International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

A REVIEW ON DRUG DESIGNING, METHODS, ITS APPLICATIONS AND PROSPECTS.

Gunjan Kalyani*[1],
Dinesh Sharma[1], Yogesh Vaishnav[2], Vishal S. Deshmukh[2]

[1]Royal College of Pharmacy, Raipur
[2]Shri Rawatpura Sarkar Institute of Pharmacy, Behind power grid, Kumhari, Durg, Chhattisgarh.

ABSTRACT

Drug design is an integrated developing discipline which portends an era of tailored drug. It involves the study of effects of biologically active compounds on the basis of molecular interactions in terms of molecular structures or its physico-chemical properties involved. It studies processes by which the drugs produce their effects, how they react with the protoplasm to elicit a particular pharmacological effect or response, how they are modified or detoxified, metabolized or eliminated by the organism. Traditionally, drugs were discovered by synthesizing compounds in a time-consuming multi-step processes against a battery of in vivo biological screens and further investigating the promising candidates for their pharmacokinetic properties, metabolism and potential toxicity. Such a development process has resulted in high attrition rates with failures attributed to poor pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse effects in humans (10%) and various commercial and miscellaneous factors. The review discusses the current trend in drug design is to develop new clinically effective agents through the structural modifications of a lead nucleus. The lead is a prototype compound that has the desired biological or pharmacological activity but may have many undesirable characteristics, like high toxicity other biological activity, insolubility or metabolism problems. Such organic leads once identified, are easy to exploit. This process is rather straightforward. The real test resides with the identification of such lead compounds and the optimum bioactive positions on the basic skeleton of such leads. Today, the process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, de novo design, in vitro, in silico ADMET screening and structure-based drug design.

Correspondence Author

GUNJAN KALYANI

E - 82 Block 7, ACC Colony Jamul, Dist – Durg. Chhattisgarh. PIN – 490024

Email: kalyani.gunjan@yahoo.in

INTRODUCTION

Drugs are the chemicals that prevent disease or assist in restoring health to diseased individuals. As such they play an important and indispensable role in modern medicine. Medicinal chemistry is that branch of science that provides these drugs either through discovery or through design. The classical drugs of antiquity were primarily discovered by empirical observation using substances occurring naturally in the environment. Drugs were also prepared by chemical alteration of natural substances. An increasing understanding of the nature of disease, how cells work, and how drugs influence these processes has led increasingly to the deliberate design, synthesis and evaluation of drug candidate molecules[1].

In earlier days, purely randomized search procedures were involved in the discovery of new drugs. In such methods, the experience and the intuition of medicinal chemists were important factors to reduce the stochastic nature of search techniques. In view of ever increasing number of chemical compounds and in particular the heavier demands to be met by new chemicals randomized search is no longer effective; it is too time consuming; guarantees too little success and is too expensive. The chance of discovering a new agent has diminished to 1 in 10,000 and will decrease even further, whereas development costs have rise to more than 40 dollars per new drug [1]. This necessitated development of a new logical and scientific approach in discovery of a new drug, which is known as Drug design.

Drug design is an integrated developing discipline which portends an era of tailored drug. It involves the study of effects of biologically active compounds on the basis of molecular interactions in terms of molecular structures or its physico-chemical properties involved. It studies processes by which the drugs produce their effects, how they react with the protoplasm to elicit a particular pharmacological effect or response, how they are modified or detoxified, metabolized or eliminated by the organism [1].

Disposition of drugs in individual regions of biosystems is one of the main factors determining the place, place mode and intensity of their action. The biological activity may be “positive” in case of drug design or “negative” in case of toxicology. Thus, drug design involves either total innovation of lead or an optimization of already available lead. These concepts are the building stones upon which the edifice of drug design is built up.

Drug discovery and development is an intense, lengthy and an interdisciplinary endeavor. Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical in vitro and in vivo studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development. For the pharmaceutical industry, the number of years to bring a drug from discovery to market is approximately 12-14 years and costing up to $1.2 - $1.4 billion dollars [1].

Traditionally, drugs were discovered by synthesizing compounds in a time-consuming multi-step processes against a battery of in vivo biological screens and further investigating the promising candidates for their pharmacokinetic properties, metabolism and potential toxicity. Such a development process has resulted in high attrition rates with failures attributed to poor pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse effects in humans (10%) and various commercial and miscellaneous factors. Today, the process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, de novo design, in vitro, in silico ADMET screening and structure-based drug design.

Drug design is the approach of finding drugs by design, based on their biological targets. Typically a drug target is a key molecule involved in a particular metabolic pathway that is specific to a disease condition or pathology, or to the infectivity or survival of a microbial pathogen [1].

Some approaches attempt to stop the functioning of the pathway in the diseased state by causing a key molecule to stop functioning. Drugs may be

designed that bind to the active region and inhibit this key molecule. However these drugs would also have to be designed in such a way as not to affect any other important molecules that may be similar in appearance to the key molecules. Sequence homologies are often used to identify such risks. Other approaches may be to enhance the normal pathway by promoting specific molecules in the normal pathways that may have been affected in the diseased state.

The structure of the drug molecule that can specifically interact with the biomolecules can be modeled using computational tools. These tools can allow a drug molecule to be constructed within the biomolecule using knowledge of its structure and the nature of its active site. Construction of the drug molecule can be made inside out or outside in depending on whether the core or the R-groups are chosen first. However many of these approaches are plagued by the practical problems of chemical synthesis [1].

Newer approaches have also suggested the use of drug molecules that are large and proteinaceous in nature rather than as small molecules. There have also been suggestions to make these using mRNA. Gene silencing may also have therapeutical applications. The current trend in drug design is to develop new clinically effective agents through the structural modifications of a lead nucleus. The lead is a prototype compound that has the desired biological or pharmacological activity but may have many undesirable characteristics, like high toxicity other biological activity, insolubility or metabolism problems. Such organic leads once identified, are easy to exploit. This process is rather straightforward. The real test resides with the identification of such lead compounds and the optimum bioactive positions on the basic skeleton of such leads [1].

Historical perspective [1]

From the prehistoric times until well into the 20th century the vast majority of organic compounds originated from natural materials, often in crude mixtures. In early times, there was no possibility of understanding the nature of disease. Rather discoveries were made & preserved based upon observations of natural phenomena & the consequences of consumption of materials that alleviated distress. Of necessity the progress was disjointed & empirical.

About 100 years ago, the mystery of why only certain molecules produced a therapeutic response was satisfactorily rationalized by the idea of Langley & Ehrlich that only certain cells contained receptor molecules that served as host for the drugs. The resulting combination created a new super molecule that had characteristically new properties producing a response of therapeutic value. One extension of this view was that, the drug was a key that fit the target specifically & productively like a corresponding lock. When fit was appropriate, a positive (agonist) pharmacological action followed analogous to opening a door. In other cases, a different kind of fit blocked the key so that the naturally intended key could not be inserted & antagonist action resulted so that figurative doors could not be opened.

The drug & its receptor (whose molecular nature was unknown when theory was promulgated) were believed to be rigid molecules precrafted to fit one another precisely. Most commonly, receptors are transmembranal glycoprotein accessible from the cell surface whose drug compatible region contains certain specific amino acids arranged in 3D-space.

The main modern difference from the classical picture, other than identifying the chemical nature of the receptor & how it interacts with how ligand, is the realization that neither drug nor the receptor need to be rigid. The opposite extreme to lock & key is the zipper model. In this view, a docking interaction takes place (much as the end of a zipper joins the talon piece) &, if satisfactory complementarity is present, the two molecules progressively wrap around each other & accommodate to each others steric needs.

Earlier it was also noted that enzymes could be modulated for pharmacological benefit. Enzymes share many characteristics with glycoprotein receptors except that they assist in the performance of chemical reactions on their substrates so that the interaction is intrinsically

more information rich than is the receptor –ligand interaction (which leaves the ligand unchanged). Disease frequently results from excessive enzymatic action so selective inhibition of these enzymes of these enzymes is therapeutically useful. Much later it was discovered that other classes of receptors existed.

Overtime it became apparent that DNA & RNA can also be receptors & that the technology needed in order to design ligands for these macromolecules differs in detail from that needed to design ligands for receptors. The earliest applications of DNA ligating lie in inhibiting its formation & function so that cell death was the expected result.

Until the mid 1970s known drug targets were primarily neurotransmitter receptors on the cell surfaces. Since that time, a wealth of information has been uncovered & many other choices are now available. Clearly, cellular receptors & enzymes make up the bulk of the targets favored at this time.

Even with the advantage of all this accumulated knowledge it is certain that many macromolecules must be investigated before a marketable version can be found. Several million compounds must be screened in order to a 1000 or so that have approximately correct characteristics & only a few of these successfully advance through analoging & biotesting to produce a dozen suitable for clinical study. The pace of screening has been accelerated dramatically in recent years. The application of high throughput screening methods has required rapid synthesis of large arrays of compounds suitable for screening. This in turn has led to the introduction & widespread acceptance of combinatorial chemical methods. It is important at this stage to emphasize that priority of discovery is essential not only for valid commercial reasons but also because drugs relieve suffering and delay is undesirable for humanitarian reasons. Thus, we rarely are able to pursue perfection. The motto of medicinal chemists is, instead, 'good enough-soon enough'.

Criteria for compounds to become drugs [2]

In order to be successful, one should know what gold looks like before panning. Drug seeking is analogous – it is essential to have a good idea of what kind of molecules are likely to become drugs before beginning. The normal preferred means of administration of medicaments is oral. Whereas there are no guarantees & many exceptions, the majority of effective oral drugs obey the Lipinski rule of fives. The data upon which this rule rests is drawn from 2500 entries extracted from the US adopted Names, the world Drug lists, & the internal Pfizer compound collections. There are 4 criteria:

1. The substance should have a molecular weight of 500 or less.
2. It should have fewer than 5 hydrogen-bonds donating functions.
3. It should have fewer than ten hydrogen-bond donating functions.
4. The substance should have a calculated Log p between approximately -1 to +5.

In short the compound should have a comparatively low molecular weight, be relatively non- polar & partition between an aqueous & a particular lipid phase in favor of the lipid phase but, at the same time, possess perceptible water solubility. There are many biologically active compounds that satisfy these criteria that fail to become drugs but there are comparatively few successful orally active drugs that fail to fit. Thus, this is a helpful guide but not a law of nature.

These criteria put in semi-quantitative terms a great deal of accumulated observations & rationalizations. For absorption & tissue distribution, a drug must be absorbed through a succession of lipid bilayers before reaching its target. Drugs must be able to pass through barrier rapidly enough to allow therapeutic concentrations to build up. As diffusion is a logarithmic function of size & shape, comparatively compact molecules of modest molecular weight are most suitable.

There must, in addition be sufficient water solubility for dissolution & transport to take place. This correlates reasonably well with the capacity to donate & to accept a moderate number of hydrogen bonds. The semi-quantitative aspects of the Lipinski's rule address the few question of how much is enough. In this sense, the rules embody

useful aspects of Hansch quantitative structure-activity equations.

Methods of lead discovery [2]

There are several approaches which can be employed for lead identification. In order to identify a lead nucleus in a given series, the whole series should be analysed for a particular biological activity. Once the lead is identified, it can be structurally modified to improve its potency. There is a difference between terms, activity and potency. Activity is a particular pharmacological activity while potency is the strength of that effect. Following are some of the important methods which can be used for lead identification:

1. Random screening
2. Non random screening
3. Drug metabolism studies
4. Clinical obsrevations
5. Rational approaches to lead discovery

Random Screening

In this method, all compounds (including synthetic chemicals and natural products of plant, marine and microbial origin) from a given series are tested. Besides the age old examples of morphine, cocaine, digitalis, nicotine, muscarine, tubocurarine, and quinine etc. recently anticancer agent taxol and anti malarial agent artemisinin have been discovered from plant source. Inspite of budgetary and manpower overuse, this method have been used to discover drugs or leads that have unexpected activities.4 Antibiotics like, streptomycin, tetracyclines, fungal metabolites like lovastatin and cyclosporine were found out by this method. Similarly potent anticancer agent, euracin A was obtained from a marine cyanobacterium.

Non Random Screening

It is a modified form of random screening which was developed because of budgetary and manpower restrictions. In this method, only such compounds having similar structural skeleton with that of lead, are tested.

Drug Metabolism Studies

Metabolism of drug occurs as an attempt by metabolizing enzymes to cut short the period of stay of the drug in the body. Structural modifications (i.e. metabolic biotransformation) are done in drug molecule by the enzymes to increase its polarity.

Clinical Observations

Many times drug possesses more than one pharmacological activity. The main activity is called as therapeutic effect while rests of the actions are known as side-effects of the drug. Such drug may be used as lead compound or structural modifications to improve the potency of secondary effects.

Sulphonamide oral hypoglycemic arose directly from the clinical observations, in 1942, that a sulphathiazole derivative, which was being used specifically for treating typhoid, lowered the blood sugar drastically. The pronounced hypoglycemia exerted 5 – isopropyl – 2 sulphanilamido – 1, 3, 4 – thiadiazole indicated that an aryl sulphonyl thiourea moiety ($ArSO_2$ – NH – c (= N) – s) present in thiadiazole is responsible for their blood glucose lowering effect. This observation led to development of carbutamide through of thiazole ring to give a thiourea moiety in which = S was then replaced by = O.

Example: the antihypertensive activity of clonidine was revealed during the clinical trials carried out to verify its predicted vasoconstrictor activity. Sildenafil citrate (Viagra) is yet another example whose penis erectile property was revealed clinical trials to screen its antianginal activity.

Rational approaches to drug discovery

The knowledge about the receptors & their mode of interaction with the drug molecules plays an important role in drug design. This knowledge may be used to develop conformationally bioactive skeletons having exact 3-D complementarity to a receptor. Greater potency, higher selectivity & less adverse effects are expected by reducing flexibility of drug structure.

For example: replacement of a terminal N, N-diethyl amino group by piperidino exploits the decreasing valency angle at the tertiary nitrogen of the latter so that access of the basic group to anionic sites might be improved. This modification leads to the development of major tranquilizers, local anesthetics, antihistaminics & spasmolytics.4 Incorporating a rigid leads to altered

pharmacokinetic & pharmacodynamic features due to altered pka of the amine & lipophilicity of the molecule.

This approach is of great importance in the identification of the lead nucleus. It involves the use of signs & symptoms of the disease. Most diseases, atleast in part, arise from an imbalances may be corrected by agonism or antagonism of a receptor or by inhibition of a particular enzyme. Once the each real site of such imbalance is identified, the natural enzyme substrate or endogenous substance may be used as a lead nucleus.

For example, endogenous hormones, progesterone & oestrogen were used for developing oral contraceptives. The development of an anti inflammatory drug, indomethacin from the lead nucleus, serotonin results at Merck with the belief that serotonin is a possible mediator of inflammation.

Rational drug design (RDD)

Rational drug design is a process used in the biopharmaceutical industry to discover and develop new drug compounds. RDD uses a variety of computational methods to identify novel compounds, design compounds for selectivity, efficacy and safety, and develop compounds into clinical trial candidates. These methods fall into several natural categories – structure-based drug design, ligand-based drug design, de novo design and homology modeling – depending on how much information is available about drug targets and potential drug compounds.

Unlike the historical method of drug discovery, by trial-and-error testing of chemical substances on cultured cells or animals, and matching the apparent effects to treatments, rational drug design begins with a knowledge of specific chemical responses in the body or target organism, and tailoring combinations of these to fit a treatment profile. Due to the complexity of the drug design process two terms of interest are still serendipity and bounded rationality. Those challenges are caused by the large chemical space describing potential new drugs without side-effects.

A particular example of rational drug design involves the use of three-dimensional information about biomolecules obtained from such techniques as x-ray crystallography and NMR spectroscopy. This approach to drug discovery is sometimes referred to as structure-based drug design. The first unequivocal example of the application of structure-based drug design leading to an approved drug is the carbonic anhydrase inhibitor dorzolamide which was approved in 1995.2

Another important case study in rational drug design is imatinib, a tyrosine kinase inhibitor designed specifically for the bcr-abl fusion protein that is characteristic for Philadelphia chromosome-positive leukemias (chronic myelogenous leukemia and occasionally acute lymphocytic leukemia). Imatinib is substantially different from previous drugs for cancer, as most agents of chemotherapy simply target rapidly dividing cells, not differentiating between cancer cells and other tissues.

The activity of a drug at its binding site is one part of the design. Another to take into account is the molecule's druglikeness, which summarizes the necessary physical properties for effective absorption. One way of estimating druglikeness is Lipinski's Rule of Five.

Structure based drug design (SBDD)

Structure-based drug design is one of several methods in the rational drug design toolbox. Drug targets are typically key molecules involved in a specific metabolic or cell signaling pathway that is known, or believed, to be related to a particular disease state. Drug targets are most often proteins and enzymes in these pathways. Drug compounds are designed to inhibit, restore or otherwise modify the structure and behavior of disease-related proteins and enzymes.

SBDD uses the known 3D geometrical shape or structure of proteins to assist in the development of new drug compounds. The 3D structure of protein targets is most often derived from x-ray crystallography or nuclear magnetic resonance (NMR) techniques. X-ray and NMR methods can resolve the structure of proteins to a resolution of a few angstroms (about 500,000 times smaller than

the diameter of a human hair). At this level of resolution, researchers can precisely examine the interactions between atoms in protein targets and atoms in potential drug compounds that bind to the proteins. This ability to work at high resolution with both proteins and drug compounds makes SBDD one of the most powerful methods in drug design.

Let's consider how SBDD methods have been used in designing drugs for a well known cancer-related protein complex. Two protein targets that have been studied extensively in cancer research are p53 and MDM2. These two proteins form a single p53-MDM2 complex as part of a cell-signaling pathway that regulates cell division. Mutated forms of p53-MDM2 result in various forms of tumors and cancers. Several decades of research have been aimed at designing small-molecule compounds that restore the normal function of p53-MDM2, and consequently reduce or eliminate certain forms of cancer.

One well-known anticancer drug - "nutlin" - has been developed by Roche Pharmaceuticals to restore the normal functioning of MDM2. (Technically, "nutlin" goes by the name cis-[4, 5-bis-(4-bromophenyl)-2-(2-ethoxy-4-methoxyphenyl)-4,5-dihydroimidazol-1-yl]-[4-(2 hydroxyethyl)piperazin-1-yl]methanone, but let's not get into naming conventions).

SBDD methods played an important role in this development. Figure 1 shows the 3D structure of MDM2 as derived from x-ray crystallography studies. MDM2's backbone structure and amino acid side chains are shown in multicolored stick form. Five separate "nutlin" molecules are also shown docked along the protein's surface and deeply embedded in hydrophobic pockets and cavities.

Docking ligands

One of the key benefits of SBDD methods is the exceptional capability it provides for docking putative drug compounds (ligands) in the active site of target proteins. Most proteins contain pockets, cavities, surface depressions and other geometrical regions where small-molecule compounds can easily bind.

With high-resolution x-ray and NMR structures for proteins and ligands, researchers can show precisely how ligands orient themselves in protein active sites. Open source bioinformatic tools such as VMD and NAMD, for example, help scientists examine multiple binding poses to determine which orientation is most likely to occur.

Furthermore, it's well known that proteins are often flexible molecules that adjust their shape to accommodate bound ligands. In a process called molecular dynamics, SBDD allows researchers to dock ligands into protein active sites and then visualize how much movement occurs in amino acid side chains during the docking process.2 In some cases, there is almost no movement at all (i.e., rigid-body docking); in other cases, such as with the HIV-1 protease enzyme, there is substantial movement. Flexible docking can have profound implications for designing small-molecule ligands so this is an important feature in SBDD methods.

Lead optimization [2]

After a number of lead compounds have been found, SBDD techniques are especially effective in refining their 3D structures to improve binding to protein active sites, a process known as lead optimization. In lead optimization researchers systematically modify the structure of the lead compound, docking each specific configuration of a drug compound in a protein's active site, and then testing how well each configuration binds to the site. In a common lead optimization method known as bioisosteric replacement, specific functional groups in a ligand are substituted for other groups to improve the binding characteristics of the ligand. With SBDD researchers can examine the various bioisosteres and their docking configurations, choosing only those that bind well in the active site. A few examples of bioinformatics tools that aid in lead optimization efforts are BIOSTER, WABE, and ClassPharmer Suite.

Once the lead nucleus is identified, it is easy to exploit. This process is rather straight forward. Various approaches are employed in order to improve the desired pharmacological properties of the lead nucleus. Important amongst them are:

1. Identification of active part (pharmacophore).
2. Functional group optimization.
3. Structure – activity- relationship studies.
4. Homologation.
5. Cyclization of side chain.
6. Bioisosterism.

Identification of active part (pharmacophore)

Any drug molecule consists of both, essential parts & non-essential parts. Essential part is important in governing pharmacodynamic (drug- receptor interactions) property while non-essential part influences pharmacokinetic features. The relevant groups on molecule that interact with a receptor are known as bioactive functional groups. They are responsible for the activity. The schematic representation of nature of such bioactive functional groups along with their inter atomic distances is known as pharmacophore. Once such pharmacophore is identified, structural modifications can be done to improve pharmacokinetic properties of the drug.4

Example: the presence of phenyl ring, asymmetric carbon, Ethylene Bridge, & tertiary nitrogen are found to be minimum structural requirement for a narcotic analgesic to become active. Similarly the presence of two anionic sites & one cationic site must be present in cholinergic agent. Morphine the prototype narcotic agent has a pentacyclic structure. The complexity of structure leads to appearance of several adverse effects. Hence the pharmacophore of morphine of morphine has been recognized through molecular dissection & was used to develop simpler & even acyclic analogs. For example, methadone is as potent as analgesic as morphine.4

Functional group optimization

The activity of drug can be correlated to its structure in terms of the contribution of its functional groups to the lipophilicity, electronic features of the drug skeleton. Hence, by selecting proper functional group, one can govern the drug distribution pattern & can avoid the occurrence of side effects.

For example: the amino group of carbutamide (antibacterial agent) was replaced by a methyl group to give tolbutamide (anti diabetic agent), similarly, removal of sulphonamide side-chain of chlorthiazide (an anti hypertensive drug with diuretic activity) helped to design diaoxide (an antihypertensive drug without diuretic activity).

Structure – Activity- Relationship studies

The physiological action of molecule is a function of its chemical constitution. This observation is the basis of SAR - studies. A SAR study usually involves the interpretation of activity in terms of the structural features of a drug molecule. Generalized conclusions then can be made after examining a sufficient number of drug analogs. For example, sulphonamides are found to be associated with diuretic & anti diabetic activities in addition to their antibacterial activity.

Because of hepatotoxic side-effects of hydarzines & hydrazides, structurally diversified compounds were synthesized resulting into the introduction of pargyline & tranylcypromine. Tranylcypromine was developed as a structural analog of amphetamine & is used as an antidepressant agent. Due to pronounced effect on blood pressure, the former was used as an antihypertensive agent. Further structural modification of pargyline skeleton resulted in to cyclogyline.

Homologation

The variation in the substituent can be used to increase or decrease the polarity, alter the pKa, & change the electronic properties of a molecule. Exploration of homologous series is one of the most often method used method to induce these changes in a very gradual manner.

A homologous series is a group of compounds that differ by a constant unit, generally a CH2 group. For example, the alkyl substituent of ethers, amines & amides are easily varied. Such variations help to judge the depth 7 width of hydrophobic cavity present in the target receptor.

Usually increasing the length of a saturated carbon side-chain form one (CH_3) to 5 to 9 atoms (pentyl to nonyl) produces an increase n pharmacological effects. Further increase results in decrease of activity.

This probably is either due to increase in lipophilicity beyond optimum value (decreased absorption & distribution) or decrease in

concentration of free drug (micelle formation).For example, maximum hypnotic activity is seen from 1 –hexanol to 1 –octanol. Therefore activity decreases for higher analogs.

Cyclization of side chain

Over simplification of the structure may sometimes be responsible for increased side effects & reduced activity or selectivity. Two straight forward strategies for enhancing binding affinity involve reducing conformational flexibility & incorporating substituents that bring additional binding interaction. Change in potency or in the activity spectra can be brought about by transformation of the alkyl side – chain into cyclic analogs.

For example: chlorpromazine has more neuroleptic activity than its cyclic analog. While in prochlorperazine, the antiemetic activity is greatly enhanced. Similarly partial rigidity may be incorporated by introducing a double bond, alkyne, amide or aromatic ring in a flexible side –chain.

Bioisosterism

Bioesters are the substituents or groups that have similar physical or chemical properties & hence similar biological pattern. Isosteric groups are isoelectronic in their outermost electron shell. Bioisosteric replacement may help to decrease toxicity or to change activity spectra. It may also alter the metabolic pattern of the drug. The parameters being changed are molecular size, steric shape, (bond angles etc.) electron distribution, lipid solubility, water solubility, the pKa, the chemical reactivity to cell compartments, capacity to undergo H- bonding (receptor interaction). Even if the Bioisosteric replacement is relatively minor (cl for CH_3 or vice Versa), cl may block hydroxylation, whereas CH_3 may be bio oxidized & the compound may have shorter half life. For example, tolbutamide (R = CH_3) has shorter half life than chlorpropramide (R = Cl).

Erlenmeyer defined isosters as atoms, ions or functional groups in which the peripheral layers of electrons can be considered identical. These are known as Classical bioesters. Here same valency & biological activity are important. However it is the retention of same biological activity which determines whether a group is bioisostere & not the valency. Hence non–isosteric groups can also be used as bioisosteres. They are known as non-classical bioisosteres. They do not have same number of atoms & do not fit the steric & electronic rules of classical bioisostere. But they do not produce a similarity in biological activity. Example, F has the same size as that of H2 but is more electronegative. Hence, it is used as an isosterene of hydrogen to vary the electronic properties of the drug without changing steric parameters.

More recently, Burger divided bioisostere as:

1. classical bioisosteres

(a) Univalent atoms & groups, ex: Cl, Br, NH_2, etc.

(b) Bivalent atoms and groups, ex: R-O-R, R-NH-R, R-S-R, etc.

(c) Trivalent atoms & groups; ex: -CH =, -N=, -P=, etc.

(d) Tetravalent atoms; ex: =C=, =P=, etc.

(e) Ring equivalents; ex: -CH = CH-, -S-, -CH=, -CH_2, etc.

2. non classical bioisosteres

(a) Halogens; ex: Cl, F, CN

(b) Ethers; ex: --S--, --O--, etc.

(c) carbonyl group; ex: >C=O, >S=O

(d) Carboxylic acid group; ex : –COOH, etc.

(e) Hydroxy group; ex: -OH etc.

(f) Catechol

(g) Thiourea

(h) Spacer group; ex: -$(CH_2)_3$ etc.

(i) Ionizing analogs; ex: Ar – OH+, Ar N+HSO_2 etc.

Application of bioisosterism in drug design

1. An important compound from catecholamine series is phenylephrine in which phenolic hydroxyl group takes part in H- bonding with bioactive site on the receptor. The hydroxyl group can be replaced by other group having ability to undergo H- bonding. Hence alkylsulphaniamido derivative of phenylephrine was found to retain activity.
2. Bioisosteric analogs are in neuroleptic category.
3. Bioisosteric analogs are in anti-inflammatory category.
4. A classical example of ring versus non-cyclic structure is diethylstilbestrol & estrogen (17β-estradiol).

5. Bioisosterism in anti-histaminic agents R-X-(CH_2)-Y, X= NH, O, CH_2 where, Y= N $(CH_3)_2$.
6. The non – thiazide category of diuretic agents has been developed by replacing ring SO_2 by carbonyl group. e.g., Quinazolinone derivative.
7. Metaclopramide shares features of both anticholinergic (-O- is bioisosterically replaced by NH) & antidopaminergic (anti emetic) agents. It is in fact used as antiulcer agent.
8. Pirenzipine, an antimuscarinic agent possesses structural similarity with tricyclic antidepressant agents. However it lacks antidepressant activity due to its poor penetration ability in CNS. Hence other tricyclic antidepressant agents (Doxepin & Trimipramine) are undergoing clinical investigations for antiulcer activity.
9. Replacement of the imidazole ring (prone to metabolism) of the antifungal agent ticonazole with a 1, 2, 4-triazole ring leads to fluconazole having improved stability.

Objectives behind lead optimization [2]

1. To maximize the drug's desired activity.
2. To minimize the intensity & frequency of side-effects associated with the drugs. Drugs usually have multiple pharmacological effects & the drug designer tries to improve selectivity of action. The aim should be to optimize the activity & not only to maximize the activity.

Prodrug designing

A prodrug is a pharmacological substance (drug) that is administered in an inactive (or significantly less active) form. The term prodrug was coined by Albert. Once administered, the prodrug is metabolised in vivo into an active metabolite. The rationale behind the use of a prodrug is generally for absorption, distribution, metabolism, and excretion (ADME) optimization. Prodrugs are usually designed to improve oral bioavailability, with poor absorption from the gastrointestinal tract usually being the limiting factor.

Additionally, the use of a prodrug strategy increases the selectivity of the drug for its intended target. An example of this can be seen in many chemotherapy treatments, in which the reduction of adverse effects is always of paramount importance. Drugs used to target hypoxic cancer cells, through the use of redox-activation, utilize the large quantities of reductase enzyme present in the hypoxic cell to convert the drug into its cytotoxic form, essentially activating it. As the prodrug has low cytotoxicity prior to this activation, there is a markedly lower chance of it "attacking" healthy, non-cancerous cells which reduces the side-effects associated with these chemotherapeutic agents.

In rational drug design, the knowledge of chemical properties likely to improve absorption and the major metabolic pathways in the body allows the modification of the structure of new chemical entities for improved bioavailability. Sometimes the use of a prodrug is unintentional, however, especially in the case of serendipitous drug discoveries, and the drug is only identified as a prodrug after extensive drug metabolism studies. Some prodrugs, such as Codeine and Psilocybin, also occur naturally.

Prodrug thus may be considered as drug containing specialized non-toxic protective group utilized in a transient manner to alter or to eliminate undesirable properties in the parent drug. Prodrug designing is required to overcome many formulations pharmacokinetic or pharmacodynamic drawbacks. The prominent include:

1. Unpleasant taste or odour (gastric irritation).
2. Wide range of adverse effects.
3. Shorter duration of action.
4. Instability.
5. Site non-specificity.
6. Poor absorption or distribution.
7. Some active compounds are unable to reach site of action (GABA).

Types of prodrug

1. Non intentional prodrug.
2. Carrier–linked prodrug.
3. Bioprecursor.

Non intentional prodrug

Sometimes, after administration of the drug the metabolic studies indicate the prodrug nature. It becomes accidentally evident that the activity of drug is because of its metabolite & because of the

parent drug. Examples include; anti inflammatory agent, Sulindac.

Carrier linked prodrug

It is a compound that contains an active drug linked to a carrier group that can be removed enzymatically, such as an ester which is hydrolyzed to an active carboxylic acid containing drug. The carrier group must be non-toxic & biologically inactive when detached from the drug. T should be removed easily to allow the active drug to be released in vivo. The most common reaction for activation of carrier prodrug is hydrolysis. A simple hydrolysis cleaves the transport moiety at adequate rate (example: Bacampicillin, progabide).

Activated amides, generally of low basicity amines or amides of amino acids are more susceptible to enzymatic cleavage. Phenyl carbamates (R NHCO2Ph) can also be used as prodrugs because of their susceptibility to the attack of plasma enzymes. The anticonvulsant action of progabide is a prodrug form of γ-aminobuyric acid, an important inhibitory neurotransmitter. Its lipophilicity helps it to cross blood-brain-barrier (BBB). Once it enters CNS, it is hydrolyzed to GABA.

Bioprecursor

The bioprecursor does not contain a temporary linkage between the active drug & a carrier moiety, but designed from a molecular modification of the active principle itself. It is a compound that is converted to active drug through metabolic biotransformation.4 For example: if the drug contains a carboxylic acid group, the bioprecursor may be a primary amine which is metabolized by oxidation to the carboxylic acid drug. (Example: Fenbufen, Phenylbutazone, Acetanilide etc.).

Similarly pyrrolines are the bioprecursors of GABA & its analogs. N-alkylaminobenzophenons were designed to get in vivo benzodiazepines by N-dealkylation of tertiary amines & ring enclosure. The linkage between the drug substance & the transport moiety is usually a covalent bond. Sulindac, a non-steroidal anti-inflammatory bioprecursor, gets converted to the sulphide metabolite (active drug) via sulphone.

Application of prodrug concept

1. Increasing absorption of drugs: example: ampicillin esters.
2. Improve site specific delivery; example: epinephrine.
3. Prolongation of drug action; example: testosterone.
4. Decreased side-effects & toxicity; example: NSAID.
5. Improve taste & color; example: Chloramphenicol palmitate.
6. Delivery to brain; example: dopamine to L-dopa. L-dopa to its methyl ester.

Drawback of prodrug approach

1. The prodrug generates toxic metabolites which are not generated by parent drug.
2. Increased consumption of glutathione during conversion of prodrug to active metabolite may leave vital cells unprotected.
3. The inert carrier moiety could not remain inert & leads to the formation of toxic metabolites.
4. The prodrug or/ & carrier moiety generate such metabolites which alter the pharmacokinetic features of the parent drug by either inducing metabolic enzymes or by competing the active drug for binding with plasma-proteins.

Examples

1. Carisoprodol is metabolized into Meprobamate. Carisoprodol is not a controlled substance in the United States, but Meprobamate is classified as a potentially addictive controlled substance that can produce dangerous and painful withdrawal symptoms upon discontinuation of the drug. Meprobamate is a sedative/tranquilizer, and Carisoprodol is also classified as a sedative/tranquilizer. It is often prescribed as a muscle relaxant.
2. Enalapril is converted by esterase to the active enalaprilat.
3. Valacyclovir is converted by esterase to the active acyclovir.
4. Fosamprenavir is hydrolysed to the active amprenavir.
5. Levodopa is converted by DOPA decarboxylase to the active dopamine.
6. Chloramphenicol succinate ester is used as intravenous prodrug of chloramphenicol,

because pure chloramphenicol does not dissolve in water.
7. Psilocybin is dephosphorylated to the active psilocin.
8. Heroin is deacetylated by esterase to the active morphine.
9. Codeine is demethylated by the liver enzyme CYP2D6 to the active morphine, as well as several other compounds that may be active in analgesia.

Soft drug concept [1, 2]

Soft drug design represents a new approach aimed to design safer drugs with an increased therapeutic index by integrating metabolism considerations into the drug design process. Soft drugs are new therapeutic agents that undergo predictable metabolism to inactive metabolites after exerting their therapeutic effect. Hence, they are obtained by building into the molecule, in addition to the activity, the most desired way in which the molecule is to be deactivated and detoxified.

In an attempt to systematize and summarize the related work done in a number of laboratories, including ours, the present review presents an overview of the general soft drug design principles and provides a variety of specific examples to illustrate the concepts. A number of already marketed drugs, such as esmolol, remifentanil, or loteprednol etabonate, resulted from the successful application of such design principles.

Many other promising drug candidates are currently under investigation in a variety of fields including possible soft antimicrobials, anticholinergics, corticosteroids, -blockers, analgetics, ACE inhibitors, antiarrhythmics, and others. Whenever possible, pharmacokinetic and pharmacodynamic properties are briefly summarized and compared to those of other compounds used in the same field.

The application of concept of 'soft drugs' is necessary to overcome & to improve:

1. Pharmacokinetic insufficiencies.
2. Transportability.
3. Site specificity.

The soft drugs are defined as "therapeutically beneficial agents characterized by a predictable & controllable in vivo metabolism to non-toxic moieties, after they achieve therapeutic role. The site specific delivery via chemical modifications involves the design of a soft drug from an inactive metabolite. The designed drug is then transformed by facile & predicted routes of metabolism ultimately resulting in the delivery of the active drug at the expected site of action. The concept was successfully applied to local delivery of steroids, drugs acting on specific areas in the eye, brain & testes.

The concept of soft drug may be applied to develop selective & safer ocular drug delivery systems for the treatment of glaucoma, ocular inflammations & infections. It was found by Bodor et al. in 1978 that diester derivatives of adrenolone have high level of ocular sympathomimetic activity due to conversion of former to adrenaline via a combined reduction-hydrolysis process in the eyes. Using same approach, tertbutaline was generated selectively in the iris-ciliary tissues by the action of reductases & esterases on ketone diester precursors of tertbutaline.

Similarly, propranolol is generated at the iris-ciliary body by the action of esterases & reductases on topically applied keto-oxime derivative of propranolol. The soft drug concept was utilized to develop loteprednol etabonate, a topical anti-inflammatory & anti-allergic agent. It is locally potent but systemically safe.

In silico based drug design [1,2]

In silico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding/ active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics.

The use of computers and computational methods permeates all aspects of drug discovery today and forms the core of structure-based drug design. High-performance computing, data management software and internet are facilitating the access of huge amount of data generated and transforming the massive complex biological data into workable

knowledge in modern day drug discovery process. The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process, from the identification of novel targets and elucidation of their functions to the discovery and development of lead compounds with desired properties. Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost. Major roles of computation in drug discovery are;

(1) Virtual screening & de novo design,

(2) In silico ADME/T prediction and

(3) Advanced methods for determining protein-ligand binding.

Significance

As structures of more and more protein targets become available through crystallography, NMR and bioinformatics methods, there is an increasing demand for computational tools that can identify and analyze active sites and suggest potential drug molecules that can bind to these sites specifically. Also to combat life-threatening diseases such as AIDS, Tuberculosis, Malaria etc., a global push is essential. Millions for Viagra and pennies for the diseases of the poor is the current situation of investment in Pharma R&D. Time and cost required for designing a new drug are immense and at an unacceptable level. According to some estimates it costs about $880 million and 14 years of research to develop a new drug before it is introduced in the market Intervention of computers at some plausible steps is imperative to bring down the cost and time required in the drug discovery process.

The crystal structure of a ligand bound to a protein provides a detailed insight into the interactions made between the protein and the ligand. Structure designed can be used to identify where the ligand can be changed to modulate the physicochemical and ADME properties of the compound, by showing which parts of the compound are important to affinity and which parts can be altered without affecting the binding.3 The equilibrium between target and ligand is governed by the free energy of the complex compared to the free energy of the individual target and ligand. This includes not only the interaction between target and ligand but also the solvation and entropy of the three different species and the energy of the conformation of the free species.

In silico ADME/T prediction

The phrase “drug-like” generally means molecules which contain functional groups and/or have properties consistent with the majority of known drugs. Lead structures are ligands that typically exhibit suboptimal target binding affinity. Studies have shown that there exists a difference between leads and drugs which can be expressed as follows: Lead structures exhibit, on average, less molecular complexity (less molecular weight, less number of rings and rotatable bonds), are less hydrophobic (lower ClogP and LogD74) and have lower polarizability (less CMR). Leads should display the following properties to be considered for further development in the drug discovery process or to be called as “drug-like”:

1. Relatively simple chemical features, amenable for combinatorial and medicinal chemistry optimization efforts;
2. Membership to a well established SAR (structure-activity relationship) series, wherein compounds with similar structures exhibit similar target binding affinity;
3. Favorable patent situation; and
4. Good ADME (absorption, distribution, metabolism and excretion) properties.

Leads discovered using virtual screening and de novo design methodologies needs to be optimized to produce candidates with improved bioavailability and low toxicity. Studies have indicated that poor pharmacokinetics and toxicity are the most important causes of high attrition-rates in drug development and it has been widely accepted that these areas should be considered as early as possible in the drug discovery process, thus improving the efficiency and cost-effectiveness of the industry. Resolving the pharmacokinetic and toxicological properties of drug candidates remains a key challenge for drug developers. Evaluation of drug-likeness involves prediction of ADMET (absorption, distribution, metabolism, excretion,

toxicity) properties and these predictions can be attempted at several levels:

1. In vitro–in vivo using data obtained from tissue or recombinant material from human and pre-clinical species.
2. Inter-species, in vivo-in vivo using data from pre-clinical species.
3. In silico or computational predictions projecting in vitro or in vivo data.

In silico prediction of drug-likeness at an early stage involves evaluation of various ADMET properties using computational approaches like QSAR or molecular modeling. A number of studies have been performed to find out the properties which make a drug distinct from other chemicals. Availability of large databases of drug or drug-like molecules, e.g. CMC (Comprehensive Medicinal Chemistry), MDDR (MACCS-II Drug Data Report), and WDI (World Drug Index) provides useful information about the properties of drugs.

The most influential study of "Lipinski's rule-of-five" identifies several critical properties that should be considered for compounds with oral delivery as concern. A deeper understanding of the relationships between important ADME parameters and molecular structure and properties is needed to develop better in silico models to predict ADMET properties. Some of the ADME properties evaluated using in silico models are; intestinal permeability, aqueous solubility, human intestinal absorption, human oral bioavailability, active transport, efflux by P-glycoprotein, blood-brain barrier permeation, plasma protein binding, metabolic stability, interactions with cytochrome P450s and toxicity.

Computer assisted design [1, 2]

Computer-assisted drug design (CADD), also called computer-assisted molecular design (CAMD), and represents more recent applications of computers as tools in the drug design process. In considering this topic, it is important to emphasize that computers cannot substitute for a clear understanding of the system being studied. That is, a computer is only an additional tool to gain better insight into the chemistry and biology of the problem at hand.

In most current applications of CADD, attempts are made to find a ligand (the putative drug) that will interact favorably with a receptor that represents the target site. Binding of ligand to the receptor may include hydrophobic, electrostatic, and hydrogen-bonding interactions. In addition, solvation energies of the ligand and receptor site also are important because partial to complete desolvation must occur prior to binding.

This approach to CADD optimizes the fit of a ligand in a receptor site. However, optimum fit in a target site does not guarantee that the desired activity of the drug will be enhanced or that undesired side effects will be diminished. Moreover, this approach does not consider the pharmacokinetics of the drug. The approach used in CADD is dependent upon the amount of information that is available about the ligand and receptor.

Ideally, one would have 3-dimensional structural information for the receptor and the ligand-receptor complex from X-ray diffraction or NMR. The ideal is seldom realized. In the opposite extreme, one may have no experimental data to assist in building models of the ligand and receptor, in which case computational methods must be applied without the constraints that the experimental data would provide.

Based on the information that is available, one can apply either ligand-based or receptor-based molecular design methods.

The ligand-based approach is applicable when the structure of the receptor site is unknown, but when a series of compounds have been identified that exert the activity of interest. To be used most effectively, one should have structurally similar compounds with high activity, with no activity, and with a range of intermediate activities. In recognition site mapping, an attempt is made to identify a pharmacophore, which is a template derived from the structures of these compounds. It is represented as a collection of functional groups in three-dimensional space that is complementary to the geometry of the receptor site.

In applying this approach, conformational analysis will be required, the extent of which will be dependent on the flexibility of the compounds

under investigation. One strategy is to find the lowest energy conformers of the most rigid compounds and superimpose them. Conformational searching on the more flexible compounds is then done while applying distance constraints derived from the structures of the more rigid compounds. Ultimately, all of the structures are superimposed to generate the pharmacophore. This template may then be used to develop new compounds with functional groups in the desired positions. In applying this strategy, one must recognize that one is assuming that it is the minimum energy conformers that will bind most favorably in the receptor site. In fact, there is no *a priori* reason to exclude higher energy conformers as the source of activity.

The receptor-based approach to CADD applies when a reliable model of the receptor site is available, as from X-ray diffraction, NMR, or homology modeling. With the availability of the receptor site, the problem is to design ligands that will interact favorably at the site, which is a docking problem.

An example of CADD [1]

Carbonic anhydrase

Carbonic anhydrase catalyzes the reaction $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$, the hydration of some aldehydes and ketones, and the hydrolysis of alkyl and aryl esters. It is a zinc-containing enzyme of about 30,000 daltons, and the three-dimensional structure has been characterized by X-ray diffraction. Physiologically, carbonic anhydrase is involved in gastric, urinary, pancreatic, lacrimal, and cerebrospinal secretions. Inhibitors of carbonic anhydrase include aromatic and heterocyclic sulfonamides, and some of these compounds have found application as diuretics.

Both traditional QSAR and computer graphical methods have been applied to the development of sulfonamides and other compounds as inhibitors of carbonic anhydrase. For example, Hansch et al developed a QSAR based on the binding constants of 29 phenylsulfonamides to the enzyme. The equation that was derived was the following:

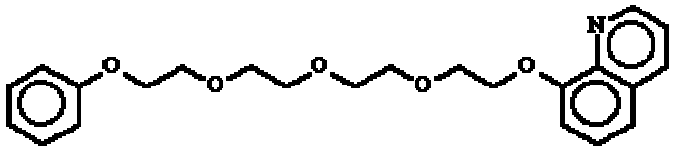
$$\log K = 1.55\sigma + 0.64 \log P - 2.07I_1 - 3.28I_2 + 6.94$$

where K is the binding constant, I1=1 if X is meta and 0 otherwise, and, I2 = 1 if X is ortho and 0 otherwise.

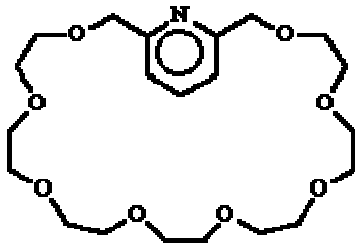
The negative coefficients of I1 and I2 suggest that they account for unfavorable steric effects when substituents are in the meta or ortho positions. Binding is favored by electron-withdrawing substituents, which is consistent with the hypothesis that the ionized form of $-SO_2NH_2$ binds to the zinc in the active site of carbonic anhydrase.

The active site is a cavity approximately 12 Angstroms deep with a zinc atom (magenta) near the bottom of the cavity. The active site is divided into a hydrophilic half (blue) and a hydrophobic half (red). In the complex, the inhibitor appears to be bound such that the sulfonamide moiety occupies the fourth coordination site of the zinc atom, with the other three sites being occupied by histidine residues. For subsequent discussion, note that the active site is much larger than is required to accommodate an inhibitor of this size.

Receptor-based drug design incorporates a number of molecular modeling techniques, one of which is docking. The Kuntz research group applied their DOCK program to the identification of compounds that may inhibit carbonic anhydrase. Structures of two of the candidates are shown below.



1-phenoxy-11-(8-quinolyloxy)-3,6,9-trioxaundecane



2,6-pyrido-27-crown-9

These molecules are considerably larger than the arylsulfonamides that traditionally are used as carbonic anhydrase inhibitors. In fact, no

arylsulfonamides were identified as potential inhibitors in this study. These results probably arise because scoring of candidates was based on the size and shape of the molecules. These large candidates can engage in a greater number of favorable interactions within the large carbonic anhydrase active site than can the smaller arylsulfonamides. More recent versions of DOCK allow scoring based on force fields, which include both van der Waals and electrostatic interactions. These results with DOCK illustrate the potential for programs such as this one to search objectively for ligands than are complementary to receptor sites, thereby assisting researchers in identifying potential drugs than may be considerably different from existing drugs. As yet, the efficacy as drugs of these candidates identified by DOCK has not been demonstrated.

Intelligent drug design [1]

Intelligent drug design, also known as molecularly targeted drug design, is a new way to confront cancer. It involves altering existing drugs or building entirely new drugs that will zero in on those distinct mutations. Current anticancer drugs attack cancer cells based on features that are also found in normal cells. The drugs therefore kill normal cells, too, causing side effects and limiting their use in patients.

By designing drugs that attack only the culprit mutations, these 'intelligent drugs' will leave normal cells unharmed, so patients should experience less toxicity and fewer side effects from the drug.

Examples of designed drugs [1]

1. Cimetidine, the prototypical H2-receptor antagonist from which the later members of the class were developed.
2. Dorzolamide, a carbonic anhydrase inhibitor used to treat glaucoma.
3. Many of the typical antipsychotics.
4. Selective COX-2 inhibitor NSAIDs.
5. SSRIs (selective serotonin reuptake inhibitors), a class of antidepressants.
6. Zanamivir, an antiviral drug.
7. Enfuvirtide, a peptide HIV entry inhibitor.
8. Probenecid.
9. Non benzodiazepines like Zolpidem and Zopiclone.

ACKNOWLEDGEMENTS

Author (Gunjan Kalyani) would sincerely thank Principal, my teacher **Vishal S. Deshmukh Sir, Yogesh Vaishnav Sir,** and Ajit Pandey Sir, staff & lab technicians SRIP, Kumhari, Durg, C.G. for their contribution in carrying forward the research work, for providing the necessary support, for his kind and valuable guidance, and for providing basic facilities in lab.

REFERENCES

1. http://www.google.co.in/wikipedia.com
2. Kadam S. S., Mahadik K. R., Bothara K. G., Principles of Medicinal chemistry, 2006 edition, Nirali prakashan, Pune, Pg-393 to 420.
3. Thomas J. Perun, Catherine Lamb Propst, Computer-aided Drug Design: Methods and Applications, Published by Informa Health Care, 1989 editon, pg - 19.
4. Povl Krogsgaard-Larsen, Tommy Liljefors, Text book of drug design & discovery Ulf Madsen, Third Edition 2006 edition, pg- 1 to 35, 93,127.
5. Greer J, Erickson JW, Baldwin JJ, Varney MD (1994). "Application of the three-dimensional structures of protein target molecules in structure-based drug design". J Med Chem. 37 (8): 1035–1054.
6. Silverman B. Richard, Organic chemistry of drug design & drug action, Academic press, second edition 2004, pg – 1 to 29,51,66,75.
7. Valentino J. Stella, Ronald T. Borchardt, Michel I. Hageman, Rezr Oliyai, Prodrug Challenges & rewards, part-1, Springer, AAPS press, Pg-3.
8. PAREXCEL'S pharmaceutical R & D statistical source book, 2001, pg -96.

*****