

## **Affordable Drugs: An Indian Effort. “Generic to Designer drugs”**

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### **Abstract:**

This review discusses the progress of India from being one of the largest producers of generics to its coming of age and initiating novel drug development programs such as the Open Source Drug Discovery for tuberculosis. A few groups have also begun to emerge which focus their research on rational or structure based drug design. We discuss here some of the ongoing efforts in drug discovery in India primarily in national research institutions and academia.

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Drug discovery is an innovative process of finding new cures for a particular diseased condition. It can broadly be classified into two major categories: non-target based empirical drug discovery and target-based rational drug discovery. The empirical or classical drug discovery approach has largely been dependent on serendipity and relies on physiological readouts of phenotypes and amelioration of disease in a particular assay system. For instance, historical discovery of penicillin to discovery of modern day Viagra have been the result of good fortune. A rational target-based drug discovery approach, on the other hand, begins with identification of a possible target with a putative role in a medical condition. Technological advances in the field of genomics, proteomics and computational tools have been the main driver of target-based drug discovery over the last few decades. A few drugs developed by this approach worldwide are captopril (an inhibitor of angiotensin converting enzyme and used for hypertension), dorzolamide, (a carbonic anhydrase inhibitor for glaucoma), saquinavir (protease inhibitor for treatment of AIDS) and zanamivir (neuraminidase inhibitor against influenza).

Explosion of genome wide information of various organisms due to improvements in sequencing technologies has led to a large repertoire of identifiable drug targets. However,

dissecting “druggable” targets from the potential ones is still a big challenge (Hopkins and Groom, 2002; Russ and Lampel, 2005). These drug targets may be proteins or nucleic acids involved in regulation, signaling or different metabolic conditions. Key enzymes in biochemical and regulatory pathways, as well as virulence factors of infectious organisms act as potential drug targets. Biochemical and structural knowledge of the target helps understand the mechanism of action of the disease and in the design of specific compounds to hit the target. Although several groups are involved in collaborative and complementary approaches to identify drug targets and their three-dimensional structure determination to aid drug design, rational target based drug design in India still awaits major conceptual breakthroughs. One of the major focus areas of India’s efforts in drug discovery over the years has been the development of cost effective and innovative processes for generic drugs. Domestic pharmaceutical companies have traditionally thrived on innovating process patents and by using low labor and research costs to export generic drugs to developed-country markets, including the US. Drug prices in India are hence much lower than the prices of similar drugs in several countries including the United States, United Kingdom as well as Pakistan and Indonesia (Agarwal, SP et al., 2007). Today, India has the largest number of

FDA approved manufacturing facilities in any country outside the United States. Several low cost and commercially viable technologies for a wide range of essential drugs, such as anti-cancer, anti-virals, anti-bacterials, anti-glaucoma, anti-inflammatory, analgesics, and cardio-vascular drugs, among others have been developed. An important contribution of Indian generics industry has been in bringing down the cost of antiretroviral therapy for HIV/AIDS treatment by the efforts of Indian Institute of Chemical Technology (IICT), Council of Scientific and Industrial Research (CSIR) and Indian pharmaceutical companies like CIPLA, which has made drugs available to more than 250,000 HIV patients in poor countries at very cheap rates. India has been a major exporter of relatively cheap Active Pharmaceutical Ingredients (APIs) and pharmaceutical formulations of several medicines, notably vaccines and antiretrovirals (ARVs).

### **GENERICS IN INDIA: A HISTORICAL PERSPECTIVE**

A number of research institutes in India are involved in the development of new technologies and process formulations for the production of generics. These technologies have enabled the Indian drug industry to emerge as one of the largest producers of generic drugs in the world. India has an estimated 24,000 drug manufacturers (DOCP Annual Report (2007-2008)). Together they produce output valued at nearly US \$18 billion, accounting for nearly 1

percent of our GDP. Indian pharma industry stands today 4th (8%) in terms of volume and 13th in terms of value of production having nearly 22% of the global generic market (<http://www.indiainbusiness.nic.in/industry-infrastructure/industrial-sectors/drug-pharma.htm>). Besides exporting pharmaceutical products to a large number of developed countries like US, UK, Germany, Russia and Japan, India is also a low-cost supplier of generic drugs to several less-developed countries, like Nigeria, Vietnam, Sri Lanka, Pakistan, Bangladesh and Nepal. However, in spite of our tremendous growth in production of generics and new process formulations, share of India's pharmaceutical industry in the world market is a mere 1.3% of a global sale of nearly US \$785 billion. The scenario is expected to improve as leading industries acquire skills and confidence to engage in high-end, new drug discovery research and technology development.

The Indian Patent Act of 1970 replaced the Indian Patents and Designs Act of 1911 and brought in major changes. The Patents Act of 1970, which was implemented in 1972, did not allow product patents on foods, pharmaceuticals and chemicals but allowed only process patents in these categories. The outdated Act of 1911 had previously allowed patenting of pharmaceutical products, resulting in very high price of medicines as controlled by MNCs. The changes in the 1970 Patents Act gave an impetus to formulation of new process technologies and

reverse engineering methods for manufacture of various essential medicines and ensured that these could be available to people at affordable prices (Thomas, 2006). Other changes brought in the Act included reduction in the time period for patents from 16 years to five years (from the date of patent granting or seven years from the date of patent application) and mandatory domestic manufacturing within three years from the date of sealing of the patent. In addition, a Drug Price Control Order (1970) was also introduced to control the maximum price that could be charged for any drug.

The government support in the area of healthcare had begun with the set up of drug manufacturing companies in the public sector, Hindustan Antibiotics Limited (HAL) (1954) and Indian Drugs and Pharmaceuticals Limited (IDPL) (1961). The production of modern medicines in India, however, dates back to more than a century. Pharmaceutical companies with a long history in India include Bengal Chemicals & Pharmaceuticals Limited (BCPL), established in 1901, Alembic Chemical Works (1907) Bengal Immunity Ltd. (BIL) (1919), and Smith Stanistreet Pharmaceuticals Ltd (SSPL). Many of these pharmaceuticals were nationalized and some are involved in the production of pharmaceutical products, major bulk drugs and chemicals using indigenous technologies even today. In addition, there are a number of pharmaceutical manufacturing units under the control of state governments, such as Goa

Antibiotics Ltd., Karnataka Antibiotics and Pharmaceuticals Ltd., Rajasthan Drugs and Pharmaceuticals Ltd etc (Maggon, 2003). National R&D institutions were set up by CSIR, namely,; National Chemical Laboratory in Pune in 1950, Central Drug Research Institute in Lucknow in 1951 and Indian Institute of Chemical Technology (IICT), formerly known as Regional Research Laboratory (RRL), in Hyderabad in 1956, all of which have a wealth of scientific knowledge and are staffed by highly qualified scientists. Over the years, NCL, CDRI and IICT have developed many process technologies for various drugs and these have been widely used by many top as well as small-scale pharmaceutical companies in India.

NCL has been developing many significant technologies for use in manufacture of various drugs. The organic synthesis group at NCL has developed cost-effective processes for anti HIV-agents viz., AZT, stamuvudine, lamuvudine, cardiac and antimalarial drugs etc. Among successful technologies developed by NCL include manufacture of epibatidine, a painkiller with potency 200 times higher than morphine and a new process using zeolite catalyst for the manufacture of ranitidine, an anti-ulcerant and one of the world's largest selling drug. Alternate and cost-efficient process technologies have also been developed for including etoposide, atorvastatin, irinotecan, neverapine, ziprasidone, cisapride, celiprolol and many other important drugs. A new process for Biotin, one of the B-

complex group of vitamins has also been developed. NCL is also exploring synthesis and purification methodologies for chiral molecules as drugs. Some important industrial partners where technologies have been transferred for manufacture and marketing include Alembic, Cadilla, CIPLA, Dabur, Emcure, Glenmark, Godrej Agrovat, Lupin, Orchid Chemicals, Ranbaxy, RPG Life Sciences etc.

CDRI has helped develop over 100 technologies for bulk drugs, drug intermediates, and biological / fermentation products. Apart from successes in new drug or technology development, CDRI has also been involved in development of many generics marketed by leading pharmaceuticals companies viz., acyclovir (Ranbaxy), ephedrine hydrochloride (Malladi Drugs & Pharmaceuticals), dextropropoxyphene hydrochloride (Wockhardt), pyriethoxin (Themis Chemicals), clofazimine (Astra-IDL), 5,6-dimethylbenzimidazole (K. Methaqualone & Chemicals), N-methyl piperazine, artemether (IPCA Labs). Today, CDRI's technology partners is a long list of industries which includes CIPLA, Dabur, Hindustan Latex, Lupin, Nicholas Piramal, Torrent, Unichem and many others.

IICT has played a major role in drug development and placing India in the global generic map. Among major drug development approaches, IICT has developed an innovative and cost-effective approach to AZT and subsequently to other HIV-inhibitors such as

betzalladines, calanolides, mischellamines and HIV protease inhibitors. They also developed technologies for many other drugs like diazepam; salbutamol; anti-cancer drugs viz., vinblastine, vincristine, etoposide; anti-diabetic drug tolbutamide; anti-inflammatory drugs enfenamic acid, flurbiprofen; enalapril for high blood pressure and antibacterial agents viz., sultamicillin, azithromycin, norfloxacin and ciprofloxacin. In addition, process for pentamidine for the cure of Kala azar (visceral leishmaniasis) was also developed at IICT and transferred to CIPLA. IICT has also developed docetaxel, Taxol C-13 side chain (API intermediate) and several new anti-cancer agents. Among other technological achievements, IICT is also making efforts towards development of technology for cost-effective synthesis of pure chiral compounds. Timolol maleate, an anti glaucoma agent was the first chiral drug successfully developed at IICT in 1989 and transferred to FDC Ltd. IICT has a long list of technology partners which includes Armour Chemicals, Bombay Drug House, Cadilla, Cheminor Drugs, CIPLA Coromandal Pharma, Dabur, IDPL, Indus Pharma, Lupin, Nicholas Piramal, Ranbaxy, Reddy's Laboratories, Sun Pharmaceuticals, Torrent Pharmaceuticals, Unichem, Zydus and many others.

Powered by its achievements in developing novel reverse engineering methods, India is today a world leader in the generics market

worldwide. Over 100 major drugs and more than 300 bulk drugs are produced in India using totally indigenous cost-effective technologies. The previous patents regime facilitated the development of the Indian generics industry by encouraging reengineering of existing high-priced drugs by alternate processes. Along with several “path-breaking” technologies developed by national R&D institutions and transferred to pharmaceutical companies for use in large scale development, research at leading pharma centres also enhanced capabilities in reverse engineering methods for large scale production of pharmaceutical products. Under its WTO commitments, however, India has changed its patent regime to allow patenting rights to both products as well as processes. Indian drug companies can no longer sell drugs patented by other firms unless they are licensed to do so. Driven by the change to a product patent regime and the opportunities offered in the international market, the mindset of R&D in India is shifting towards innovative research. While the initial focus of most of the national R&D institutions was development of reverse engineering capabilities for generics, there is a concerted effort towards discovery and development of newer therapies, many of which are in later stages of development or clinical trials.

#### **EARLIER AND PRESENT PHASE OF DRUG DISCOVERY IN INDIA**

The discovery of urea stibamine and its effective use for the treatment of kala azar in the 1920s by

Prof. U.N. Brahmachari marked one of the first major success stories of modern drug development in India. India has since come a long way with several groups of scientists in national R&D institutions and in academia developing cost-effective drugs and technologies for various ailments affecting the local population.

Centchroman, a non-steroidal oral contraceptive for women was developed by CDRI in the 1980s. Centchroman acts by modulating the balance between estrogen and progesterone at the uterine level without interfering with their synthesis or blood levels and was the first anti-implantation agent approved for clinical use in the world. Centchroman offers a combination of weak estrogenic and antiestrogenic properties that inhibits the fertilized ovum from nidation and thus prevents pregnancy. Since it does not disturb the endocrine system, it is considered much safer than other steroidal alternatives. Centchroman is a popular contraceptive for women and has been in the market in India for many years under its trade name ‘Saheli’.

$\alpha,\beta$ -Arteether, a semi synthetic derivative of artemisinin, developed at CDRI in collaboration with Central Institute of Medicinal and Aromatic plants (CIMAP), Lucknow is useful as a second line of treatment for chloroquine-resistant *P. falciparum* malaria including cerebral malaria. The drug has been licensed to Themis Chemicals Ltd., Mumbai and is being marketed as an injectable formulation,

under the trade name 'E-Mal'. Post marketing surveillance data of Arteether has indicated no drug related side effects so far. Another anti-malarial that has undergone extensive trials and is currently being marketed is the primaquine derivative bulaquin. Bulaquin is used in combination with chloroquine as anti-relapse, antimalarial for prophylactic against *P. vivax* malaria and does not have the methaemoglobinaemia or leukopenia side effects caused by primaquine.

Taking a lead from ancient Indian system of medicine of Ayurveda, scientists at CDRI identified guggulsterones from the extracts of plant *Commiphora mukul* (commonly known as "Gugglu") for its cholesterol lowering properties. The fraction containing active constituents is currently marketed as 'gugulip' by CIPLA. A synthetic substitute for guggulip that acts through FXR nuclear receptor to inhibit bile acid mobilization in liver has now been developed and is currently under Phase III clinical trials. Another molecule, Picroliv, a hepatoprotective agent of plant origin has been developed. Picroliv has antiviral and immunostimulant activities. It is devoid of any significant Central Nervous System (CNS), cardiovascular system (CVS), autonomic and other systemic activity. Phase III clinical trials are in progress in patients of tuberculosis receiving multi drug therapy and in patients suffering from alcoholic cirrhosis.

In addition, several R&D programmes, viz., the Drug and Pharma Research Programme (DPRP) of Department of Science and Technology (DST) have been initiated to boost innovative research in the area of drug discovery and development in India. DPRP aims to support development of new drug formulations and create infrastructure to facilitate drug discovery in industry as well as academia. At present there are 85 industry-institution alliances involving 60 industries and 38 public funded institutions. This program funds 85 research projects at 32 national R&D institutes and 38 research projects to industry. Collaborative efforts in the area of drug and vaccine development for various diseases like malaria, tuberculosis, cardiovascular diseases, hepatitis B, dengue etc are being pursued under this programme.

New Millennium Indian Technology Leadership Initiative (NMITLI) program of CSIR also aims to boost partnerships between public and privately funded R&D institutions. About 57 projects developed under the NMITLI program over the years involving 80 industry partners and 270 R&D groups from different institutions are currently being pursued. Of these, 11 projects with a total outlay of nearly Rs. 100 crores (US \$20 million) are aimed specifically towards drug development programmes. Some of the products developed under the NMITLI programme have been approved as Investigational New Drug (IND) and are at different stages of clinical studies.

No preventive or curative therapy exists for psoriasis except the symptomatic management. Based on the traditional knowledge and using the reverse pharmacology approach, a single plant based oral herbal formulation has been developed under NMITLI collaborative programme which includes Lupin Laboratories as the industrial partner and CDRI and NIPER, Mohali as the partners from national R&D institutes. After extensive safety, efficacy, toxicology and pharmacokinetic studies, the formulation has been filed as an IND, a first in the country for an herbal based formulation. The formulation has cleared phase II clinical studies and is currently undergoing phase-IIb & III studies.

In a successful collaboration under the NMITLI programme, the expertise of 12 institutional partners and Lupin Laboratories as the industry partner were synergized for the development of new targets, drug delivery systems, bio enhancers and therapeutics. A new chemical molecule, Sudoterb (LL-3858), has been developed for the treatment of tuberculosis. The molecule is active alone and also compatible with the four-drug combination therapy of tuberculosis and works well replacing some of the drugs from the standard tuberculosis regimen. The molecule is less toxic, clears the total infection within two months and no recurrence has been observed in animal studies. This would be one of the first global successes achieved in developing a new therapeutic

molecule for tuberculosis in more than 40 years. An Investigational New Drug (IND) application for the molecule has been cleared and clinical trials are in progress.

Another achievement under the NMITLI scheme has been the development and improvement of lysostaphin as a biotherapeutic under collaborative efforts of Institute of Genomic and Integrative Biology (IGIB), CDRI and Bharat Biotech. Lysostaphin is a 27-kDa-glycylglycine endopeptidase produced by *Staphylococcus simulans* that kills all other strains of *Staphylococcus* by cleaving the penta-glycine bridges linking the peptidoglycans in their cell wall. Due to its rapid and unique mechanism of action, lysostaphin has the potential to substitute and/or augment antibiotics as the first line of treatment for *S. aureus* infections. The development of lysostaphin as a therapeutic agent has had a major limitation over the years due to potential immunogenicity of a parenterally administered protein and the impurity of lysostaphin preparations. Under the NMITLI program, the technology for recombinant mature lysostaphin was developed at IGIB and licensed to the Bharat Biotech to obtain lysostaphin that is > 90% pure, free from pre-pro and pro-lysostaphin and that can be produced in large quantities from *E. coli*. This overcomes all problems of potential immunogenicity from impure protein therapeutic. The development is protected as a



product patent in several countries. The studies have shown the drug to be efficacious in controlling topical infection caused by drug resistant strains of *Staphylococcus aureus*. Elaborate pre-clinical animal studies on lysostaphin gel and cream formulations indicated no adverse pharmacological and toxicity effects. The formulation was the first biotech molecule to clear IND in India. Presently, the formulation is undergoing Phase II clinical trials in patients.

Furthermore, poly herbal formulations, for diabetes, arthritis and hepatocellular protection, have been developed under the NMITLI program based on traditional medicinal system and following reverse pharmacology approach using modern science and medicine tools. Phase III clinical studies have recently been concluded on many of these molecules and the results are being analyzed.

### **Recent research activities towards Drug Targets Discovery and Development**

Subsequent to the enthralling success of generics as well as some new product development, a large number of institutes and individual investigators are making remarkable achievements today to find specific inhibitors or lead compounds against various diseases. Many new candidate drugs and nutraceuticals have been developed indigenously. Several institutes and groups in the country are focused to new product development in the areas of cancer,

diabetes, osteoporosis, malaria, leishmaniasis, tuberculosis etc. 'Bio-therapeutics' or use of synthetic or naturally occurring biological compounds, small molecules and their derivatives for therapeutic purposes is also being developed. While it is not possible to elaborate on basic research activities of a large number of groups actively involved in dissecting the molecular and genetic basis of various diseases, some of the recent research activities through institutional or individual efforts that have led to or have a strong potential of being a candidate drug molecule are briefly described in this section.

**Peptides** and peptidomimetics have a strong potential for use as novel drug therapeutics. Synthetic peptides are being studied at the Institute of Science (IISc), Bangalore to dissect the rules governing formation of secondary structure elements in proteins. Several synthetic peptides have been constructed using unnatural amino acids to introduce local constraints and understand peptide folding and stability in greater detail. Insights from these studies have been used in design of synthetic interface peptides, which can potentially impede subunit association of triosephosphate isomerase, a potential drug target of *Plasmodium falciparum* (Singh et al., 2001). In addition, studies on peptides from venom extracts of marine cone snails, *Conus amadis* to explore the potential of development of new pharmacophores are being

carried out (Sudarslal et al., 2003). In a separate study carried out at CDRI, synthetic peptides for use as antifungal agents with high efficacy in combination with standard antifungal agents have been identified (Kundu et al., 2002). Several other groups have now begun to explore role of peptides as potential inhibitory molecules. Investigators at CCMB are currently studying the antibacterial role of synthetic human beta-defensin analogs, which exhibit activity against *Escherichia coli* and *Staphylococcus aureus* (Krishnakumari et al., 2006). The antibacterial activities were attenuated in the presence of increasing concentrations of NaCl and divalent cations such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with the site of action being the bacterial membrane. Since this research has important impact in the development of antibacterial peptides as therapeutic agents, a US patent has been secured. In another recent report, a synthetic peptide derived from the leucine zipper motif of hemolysin E of *E. coli* has been shown to inhibit the hemolysin activity against human red blood cells (Yadav et al., 2008). This peptide has been modified by substitution of leucine residues at 'a' and/or 'd' position of the heptad repeat with single or double alanine residues, thereby reducing the toxicity of Leucine Zipper Peptide (LZP) against human red blood cells (hRBCs), as it impaired the binding and localization of LZP to hRBCs.

### **Proteins as biopharmaceuticals:**

A significant step in the use of proteins as therapeutics has been the successful development and marketing of clot-dissolving agents viz., streptokinase. IMTECH, Chandigarh has successfully developed a cost-effective technology for production of therapeutic grade streptokinase by biotechnological means and transferred the technology to Cadilla for production and marketing in India as 'STPase'. IMTECH also developed an engineered clot-specific streptokinase that becomes active only when it encounters the blood clot. The technology was transferred to Nostrum Pharmaceuticals for worldwide clinical development and marketing. In addition, Bharat Biotech and Shantha Biotech have also independently developed recombinant streptokinase for therapeutic use and are currently being marketed as Indikinase and Shankinase, respectively.

The development of lysostaphin as a therapeutic agent against *Staphylococcus* infections has already been described. Another protein with important therapeutic role, recombinant epidermal growth factor is being successfully used for treatment of for Diabetic Foot Ulcers as well as for burns and skin grafts. This recombinant protein was developed at IGIB and is being marketed by Bharat Biotech as 'REGEN-D'. In other significant achievements, several groups from different institutes have

identified various protein molecules as biomarkers which may help in the early diagnosis of various types of cancer.

**Natural compounds:** In recent leads, some natural products (dihydrobetulinic acid, luteolin, diospyrin and indolyl quinolines) were identified as inhibitors of leishmanial topoisomerases at IICB. In addition, researchers at IICB found herbal formulations obtained from *M. koenigii* and *Tribulus terrestris* to be useful for the treatment of prostate cancer and is currently being marketed under the brand name 'Prostaly'n'. Another molecule isolated from the flowers of *Woodfordia fruticosa* at IICB has been found to be useful against peptic ulcers. A patent application has been filed in several countries.

In a collaborative effort from scientists at National Centre for Cell Sciences (NCCS), National Institute of Oceanography (NIO) and International Centre for Genetic Engineering and Biotechnology (ICGEB), two anti-malarial compounds from mussels, 'NIO-1' and 'NIO-2' have been found to have very specific activity against *Plasmodium falciparum*. The license to commercialize the drug process has already been transferred to a Mumbai-based company Shreya Life Sciences.

## TOWARDS RATIONAL DRUG DESIGN

Worldwide, several groups are making inroads into tackling various diseases by development of

novel inhibitors or lead molecules through rational drug design efforts. This involves computer-aided drug design methods including structure based drug design (SBDD), ligand-based drug design, de novo synthesis for the drug targets identification etc. In India, a major effort of majority groups in rational drug design is focused towards identification and discovery of potential targets for further use in drug development processes. Many companies have initiated work towards creating integrated in-silico drug design tools, molecular docking and virtual high throughput screening to help reduce the discovery time. There are large number of Contract Research Organizations (CROs) in India which are getting involved in various stages of drug discovery giving a significant thrust to the pharma companies to achieve their goals (Clark and Newton, 2004). Many of these companies have also been recognized by Department of Scientific and Industrial Research as premier R & D centres. Some of the CROs based in India and their specializations are summarized in Table 1. Although not an exhaustive list, the table provides an overview of various stages of drug development that are being aided by an increasing number of CROs and expertise of talented researchers available therein.

### Computational efforts:

A major collaborative effort for drug target identification and drug development using computational approaches has been initiated as a

collaborative project among several CSIR labs. This project aims to develop new software and strategies to enable identification of therapeutic targets; to design and develop new tools for predicting toxicity and drug response in-silico; and to generate qualified and trained IT professionals for pursuing research in the area of bioinformatics. As ongoing efforts of this project, many non-coding DNA structural motifs have been validated experimentally as potential targets (Verma et al, 2008, Thakur et al, 2008). Novel approaches have been developed to identify protein targets in infectious organisms. One such approach uses the invariant peptide approach. Invariant peptides with an evolutionary pressure in at least seven organisms were identified and found to act either as 'functional signatures' or fold determinants in several proteins (Prakash et al., 2004; Prakash et al., 2005). Invariant peptide regions of functionally important proteins of *M. tuberculosis* are being explored as potential drug targets. In another study, unstructured regions in essential proteins have been proposed to be important functional regions that may be targeted for drug development (Debasis Dash, personal communication).

### **Synthetic small interfering RNAs (siRNAs)**

siRNAs have found wide applications in therapeutics since these can direct the cleavage of perfectly complementary target mRNAs and knock down the expression of a gene in a highly specific manner. A natural analog of siRNAs,

microRNAs (miRNAs) down regulate targets with incomplete complementarity. miRNAs can sequester targets in silenced complexes and may be capable of simultaneously shutting down many members of a biological pathway and hence can be of potential therapeutic use. Role of miRNA in host-pathogen interactions and their potential use in therapeutics are being explored and have been reviewed recently (Scaria et al., 2007). Efforts are underway at many academic and pharmaceutical research organizations in inter-institutional collaborative programmes to explore such methodologies in the field of drug discovery.

### **Structure based drug design (SBDD)**

Structural biology forms an integral part of rational computer aided drug design methods. The main approaches to determine three-dimensional structures are X-ray crystallography and NMR. Macromolecular crystallography took early shape in India primarily through the crystallography work on lectins in the initial years and has been reviewed recently (Vijayan, 2007a; 2007b). Over the years, as many as 20 research institutions now have macromolecular crystallography as one of the major tenets in tackling various biological problems. Many of these groups target SBDD for identified protein targets as a primary objective of their research goals. A number of research groups are addressing problems associated with infectious diseases relevant to India. One of the major

successes emanating from the relatively new field of SBDD efforts in India, however, is on phospholipase A2 (PLA2).

PLA-2 is an enzyme involved in production of eicosanoids. It catalyzes the hydrolysis of the sn-2 ester linkage of glycerophospholipids to release fatty acid and lysophospholipid. Any imbalance in the physiological concentrations of eicosanoids is associated with various diseases viz., inflammation, rheumatism, arthritis and asthma. Structures of several isoforms of snake venom PLA2 (reviewed recently in Singh et al., 2007) and bovine phospholipases (Sekar et al., 1997) are available. Crystal structures of PLA2 in apo form and in complex with various natural compounds viz., anisic acid, atropine, aristolochic acid, tocopherol (vitamin E), substrate analogs, indole derivatives and several NSAIDs, viz., indomethacin and aspirin are available. The structure of PLA2 has revealed that the substrate binding site consists of a large linear hydrophobic channel ending into the active site consisting of residues His48, Asp49, Tyr52 and Asp99. The binding site can be divided into six subsites. Detailed structural analysis of different complexes revealed that most of the currently available inhibitors bind to only a few of these subsites resulting in poor potency. The structural analysis of specifically designed peptide LAIYS in complex with PLA2 confirmed that the hydrophobic residues of the peptide interact with the hydrophobic channel of the binding site and placed the hydroxyl group

of tyrosine for interaction with Asp49 and His48 yielding a binding affinity of approx  $8.8 \times 10^{-9}$  M. Further modification of this peptide to FLSYK in order to exploit electrostatic interactions between the peptide and active site has resulted in a binding affinity of  $1.1 \times 10^{-9}$  M for the peptide inhibitor (Chandra et al., 2002). The design of this highly specific peptide inhibitor is exciting and instills confidence in indigenous SBDD efforts towards other protein targets in the recent future.

Apart from the SBDD studies on pain and inflammation, a few groups are addressing molecular mimicry and antibody maturation (for instance, Jain et al., 2001). However, structure based drug design in India is largely aimed towards pathogenic organisms prevalent in tropical countries, particularly India; *Mycobacterium tuberculosis*, *Plasmodium falciparum*, *Salmonella typhimurium*, *E. histolytica* and *L. donovani* and HIV to name a few. A large number of these efforts have been focusing on drug target identification and structural characterization that may serve as templates for SBDD in subsequent stages. Three-dimensional structures of Triose phosphate isomerase (Velanker et al., 1997; Parthasarthy et al., 2002; Eaazhisai et al., 2004a) and adenylosuccinate synthetase (Eaazhisai et al., 2004b) of *P. falciparum*, important drug targets, in complex with several ligands and substrate analogues have been obtained in order to obtain leads into design of therapeutics. Fatty

acid biosynthesis and its inhibition by triclosan is being investigated in detail to improve the specificity of antimalarials (Surolia and Surolia, 2001; Suguna et al., 2001). In addition, peptide derivatives have been designed for inhibition of plasmepsins, key enzymes in the life cycle of *Plasmodium* and their inhibitory effects being investigated to further aid drug development against this pathogen (Kesavulu et al., 2005). Several potential inhibitory molecules are being explored through crystallography complexes against HIV protease for design of specific inhibitors (Pillai et al., 2004; Das et al., 2007; Bihani et al., 2008). Structural efforts into protein targets of *Leishmania donovani*, the causative agent of kala azar, *S. typhimurium* and *E. histolytica* have also been initiated and would be important steps in drug design against these pathogens.

### ***Mycobacterium tuberculosis*, a major threat in India: structural efforts**

The crystal structure of RecA from *Mycobacterium tuberculosis* (Datta et al, 2000), a protein involved in homologous DNA recombination and repair was one of the first structures of a mycobacterial protein from India. Over the last decade, several groups have taken up the structure determination of proteins from this dreaded pathogen, causative agent of largest number of deaths by a single infectious organism worldwide. At present, India alone accounts for nearly 10% of all available crystal

structures of *M. tuberculosis*. Structures of many important drug targets of *M. tuberculosis* viz., uracil-N-glycosylase (Kaushal et al., 2008), Pantothenate kinase (Das et al., 2006), chorismate mutase (Qamra et al., 2006), NAD<sup>+</sup> dependant DNA ligase (Srivastava et al., 2005), lysine  $\epsilon$ -amino transferase (Tripathi et al., 2006), polyketide synthase (Sankaranarayanan et al., 2004), peptidyl tRNA hydrolase (Selvaraj et al., 2007; Pulavarti et al., 2008) to name a few have been determined in India. The design of specific inhibitors as potent lead molecules for drug development is being carried out in several labs.

One protein target of *M. tuberculosis* against which SBDD has led to identification of specific inhibitors with IC<sub>50</sub> in low micromolar ranges is the NAD<sup>+</sup> dependent DNA ligase (Srivastava et al., 2005). NAD<sup>+</sup> dependent DNA ligases are present in bacteria and certain entomopox and mimi viruses. They are essential for bacterial growth and hence considered as important drug targets. Glycosyl ureide, glycofuranosylated diamines and tetracyclic indole derivatives have been identified as novel inhibitor molecules that specifically inhibit the *M. tuberculosis* NAD<sup>+</sup> dependent ligases over other bacterial ligases. Further analysis of its binding pockets and interactions with bound water networks is being carried out to further improve the inhibitor selection. In addition, the group has identified that BRCT-domain of DNA ligase is essential for the bacterium's survival and is currently

carrying out molecular docking studies to explore inhibitors against this domain as well.

### **OPEN SOURCE DRUG DISCOVERY: A PARADIGM SHIFT**

The Council of Scientific and Industrial Research in India has recently initiated a novel comprehensive drug discovery programme termed 'Open Source Drug Discovery for Tuberculosis' (OSDD) (<http://www.osdd.net/>), a network project encompassing R&D institutions, academic institutes, Universities and Industries in a collaborative mode (refer Commentary by Singh, 2008). This project aims to employ modern genomic, proteomic and informatics technologies with a strong inclination to apply a concerted effort to address the Drug Discovery programme against tuberculosis. The project draws strengths from the success of Open Source models for IT (e.g., World Wide Web Technology, GNU/Linux Operating System) and Biotechnology (e.g., Human Genome Sequencing). The Government of India has committed Rs. 150 crores (US \$30 million) towards this project. An equivalent amount of funding is expected to be raised from international agencies and philanthropists. Nearly 50 crores (US \$10 million) has been already released for Phase I of the project.

Nearly one-third of the world's population is presently infected with tuberculosis with India

being the highest TB burden country globally. OSDD provides an open platform to "collaborate, share and discover" and also provides tools for the same. OSDD contains a comprehensive TB database called Mtb Sysborg, which provides extensive information related to the complex biology of the pathogen, drugs and their interaction with different proteins, protein-protein interactions etc. It also provides computational resources at one unique platform to aid the initial stages of target identification and drug discovery (<http://crdd.osdd.net/>). Open bookmarks and TBprints archive are other tools to share discussions, online resources as well as to provide published and unpublished data related to drug development against tuberculosis. Current developments in the project includes several active sub-proposals to identify drug targets, virtual screening and in silico toxicity work.

The OSDD programme has been divided into several research packages to address various stages of a Drug Discovery process. It aims to encourage and support ideas and sub-proposals from the scientists at all stages of this process in an open source mode. Since a Drug discovery program would require expertise from scientists, doctors and technocrats from diverse schools of thoughts and disciplines, current partners in the OSDD program are from diverse research fields and cutting across barriers of university, industry and institutional structures. This novel way of recognition serves as an important platform for

multi-partnership in OSDD and enriches the OSDD project. The work for drug discovery and development is proposed to be shared with Academia, University students and CRO's at much lower cost and at a much varied platform. These sub-projects with defined short goals can hence be implemented at minimal costs and will also be a matter of pride and challenge for the brilliant young students contributing in various stages of the project.

Protection in OSDD is through clickwrap and copyleft protection. Therefore, the processes of sharing knowledge and encouragement of growth of ideas can go at fast pace. All IPR rights will be shared among the OSDD community whose contributions are monitored by a microattribution system. The new chemical entity (NCE) generated under OSDD will be undertaken for clinical trials using a consortium of Pharma industries that will be given manufacturing rights. The IPR would be held by CSIR which proposes to make the NCE generated by OSDD as 'generic' by sharing it with multiple pharmas. This may stimulate some healthy competition, but will help avoid or discourage sole proprietary motivation and also help to bring the cost of a potential drug molecule as low.

#### **FUTURE DIRECTIONS: PERSONALIZED MEDICINES**

The availability of human genome sequence has heralded a new era of predictive medicine

yielding an interesting aspect of future drug development and biomarker discovery. Complex disorders like epilepsy, schizophrenia, diabetes, cardiovascular disorder are some of the major public health problems even in India. It requires long term medication and leads to an expensive treatment through conventional methods. Hence, availability and affordability of drugs to combat such complex disorders is a key factor.

Pharmacogenomics, which has the potential to reveal the genetic basis of adverse drug reaction and modulating therapies to specific genetic profiles of patients, is expected to play a key role in clinical medicine in the coming years. The choice of antiepileptic drugs in patients, for instance, is based mainly on efficacy and safety. However, a section of Indian sub-population tolerates much smaller doses of antiepileptic drugs as compared to other populations with different genetic profiles. One of the aims of pharmacogenomics is to implement the concept of 'personalized medicines' by fine tuning of such drug dose adjustments to minimize side effects and improve absorption, transport and metabolism of these drugs in a population specific manner. This also raises the potential of making affordable drugs in near future by reviving withdrawn drugs for use by specific sub-populations only thereby also minimizing their side effects. In order to keep the low cost or withdrawn drugs in the market, identification and development of drug response biomarkers would also be of immense therapeutic potential.



The biomarkers identified in this respect would not only help in diagnosis of disease conditions and predict efficacy of different drugs but also in prediction of risk of disease in some populations.

In an effort to generate a map of genetic structure of Indian population which will help compare the same with other populations, mapping of single nucleotide polymorphisms (SNPs) in 55 diverse populations of India was carried out (Indian Genome Variation Consortium, 2008). This is a significant achievement wherein comprehensive analysis of the Indian population with respect to disease predisposition and drug response genes has been carried out. Details of this study are available in the form of a database, IGVdb (<http://www.igvdb.res.in/>). This database would be used to provide basis for epidemiological data to differentiate patients on the basis of their response to a drug. This would be of immense value in pharmacogenomics or predictive medicine & drug response in the country. A study conducted on Indian population to monitor the response of drug salbutamol revealed a significant genotypic association of a non synonymous SNP (Kukreti et al., 2005). Populations having AA genotype at nucleotide position 46 of beta adrenergic receptor gene were poor responders of the drug while the population with genotypic frequency GG were good responders. In another recent study, a functional polymorphism at codon 158

(Val/Met) (due to G/A transition) in catechol-*O*-methyltransferase (*COMT*) gene is found to be very important for the metabolism of catecholamines and catechol estrogens. Val/Val genotype was found to have a three–four fold higher activity of the *COMT* enzyme than Met/Met genotype. In clinical based studies there are evidences that the Val158Met polymorphism could be related to response to typical and atypical antipsychotics (Illi et al., 2007). In a study conducted on south Indian population, a plausible link between response to risperidone and specific polymorphisms located within the *COMT* gene (Gupta et al., 2009) were found. Furthermore, data for the Indian population for *COMT* SNP at codon 158 (Val/Met) showed variable allelic frequencies (Kukreti et al., personal communication) among the studied 24 populations (Figure 1). This data may provide a framework for designing future epidemiological studies to identify subpopulations with differential response to a given drug. Such studies would also open a new era of preventive medicine away from curative medicine.

## CONCLUSIONS

India's R&D strengths in the last few decades have been in the fields of organic synthesis, purification, extraction and structure elucidation of small molecules. This has powered India to be a world leader in the production of generics and a provider of affordable drugs the world over.

Indian national R&D institutions and pharma companies have acclaimed huge successes by doing reverse engineering and at the same time have acquired skills and confidence to engage into high-end research and technology development. Several programs have been developed to bring industry and national R&D laboratories at a common platform to complement each other and implement this newly found confidence into the drug discovery and development process. Despite initial successes in new drug development, generics will continue to be one of the major strengths of Indian pharma industry in the coming years. The recent economic recession and stringent regulatory action for approval of new drugs are among some of the factors that will push pharma industries towards generics products. Moreover, an estimated \$62 billion worth of drugs are expected to be out of patent during 2006-2010 including some of the 'blockbuster drugs' such as Merck's simvastatin, GSK's fluticasone, Pfizer's cetirizine and GSK's carvedilol etc (<https://www.prescriptionsolutions.com/c/rxnews/rxnews.asp>). This opens up the market for important drugs that can be made available as generics and the Indian pharma industry would help in bringing down the cost by taking up the production of these blockbuster drugs.

India's foray into drug development and patent reforms in the 1970s helped achieve affordable healthcare to its people. Under the new IPR regime since 2005, following the patent

amendment as per WTO agreement, India has now included product patents along with process patents. The new patents regime also allows for patenting of herbal preparations. In order to exploit traditional medicinal knowledge for cure of various diseases, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy-(AYUSH) in collaboration with CSIR is creating a Traditional Knowledge library. This would not only help in (re)exploring traditional medicines as pharmacophores but also help in preventing patenting of prior knowledge by MNCs. In addition, India has reinvigorated its research programs in the field of herbal medicines and formulations to develop products with IPR potentials at affordable costs. Several new avenues of drug development are also being explored. Increased attention is being given to new and upcoming fields to develop generic versions of 'biotherapeutics' a relatively new class of medicines based on proteins/biomolecules made by living cells. Many genetically engineered protein molecules are increasingly being used to treat diabetes, arthritis, cancer, and other diseases. A field emerging strongly as an interface of biology and engineering, 'synthetic biology' aims at design of new and natural biological systems to synthesize small molecules or their precursors in designer biological systems for high efficiency and accuracy. Several groups in India have begun to explore synthetic biology as an important tool to manufacture cost-effective

drugs. Combined with the visionary and revolutionary projects such as Indian Genome Variation and Open Source Drug discovery, India is making its presence felt in the global scientific map. While the former aims to implement personalized medicines for various Indian populations, the latter addresses a menace affecting a sizeable portion of its population. The national laboratories and academic institutions in the country will have to come forward to take new and more aggressive research initiatives and play a major role to bring in the next revolution in the Indian pharmaceutical sector to ensure affordable healthcare is maintained as a right of all.

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**Table 1.** Some leading CROs in India and their major activities. More than 40 CROs are approved by DSIR. Complete list is available at DSIR Annual Report, 2007-08.

<b>Name of Contract Research Organization</b>	<b>Services provided</b>
Alembic Research Center	The services provided are in the domains of Chemistry, Formulations, Pre-clinical Bioequivalence / Bioanalytical studies
Aurigene	Offers services in protein sciences, structure based drug design, medicinal chemistry and natural product screening and synthesis
Bharat Biotech	Manufacturing capabilities in vaccines and biotherapeutics
Chembiotek Research International	Offers services in medicinal chemistry, chemical process development, custom synthesis and analytical chemistry along with assay development, cell and molecular biology methods, in vivo pharmacology and computational biology
Clinigene	Offers services in Phase I-IV clinical trials and studies for novel/generic molecules to international pharmaceutical majors
Discovery Partners International	Provides services in areas such as target characterization, library design and synthesis, high throughput and high content screening, lead generation and optimization, gene expression and protein crystallization
GVK Biosciences	Services in informatics, medicinal chemistry and clinical R&D
Jubilant	Jubilant and its subsidiaries (Jubilant Organosys, Jubilant Biosys, Jubilant Chemsys, Jubilant Clinsys) provide a complete range of manufacturing of small molecule, clinical trials and management services
Premas Biotech	Offers life science research solutions (Cloning, Expression, Protein Purification, Biochemical & Cell Based Assays, Reporter Gene Assays, Characterization Studies) along with Protein Production & Manufacturing Services
Reliance Clinical Research Services	Provides preclinical services, Phase I Clinical Trials, Phase II-IV clinical trial management, monitoring and training
Shantha Biotech	Services in the fields of gene cloning, monoclonal and polyclonal antibody development, fermentation, recombinant protein expression and purification, optimization of fermentation processes
Siro ClinPharma	Services in different phases of clinical trials and in all key areas of the drug development process.
Strand Solutions	Offers value-added technology services in the field of bioinformatics, cheminformatics, data management and automation
Syngene	Offers services in synthetic chemistry and molecular biology for early stage drug discovery and development
Veeda	Full service global CRO specializing in the early clinical development of drugs.

### Legends to the Figure

**Figure 1:** Gradient map showing distribution of allelic frequencies associated with COMT (rs4680) (G/A) in Indian populations. The color gradient (light to dark) depicts the range of observed frequency from minimum to maximum.

