. Scoring is based on a combination of intra- and intermolecular force-fields terms.

APPLICATIONS

Design of HIV-1 protease inhibitors:

An impressive example of the application of structure-based methods was the design of an inhibitor of the HIV protease. This enzyme is crucial for the replication of the HIV virus and so inhibitors may have therapeutic value as anti-AIDS treatments. The design of orally active HIV-1 protease inhibitors[50-57] has been shown in fig. 3. Design of peptidomimetics, saquinavir[58], ritonavir[59-61] and indinavir[62]; and non-peptidomimetics, nelfinavir[63] are the other examples of this approach.

Design of bradykinin receptor antagonists, aldose reductase inhibitors and catechol-O-methyl transferase (COMT) inhibitors:

Bradykinin receptor antagonists have their utility in the treatment of inflammatory disease. Design of 9-deazaguanine derivatives[64,65] is an example of this approach. Aldose reductase inhibitors[66-69] are used in diabetes mellitus. Examples of this approach are lidorestat and tolrestat[70].

Catechol-O-methyl transferase inhibitors[71,72] are adjuncts to L-dopa therapy of Parkinson's disease. Examples are nitecapone and entacapone.

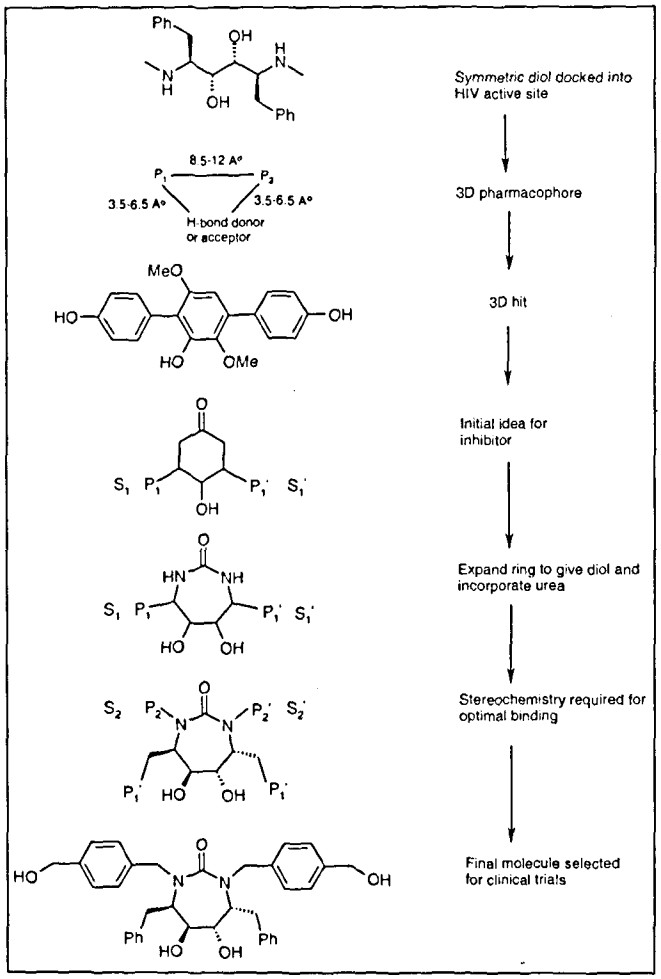

Fig. 3: Flow chart showing the design of novel orally active HIV-1 protease inhibitor

There are a number of applications of this approach in the design of many other categories of compounds like, purine nucleoside phosphorylase inhibitors[73,74]; thrombin inhibitors[75,76]; thymidylate synthase inhibitors[77]; carbonic anhydrase II inhibitors[78,79]; antitrypanosomiasis[80] (sleeping sickness); immunomodulators (immune disease) and anti-influenza viral drugs[81], rhinoviral capsid-binding inhibitors[82,83] (antivirals), estrogen receptor antagonists[84] and antifungal agents[85].

LIMITATIONS

Ligand design methods are still rather slow and inefficient; they generate small numbers of good ideas. Most methods are simplistic; they use simplified models for the ligand receptor system, and simple scoring functions. These methods are hard or impossible for the non-expert to use.

FUTURE PROSPECTS

These methods can be useful in the drug discovery process. In the coming years, it will be important for *de novo* methods to improve in several areas. They must become much faster and efficient; they must use more realistic models for the receptor and better scoring functions; and they must take synthetic accessibility into consideration. A good ligand is not necessarily a good drug. As our understanding of various disciplines like pharmacology, toxicology, metabolism, basic biology etc. becomes more sophisticated, it will, perhaps, be possible to create *de novo* ligand design tools that anticipate some of the downstream development issues and suggest actual drugs.

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