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RATIONAL DRUG DESIGNING STRATEGIES FOR *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Despite the availability of various techniques for diagnosis; the presence of improved and modified version of vaccines and the existence of more than a dozen of drugs, tuberculosis still remains as a significant infectious disease. The publication of complete genome of Mycobacterium tuberculosis has lead to the development of new genetic tools to ascertain the functioning of individual genes, leading to subsequent identification and validation of potential drug targets. With the help of Rational Drug designing, a computer-aided approach to find molecules with desired chemical and geometric properties that bind in a receptor cavity of specific target protein. It is hoped that promising new drugs for the effective treatment of not only TB also for MDR, XDR, HIV and persistent TB will be possible in the near future.

KEYWORDS: *M. tuberculosis, Drug, Genome, Protein, Structure-based drug design.*

INTRODUCTION

Although, Tuberculosis (TB) proved to be a controllable, preventable and curable disease, it still remains as a leading cause for mortality. The emergence of multi-drug resistant (MDR) TB defined as strains which are resistant to the two most potent anti-TB drugs; isoniazid (INH) and rifampicin (RIF) and its association with HIV/AIDS has worsened the situation. The present day enormous concern about extensible drug resistant (XDR-TB) defined as MDR-TB that is resistant to second-line TB drugs- fluoroquinolones and at least one of three injectable aminoglycosides- Capreomycin, Kanamycin or Amicacin has further compounded the effect. In 2008, an estimated 11.1 million people were living with (active) TB, including 9.4 million new cases and 1.8 million TB deaths. [1] Globally, it is estimated that 3.3% of all new TB cases had MDR-TB in 2009. There were an estimated 4,40 000 cases and 1,50 000 deaths caused by MDR-TB occurred in 2008.[2] There were an estimated 25,000 cases of XDR-TB emerging annually.[3]

Tuberculosis can be cured with the current therapy, the six months needed to treat the disease is too long, and the treatment often has significant toxicity. These factors make patient compliance to therapy very difficult, and this noncompliance frequently selects for drug-resistant TB bacteria. One of the drawbacks of existing TB drugs is that they target only against the actively growing bacteria and not towards persistent or latent bacilli and another being the lack of efficient drugs for the treatment of M/XDR-TB and HIV-TB. In spite of the fact that the second-line drugs were shown to have high activity against tubercle bacillus and useful for the treatment of drug-resistant TB and were effective against MDR-TB, many of them are under clinical trails. This problem clearly demonstrates the need for a re-evaluation of our knowledge of the current TB drugs, chemotherapy, the need for new and better drugs that are not only active against drug-resistant TB, also more importantly shorten the requirement for six months of therapy.[4] In order to overcome the drug-resistant problem, it is important to develop new drugs that inhibit novel

targets that are different from those of currently used drugs. To avoid significant toxicity, the targets of inhibition should be present in bacteria but not in the human host. Although modification of existing drugs for improved half-life, bioavailability, or drug delivery may be of some use, agents obtained by this approach may have a cross-resistance problem, as seen in the new rifamycins or quinolones. Similarly, targeting existing TB drug targets for drug development may be of limited value because of potential cross-resistance. New drugs that inhibit novel targets are needed. Hence, there is a desperate need for better drugs against M/XDR, HIV and persistent TB. [5] This brief review provides useful information regarding computer based drug designing strategies in TB. This is the important area where progress for TB is still at its infancy, unlike other diseases there are very few drugs designed using rational drug design in the field of TB. Hopefully drugs designed using these strategies will be used to develop drugs for treatment of TB in future, more particularly will provide solution to the currently facing problem of drug resistance.

History of chemotherapy in TB

The chemotherapeutic era in TB began in the year 1944 with the advent of streptomycin, followed by p-aminosalicylic acid (PAS, 1946), isoniazid (1952), cycloserine (1955), kanamycin (1957), rifampicin (1965), ethionamide (1966), ethambutol (1968) and pyrazinamide (1970). The golden era of TB drug discovery was during 1950-70, most of the TB drugs in use today were discovered in this period, except the broad-spectrum quinolone drugs, which were developed in 1980s. During the late 1980s, the second-line drugs like moxifloxacin and gatifloxicin were discovered. [6] (**Figure-1**)

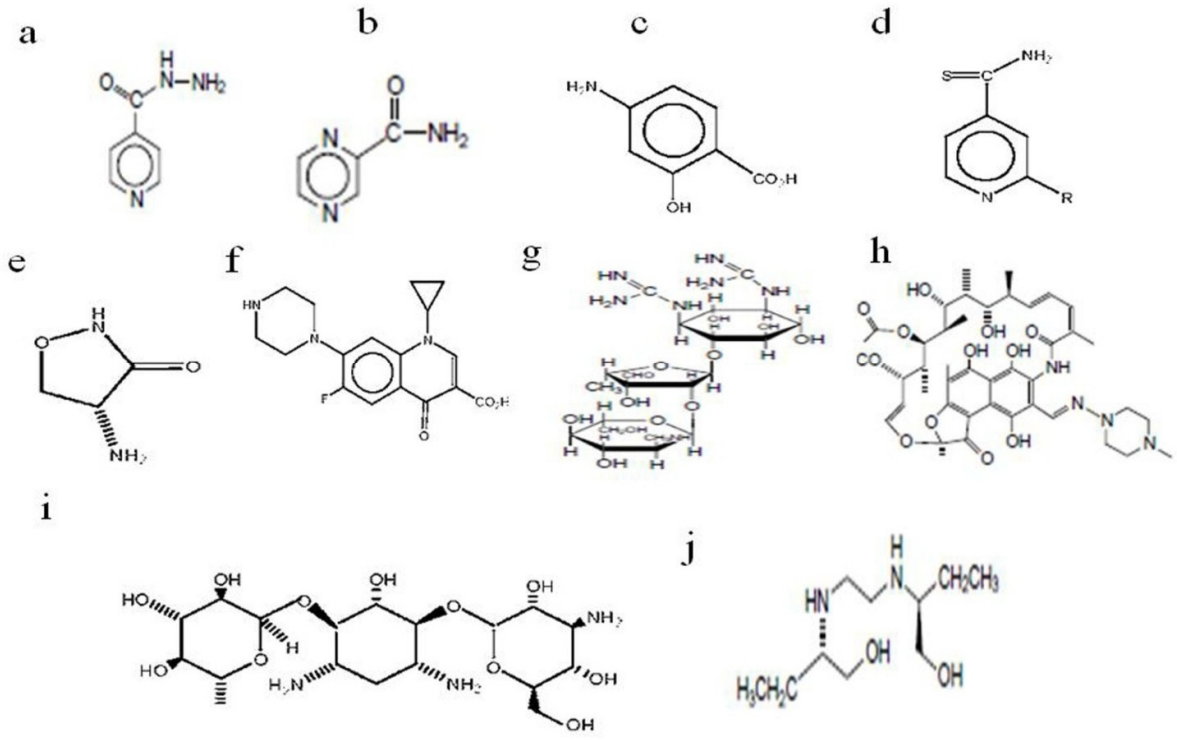

Figure-1: Structures of common TB drugs. a) Isoniazid b) Pyrazinamide c) p-Aminosalicylic acid d) Ethionamide e) Cycloserine f) Ciprofloxacin g) Streptomycin h) Rifampicin, i) Kanamycin, j) Ethambutol

Promising drug candidates

Some of the promising candidates that have passed preclinical development and are close to entering clinical trials or those that are clinically used to treat other disease conditions but happen to have antituberculous activity are Oxazolidinones (Linezolid), New Fluoroquinolones, Phenothiazines, Riminophenazine Derivatives: Clofazimine, Azole Drugs, Nitro-Containing Drugs, Nitroimidazopyran (PA-824), Peptide Deformylase (PDF) inhibitors (**Figure-2**). The most promising lead compound to have emerged is the Nitroimidazopyran PA-824. It is active against both TB and MDR-TB. The compound works by a novel mechanism, affecting protein and lipid synthesis. However, very less number of new TB drugs have been developed during the last 30 years, and those who act against resistant are few. The TB drug development processes should aimed at developing drugs with desired profiles and properties like rapid bactericidal and sterilizing activity, thus killing all TB populations including MDR-TB isolates.[5]

Newer drugs can be generated in following ways (a) an existing drug can be chemically modified to improve its pharmacokinetic and pharmocodynamic properties leading to the generation of analogues or derivative. (b) New lead molecules must be discovered either by random screening with a detailed knowledge of a specific target by rational design. [7] The recent breakthrough in the technological advancements in the fields of molecular and computational biology has opened new windows to pin hopes to remove this ancient scourge at least by the turn of this century. During the last years, we have witnessed a huge progress in the development of new methods in the fore mentioned fields especially the publication of complete genome of *M. tuberculosis* has lead to the development of new genetic tools to ascertain the functioning of individual genes, very large number of protein sequences has become available. This has improved the tools for rational drug design (RDD) significantly.

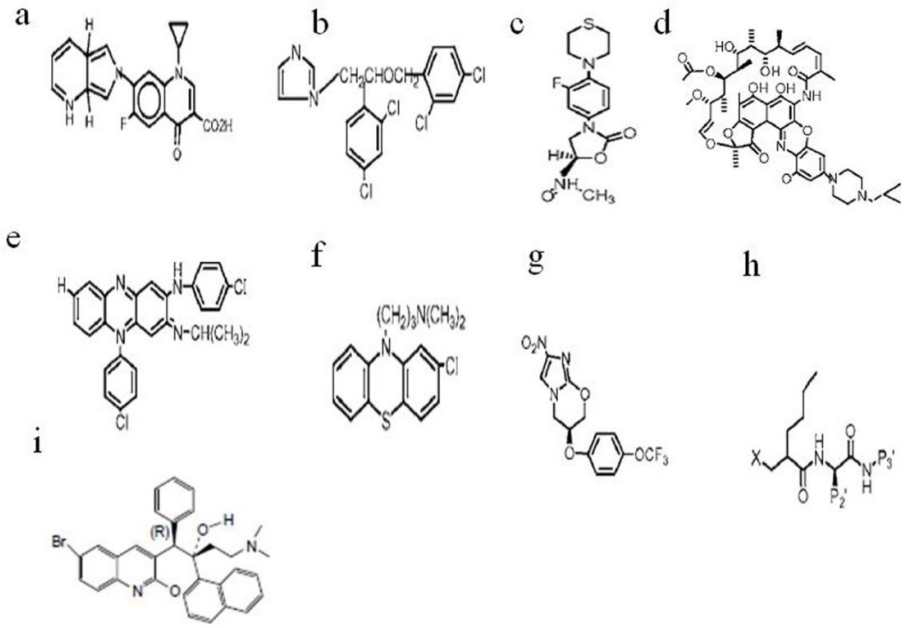

Figure-2: Structures of potential TB drug candidates. a) Moxifloxacin b) Miconazole c) Oxazolidinones (Linezolid) d) Rifalazil e) Clofazimine f) Chlorpromazine g) Nitroimidazopyran (PA-824) h) Peptide Deformylase (PDF) i) Diarylquinoline

Rational drug designing

It is basically a computer-aided molecular modeling. It is an iterative process. If it is based on the knowledge of three-dimensional (3-D) structure of the target proteins of interest, it is called as structure based drug design or Target based drug design. Such knowledge allows us to design molecules capable to bind the receptor so as to maximize the drug affinity and specificity towards the target. It is heavily dependent on computational chemistry techniques, and advances in RDD are tightly coupled to advances in new algorithms for computer-assisted molecular modeling (CAMD). Computer Aided Drug Design or Computer-assisted drug design also called CAMD represents more recent applications of computers as tools in the drug design process.[8]

The design is based on knowledge of the target structure. The computational power is yet insufficient to simulate complex biochemical reactions or even to monitor conformational changes of proteins caused by the binding of ligands. To design a new ligand for a biomacromolecule of interest using the structure of the target as a guide, the structure of the target must have been found with sufficient resolution to be of utility. One must then attempt to predict the bound geometry and intermolecular interactions responsible for the high binding affinity of novel potential ligands (or molecular fragments) associated with the biomacromolecular target.[9] 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. 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-D structural information for the receptor and the ligand-receptor complex from X-ray diffraction or nuclear magnetic resonance (NMR). 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. Computer algorithms (**Table 1**) have been developed over the past few years that aid in the identification of potential docking modes. These algorithms have also been used to identify, from 3-D databases, molecules that can potentially dock (and hence bind) to a biomacromolecular target. The prediction on of biological activity of a potential ligand prior to synthesis represents another essential activity for the structure-based design of new drugs. [8]

Major steps in the drug design

This involves the following: **1.** Hit identification: It constitutes the screening of large collections of compounds that interact with the biological target. Such a compound is referred to as a "HIT".**2.** Lead Generation: The chemical modification of hits done by repeated cycles of synthesis and testing their analogs to produce what is called as "leads", by appending improvement in chemical characteristics, thereby increasing their suitability as potential drugs subsequently. **3.** Lead Optimization: The optimization of leads by additional repeated modification to produce compounds with drug like characteristics, such candidates with optimized characteristics can be further used for pre-clinical and clinical development. The initial phase in drug design is screening of the lead molecules. Quantitative measurements of its ability to bind with the target protein will reveal extent of changes induced in the target which will provide ways to refine the lead molecule (**Figure-3**).

These compounds may serve as starting points for the optimization cycle using structure based drug

Table-1

Computer-based Tools for Drug Design

Name of Tool	Short Description
LUDI	Fragment-based, combinatorial search
BUILDER	Recombination of docked molecules, combinatorial search
MOLMAKER	Graph theoretical technique for 3-D molecular design
GROWMOL	Fragment-based, sequential growth
LEGEND	Atom-based, stochastic search
CONCERTS	Fragment-based, stochastic search
SPROUT	Fragment-based, sequential growth
MCSS	Fragment -based, stochastic sampling of probes
PRO-LIGAN	Fragment -based, combinatorial search

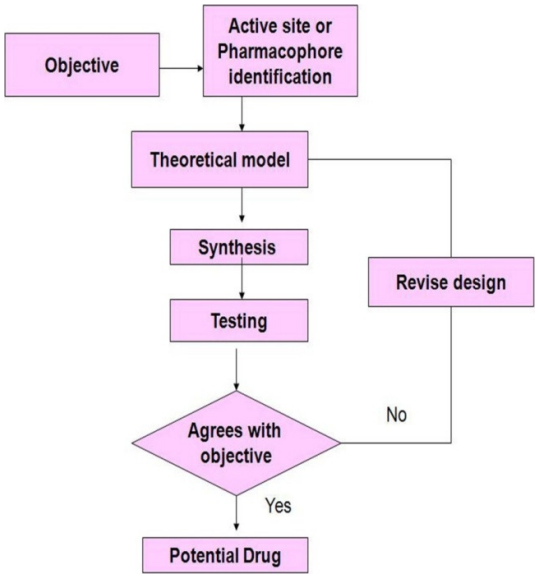

Figure-3: Rational drug designing is an iterative process

design. Once a promising compound has been designed and optimized, its activity is evaluated *in vitro* and *in vivo* systems until the designed compound exhibit the desired properties. The experimental drug is then ready for conventional drug development e.g. toxicological studies, formulation, and clinical trials etc.[10]

Quantitative structure activity relationship (QSAR)

It tries to establish a link between the ability of a molecule to perform its desired function and properties of that molecule. It involves the statistical analysis of a set of properties or descriptors for a series of biologically active molecules and a valuable time consuming alternative to labour intensive approach.[11]

Pharmocophore mapping

It is a search to identify lead compounds against a desired target. A pharmocophore is the specific 3-D arrangement of functional groups within a molecular framework that are necessary to bind to a macromolecule or an enzyme active site. [12]

Comparative molecular field analysis (CoMFA)

It is a 3-D QSAR technique based on data from known active molecules. CoMFA can be applied, when the 3-D structure of the receptor is unknown. To apply CoMFA, the activities and the 3-D structures of the molecules are needed. 3-D

structures can be determined either by measurement of X-ray analysis or by calculation from the 2-D diagram and (optionally) subsequent optimization. The aim of CoMFA is to derive a correlation between the biological activity of a set of molecules and their 3-D shape, electrostatic and hydrogen bonding characteristics. This correlation is derived from a series of superimposed conformations, one for each molecule in the set. These conformations are presumed to be the biologically active structures, overlaid in their common binding mode. Each conformation is taken in turn, and the molecular fields around it are calculated. The fields, usually electrostatic and steric (van der Waals interactions), are measured at the lattice points of a regular Cartesian 3-D grid; the lattice spacing is typically 2 Å. The "measured" interaction is between the molecule and a probe atom (an sp^3-hybridized carbon with +1 charge). [13]

Docking

It refers to the ability to position a ligand in the active or a designated site of a protein and to calculate binding affinities. Docking algorithm can be used to find ligands and binding conformations at a receptor site close to experimentally determined structures. The different conformations are generated and evaluated using computer programs. Similar calculations are performed for a set of different molecules. Those molecules giving the highest interaction energies are considered for further experimental tests. [8]

Scoring Functions (SF)

Every docking protocol has an evaluation and ranking program for the determination of best fit mode of ligand conformations defined as scoring function, predicted on the basis of the search algorithm. The scoring function should enable the distinction between the true binding modes and all the other alternative modes explored, or between active and random compounds. However, a very rigorous SF would be computationally too expensive, rendering the analysis of the several binding modes unfeasible. Hence, a number of assumptions and simplifications have to be used to reduce the complexity of the scoring functions, with a natural cost in terms of accuracy. For this reason, the lack of a suitable scoring function, both in terms of speed and accuracy, is the major bottleneck in docking. The scoring functions normally employed in protein–ligand docking are generally able to predict binding free energies within 7–10 kJ/mol, and can be divided into three major classes: force field-based, empirical, and knowledge-based.[14]

Rational Drug Design in Tuberculosis

In spite of the obstacles associated with employing an analytical approach to the design of new drugs, RDD has, nonetheless, been of enormous utility to the pharmaceutical industry. The QSAR method has played a role in the development of a number of drugs currently undergoing clinical trials and there are marketed products for which QSAR has been instrumental. A number of potential drugs that have been discovered using structure-based drug design techniques are currently under preclinical or clinical investigation for the treatment of diseases that include cancer, AIDS, rheumatoid arthritis, psoriasis, and glaucoma. With respect to TB, the progress made is still at infancy, research with this goal of identification and optimization of a lead raised against a specific target is very limited with few reports. [15], [16] Will the identified inhibitors turn out to be a successful drug in future, is the question to be answered. On the contrary, significant amount of research work has been performed during the recent years towards identification of potent drug targets against TB owing to the availability of the genome of TB.

Target Identification and Validation

The most essential task in RDD is target identification and validation which can be done by various molecular methods. The availability of the *M. tuberculosis* genome sequence opens up a new opportunity to understand the biology of the organism and provides a range of potential drug targets[17]. The recent developments in microarray technology, signature tag mutagenesis, Mycobacterial transposon mutagenesis, and gene knock-out technology provide important tools to identify new drug targets. [18]

Potential new drug targets

In choosing targets for drug development, it is

important that they may be involved in vital aspects of bacterial growth, metabolism, and viability. A list of potential targets genes is shown in **Table-2** whereby new drugs may be developed for improved treatment of TB. The problem of drug resistance can be overcome by choosing multiple targets which can be inhibited by a single drug which has been evidenced by the family of cyclopropane synthases, similarity of this family of enzymes in the mode of binding substrates and in their catalytic mechanism is clear. This makes the prospect of a single drug effective against multiple targets a strong possibility. The problem persistance can be overcome by taking enzymes of glyoxalate shunt pathway like isocitrate lyase and malate synthase as targets. [22] Recently there is launch of software called Assess Drug Target which gives more insight about the identification of genome based drug targets dedicated to TB. [25]

Table-2

Essential targets of TB

Cellular factors	Genes and proteins (target)
Seven gene families	Aminoacyl tRNA synthetases Purine ribonucleotide biosynthesis Polyketide and non ribosomal biosynthesis Fatty acid and mycolic acid biosynthesis Ser/Thr kinases and phosphatases Molybdopterin biosynthesis PE-GPRS repeats [19, 20]
Mycobacterial Persistence	RelA, [21]*icl* coding for isocitrate lyase *pca*A for proximal cyclopropane synthase gclB for malate synthase [22]
Sigma Factors	SigA, SigB, SigC, SigE, SigH, SigF [23]
Virulence Factors a)Culture filtrate proteins	HspX (Rv2031c, hspX) , Esat6/CF-10 (Rv3875, Rv3874), Glutamine synthase (Rv2220, glnA1) 19-kDa protein (Rv3763, lpqH) [23]
b) Cell surface componenets	Erp (Rv3810, *erp*), Mas (Rv2940c, *mas*), FadD26 (Rv2930,*fadD26*), FadD28 (Rv2941, *fadD28*) , MmpL7 (Rv2942, *mmpL7*), MmaA4 (Rv0642c, *mmaA4*), FbpA(Rv3804c, *fbpA*), PcaA (Rv0470c, *pcaA*), OmpA (Rv0899, *ompA*), HbhA (Rv0475, *hbhA*), LAM [23]
c)Two-Component Systems	11 two-component system homologs viz: MtrA-MtrB, SenX3- RegX3, the DevR (DosR)-DevS, PrrA-PrrB, MprA-MprB, and PhoP/PhoR[17]
d) Cell wall synthesis	KasA and KasB[24]

Also, proteins with complex architecture may be considered as potential targets for drug design was documented in another report. [26]

Structural biology

The 3-D structure of the target biomacromolecule can be determined using X-ray diffraction or NMR spectroscopic techniques. If the 3-D structure of the target is unavailable, then a hypothetical model is formulated by Homology modeling (Comparative modeling or Knowledge –based prediction) exploits the fact that evolutionary related proteins with similar sequences have similar structures. The degree of similarity is very high in the so called "core regions" comprising of secondary structural elements (α-helices and β -sheets) whereas the degree of similarity is usually low in loop regions connecting the secondary structures. In Homology modeling prediction is based on information derived from known protein 3-D structures. [27] Several softwares are available presently to predict the unknown structure of a protein of interest.

Studies being conducted by the TB Structural Genomics consortium (TBSGC) will make available the structures of many potential target proteins. Further through the focused efforts of TBSGC the number of protein structures gets accelerated effect. Interestingly, the number of TB structures in PDB continues to increase, currently (2011) being 1166 [28]; this compares with just 8 structures at the beginning of 2000. The number continues to increase everyday. These must be selected for further analysis and computational chemistry techniques applied to identify more leads.

Advantages of RDD over Traditional Drug Discovery

RDD eliminates a large amount of preliminary lab work. It reduces the cost and time which involve in conventional approach. It will produce highly specific drugs and decreases side effect caused by inappropriate binding. It will generate more potent, efficacious and precise drugs. The central aim of RDD is to help the scientists to discover "faster, cheaper, and safer" drugs.

The traditional discovery of new drugs is an empirical process that starts with a compound of marginal biological activity. This "lead" compound either is discovered serendipitously by the random screening of a large number of compounds (often obtained from libraries of previously synthesized molecules) or is obtained by preparing analogues of a natural ligand (i.e., a small molecule such as a hormone that binds to a biomacromolecule such as an enzyme). Chemical intuition and experience as well as ease of synthesis serve to suggest other closely related molecules (analogues) for synthesis. This process is iterative and continues until a compound is discovered that not only possesses the requisite activity toward the target but also possesses minimal activity toward other biomacromolecules (i.e., it is selective and nontoxic). The compound must also have a desirable duration of action in a suitable dosage form, its synthesis must not be too costly so that its use will be cost-effective, it must be patentable, etc. This process can take many years, can cost millions of dollars, and often does not result in a marketed product. [6, 27, 29]

Disadvantage

A major challenge to successful drug development remains finding analogs with satisfactory bioavailability. In spite of the power of RDD and combinatorial chemical synthesis to identify highly useful "lead" compounds needed for drug discovery. RDD and high throughput screening methods afford very tight-binding ligands, but these often have horrible pharmaceutical properties. The challenge remains to identify structures that can retain the interactions with the desired target while attenuating the non-productive interactions. Solutions to this problem are usually found empirically via multiple analog syntheses followed by high throughput screening. General solutions do not yet exist. The systematic study of how enzyme-inhibitor interactions have helped elucidate the fundamental structural biology behind enzyme catalytic mechanism and host-ligand interactions. Analysis of both the successes and failures to develop clinically useful drugs suggests strategies that might lead to more efficient future drug discovery. Alternative methods of therapy now in early stages of development may ultimately replace the ligand-target strategy employed for the past 100 years. [6, 29]

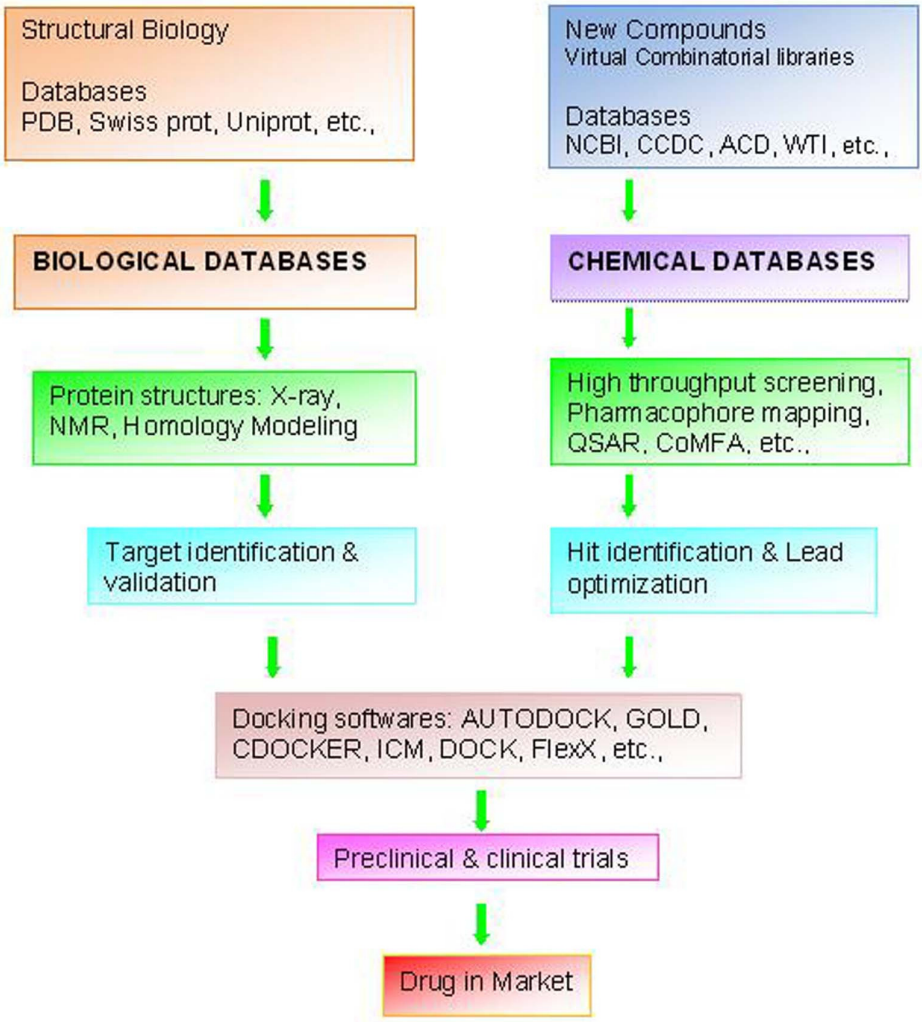

Figure-4: Overview of rational drug discovery: An Integrated multi-disciplinary approach

CONCLUSIONS

An integrated approach is required to find lead compounds active against the drug targets that have already been identified and validated. This will require input from companies and organizations that possess compound libraries and the advanced robotics and technology required for high throughput screening (**Figure-4**). The establishment of The Global Alliance for TB Drug Development (GATB)-2000 changed the landscape in TB drug development, the alliance aims to overcome the natural barriers to TB drug development by working in partnership with organizations as diverse as academic institutions, government research laboratories, non-governmental organizations, the pharmaceutical industry and contract research houses, the GATB will plug gaps in the Research and Development pipeline. According to GATB economics report, the estimated cost for the development of TB drug would be ~$100 Million.[30]

In summary, the Knowledge of the TB genome promises the introduction of an era of RDD in TB. Although significant obstacles remain, the prospects for the development of new and effective drugs against MDR, XDR, HIV and Persistent TB are much greater than at any time in several decades.

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