In pursuit of new drugs—Central Drug Research Institute

B. N. Dhawan and Zaka Imam

Introduction

The Central Drug Research Institute, established under the aegis of the Council of Scientific and Industrial Research (CSIR) in 1951, is primarily concerned with various facets of drug development. This article presents a perspective of new drug development, CDRI's current and future programmes.

New drug research

New drug discovery is often serendipitous or chance finding during screening of synthetic chemical compounds or natural products obtained from terrestrial plants and marine flora and fauna. On an average, one drug is developed out of about 10,000 compounds screened, due to high mortality of compounds during post-screening development work. It takes 10-20 years to complete these developmental studies and the cost may run to several hundred million rupees.

Efforts worldwide to develop rational drug designing are aimed at reducing the number of compounds required for synthesis and evaluation and cutting the cost and time. Based on available knowledge an appropriate structural type may be selected as a 'lead' molecule, followed by further work in three steps: (i) Design and synthesis of a few selected prototypes of lead molecule, (ii) Biological evaluation and elucidation of structure-function relationship of prototypes identifying the role of their structural parts in terms of pharmacologic and pharmacodynamic characters, (iii) Lead optimization by molecular modification of prototypes. The effects of modification are quantified and the quantitative structure–activity relationship (QSAR) is facilitated by computer. Computer is also employed for better understanding of biochemical receptor-drug interaction. Knowledge acquired through such studies helps modify the three-dimensional geometry of the drug molecule to enable high selectivity with optimum binding of the drug to the active site and in some cases response predicted by computer modelling.

During recent years it has been possible to identify critical rate-limiting enzymes or specific receptors regulating vital metabolic activities and neurotransmitter synthesis and their release. This has helped in developing a target-based approach wherein the new molecule may be an enzyme inhibitor like captopril or a receptor blocker. In many instances the possible therapeutic indications may be uncovered after biochemical studies are completed.

Once a novel compound is obtained, it is desirable to determine its spectrum of biological activity. To be able to pick up novel types of biological activity and for better extrapolation of findings to patients, constant efforts are needed to develop better animal models and in vivo test systems. After a compound is found effective during primary biological screening in animals and the results confirmed in the same and other species of animals it is followed by detailed pharmacological studies to evaluate its effect on various organ systems, pharmacokinetic studies to work out its metabolism and excretion, toxicity studies to determine its possible harmful effects, standardization and quality control and process upsaling to prepare large quantities of the compound. Activity and toxicity data of the compound are submitted to the drug regulatory authority and also to the necessary ethical committee for permission to conduct clinical studies in humans. In India, the permission for clinical trials is granted by the Drugs Controller of India (DCI).

Research and development at CDRI

CDRI annually screens about 1000 new compounds/plant extracts through approximately 100 screens (test systems) to pick up compounds with promising biological activity. These are then subjected to a detailed activity profile study, including comparison with available standard compounds and analysis of pharmacodynamic activity profile. The compounds showing clinical potential are evaluated for subacute and chronic toxicity in at least 2 animal species. Simultaneously, compound process is also upscaled to produce it in large quantities to meet requirements of toxicity testing and clinical trials in normal human volunteers and quality control criteria are laid down.

R&D programme

The Institute's multidisciplinary R&D programme is currently organized under project areas—antifertility, malaria, filariasis, leishmaniasis, immunology and microbial genetics, natural products, cardiovascular and nervous system disorders, other pharmacological studies, new drug standardization and delivery systems, fermentation technology, chemical technology, development of new leads, chemistry and biology of peptides, toxicology, clinical trials and biological screening.

Technology innovation

There are adequate facilities for development of suitable chemical/fermentation technology for known drugs and to undertake pilot plant scale production of the Institute's candidate drugs. The Institute also undertakes development of suitable process technology for known drugs, drug intermediates, biologicals and fermentation products.

R&D infrastructure

The Institute has facilities for development of a new drug from its synthesis/
isolation to clinical studies. The Institute has 18 research and development divisions manned with specialised groups of scientists and equipped with facilities to perform specific roles in the Institute’s R&D programme.

National facilities/services

Several national agencies participate in the Institute’s programmes by establishment of national facilities.

(i) ICMR Centre for Advanced Pharmacological Research on Traditional Remedies pursues biological evaluation of traditional remedies against selected diseases for which no suitable modern therapy is currently available like bronchial asthma, urticaria, hepatic disorders or where better agents are needed as in the case of leishmaniasis and filariasis. The contributions made by the centre are: development of Picrotin, a standardized extract with hepatoprotective activity from Picrotica kuruwa, the major constituent of the extract being picroside I and 2-ketopicroside; identification of antifilarial activity of Streptus asper in its 2 glucosides; standardization and quality control of specifications for Khassarutra to effect minimal variations in clinical preparations; identification of antiprotozoal activity of Crataeva nurvala in its active principle, luperol.

(ii) WHO Collaborating Centre for Preclinical Evaluation of Antimalariaals develops new drugs and other control measures against malaria by screening potential antimalarial compounds for blood schizontocidal, causal prophylactic, antirelapse and gametocytocidal activities in rodents and rhesus monkey. It imparts training in maintenance of rodent malaria parasite and primary screening of potent antimalarial compounds. It also supplies strains of rodent and simian malaria parasites to other institutions.

(iii) National Laboratory Animal Centre (NLAC) has breeding and maintenance facilities for animals belonging to 15 species including rodents, cats, dogs, monkeys and birds under standard husbandry conditions. There are also facilities for breeding specific pathogen free (SPF) and germ-free (germfree) rodents and for constant health monitoring of animals. In addition, a tissue culture laboratory and a primate behaviour facility are being developed.

(iv) Regional Sophisticated Instrumentation Centre (RSIC) has facilities like electron microscopes (SEM and TEM), high pressure liquid chromatographs, elemental analysers, gas liquid chromatograph, IR spectrophotometers (including FT IR), mass spectrometers, NMR spectrometers (H, 13C, 400 MHz FT NMR), and scintillation counters. RSIC analyses about 30,000 samples every year.

(v) National Information Centre for Drugs and Pharmaceuticals (NICDAP) caters to information needs in the area of drugs and pharmaceuticals. It possesses a computerized system for information retrieval and library management. The centre is linked with Dialog databases for online searching. It has been identified as a Centre for Biotechnology Information System Network, as an Information Centre of Department of Ocean Development and is the only WHO Collaborating Centre on Drug Information for countries of the southeastern Asia.

Achievements

Achievements vis-a-vis national objectives

Fertility regulation. The Institute’s efforts led to development of Centchroman, a safe nonsteroidal oral pill with good pregnancy protection; Isapent, a cervical dilator used in medical termination of pregnancy; and Conpax, a spermicide cream currently under clinical evaluation.

Control of tropical diseases. The major thrust areas are malaria, amoebiasis, leishmaniasis (kala-azar) and filariasis.

The Institute’s collaborative work with CIMP led to development of Artemisimine-artether which is likely to emerge as an effective antimalarial drug against cerebral and resistant cases of malaria caused by Plasmodium falciparum. An antirelapse antimalarial drug, compound 80/53, is 3-4 times less toxic than the existing antimalarial drug primaquine. Both these products are undergoing clinical assessment.

Filariasis is highly endemic in India and around 300 million people residing in filarial areas are at the risk of infection while nearly 35 million suffer from the disease. Deformities due to filariasis, once established, are irreversible. Therefore, early diagnosis of the disease is necessary for proper treatment. The conventional night blood examination for microfilareae is inconvenient for field application. The Institute has developed a filariasis skin test which is sensitive and specific and has been commercialised. Two new microfilarial drugs (81/470, 82/437) synthesized at the Institute are under detailed evaluation and Centperazine is under clinical trials.

The Institute has found a protective role of interferons in various fungal infections, including keratitis, in experimental animals and in experimental malaria in the rhesus monkey.

Liposomised drug preparations have been successfully used for drug targeting against kala-azar, chloroquine-resistant malaria and fungal infections in animal experiments.

Vaccines. The Institute has developed a vaccine against leprosy. The vaccine derived from Mycobacterium habana, an atypical strain, has been found effective and safe during preclinical studies. This vaccine can be produced at low cost because pure culture of M. habana can be easily grown in large quantities and is the only vaccine effective in the mouse foot pad test.

A surface protein from Vibrio cholerae has been isolated and its gene coding subclone. Combined vaccination with this protein (22 kD) and B subunit of V. cholerae has provided full protection in animal experiments.

Drug delivery systems. Development of long-acting injectable, skin and oral preparations has been initiated. Some dosage forms under development are: clofazamine and dapsone combined tablet for leprosy, rifampicin sustained release injectable for tuberculosis, primaquine transdermal tape for malaria, Centbrvidine long acting injectable (local
Anaesthetic) and Centhaquine transdermal tape (hypotensive).

Exploration of medicinal plants. The Institute is engaged in isolation and identification of biologically active compounds from the country’s vast plant resources. Over 3700 species of plants, including those used in traditional medicine, have been evaluated and the plants found active subjected to chemical and pharmacological investigations. Almost 100 biological test systems have been standardized and employed for screening of plant products as well as those obtained from marine organisms. These have led to identification of several active compounds: arabitosides (Nuxvartes arbortristis), arcein-1 (Arnebia nobilis), acskepin (Asclepias curcas var. indica), bacosides A B & B (Bapusa monieri), colocyn (Coeles forskohlii), coumarins (Cassia auriculata), curcumin (Curcuma longa), gugulipid (Commiphora mukul), hyatin (Cissampelos pareira), pteroclad and kuttakside (Pierolizita curvata), polyose (Tamarindus indica), psoralen (Psoralea corylifolia), punarnavosid (Boerhavia diffusa), spasmolytic sesquiterpenes (Cedrus deodara) and spermicidal saponins (Sapindus mukorossii). New biologically active compounds from plants have provided valuable leads for designing novel and more effective compounds. Besides, a large number of new and unusual structures have been discovered from plants.

Exploitation of marine flora and fauna. The Institute is coordinating a multi-laboratory national project aimed at investigating marine flora and fauna for development of new drugs. So far, 450 extracts derived from marine organisms have been screened and about 150 of these have been found to possess antifertility, antiviral and pharmacological activities. Organisms showing significant activity include the marine alga Uva fasciata and a star fish (antiviral activity), the sea-weed Padina tetrasomatica and a sponge (antifertility activity) and a coral (antihypertensive).

Import substitution through indigenous technology. Nine technologies are currently being used for commercial production of known drugs/drug intermediates.

Technologies for rural application. These include mosquito larvicide (Biocide), test paper for iodine detection in common salt, isapent (cervical dilator), palatable laxative granules, filariasis diagnostic kit and an intravascular injection device for sterilizing scamb cattle.

Products released are being marketed by companies under their trade names. (i) Centbrine, a non-steroidal oral contraceptive for women, has been introduced in the National Family Welfare Programme. (ii) Gugulipid, a hypolipidaemic product from the resin of Commiphora mukul was reported as a significant new drug introduction in the world during 1988. (iii) Isapent, a cervical dilator developed from psyllium (isapgo) seed husk. (iv) Centbrine (Biocide), INN, an oral anaesthetic for infiltration and nerve block anaesthesia. (v) Centbutindole (Biperone, INN), a neuroleptic drug. (vi) Centimizone (Mipazine, INN), an effective antihypertensive compound useful in treatment of thyrotoxicosis. (vii) Diagnostic kit for amoebiasis (extra-intestinal), a kit useful for serodiagnosis of extra-intestinal (invasive) amoebiasis, has been developed from a preparation of whole antigen of axenic Entamoeba histolytica. (viii) Diagnostic kit for filariasis, a skin test for early diagnosis of filariasis, has been developed using whole antigen of infective larvae of Brugia malayi, the human filarial parasite.

Drugs under development

In clinical trials: Centpropazine, antidepressant; Centperazine, antifilarial; Curcumin, anti-inflammatory; Choromun iodide, neuromuscular blocking agent; Consap, contraceptive cream; Compound 80/53, antirelapse antimalarial; Artemisinine artemether, antimalarial; Compound 80/574, hypolipidaemic; Picroliv, hepatoprotective; Compound 73/602, antiallergic; Compound 81/470, broad spectrum anthelmintic and Centperazine, antifilarial, are in different stages of clinical trials.

Under preclinical safety evaluation: Compound 82/4317, antifilarial; Bacosides A and B, memory improvement; Compound 78/103, antidepressant are undergoing preclinical toxicity studies.

Technology development

Bulk drugs. Processes for about 75 drugs, drug intermediates and fermentation products have been standardized and 50 of these released to industry. The following processes are currently in commercial production: Clofazimine and Ibuprofen; Paracetamol, O-2 Amino butanol and Pyrithioxin, Dextropropoxyphene hydrochloride, N-Methylpiperazine, 5,6-Dimethylenimidazole, L-Phenylephrine hydrochloride, 1-Acetylphenylcarbinol. The other processes released include those for indomethacin, amitryptiline, ampicillin, clonidine, bacampicilin, cyclophosphamide, tamoxifen, sulphamethoxazole, cyproheptadine, nitrofurans, ethacridine lactate, 1-dopamine, trimethoprim, 2-furoic acid, primaqueine, lidamidine, pralidoxime iodide, pseudoephedrine.

RIA Kit for steroidal hormone. Technology for production of antigen conjugates for steroidal sex hormones (estrogen, progesterone and testosterone) has been developed.

Technology for biocide. Technology has been developed for the production of Bacillus sphaericus spong toxin complex formulation, a mosquito larvicide, and scaled up to 1500 litre fermenter. Field trials have shown encouraging results.

Test paper for iodine detection in salt. It is a simple test paper for detection of iodine in common salt; the iodine content is determined by comparing the colour of used test paper strips with the given reference colours.

Palatable laxative granule. Technology has been developed for preparation of effervescent and non-effervescent enteric coated types of sweet and flavoured laxative granules from isapgo seed husk derived from the plant, Plantago ovata.

Basic studies

Basic research forms an integral component of each project area, and roughly accounts for 25-30 per cent of effort in each area. The major areas of basic research include: Studies on parasites particularly host--parasite reaction and metabolic and neurotransmitter pathways of the parasite; characterization of protective antigens and cloning of toxin coding genes; structure and function of biological membrane like RBC membrane; ligand–receptor interactions; QSAR and computer-modelled drug design; sugars as synthons for synthesis of biologically active compounds; phy-
siology and ultrastructure of reproductive tract; adrenergic mechanisms in cardiovascular and nervous systems.

**Significant findings**

(a) Studies on mechanisms governing phospholipid asymmetry in erythrocyte membrane have demonstrated that it is maintained primarily by an ATP-dependent aminophospholipid pump in close interaction with membrane skeleton-bilayer.

(b) Studies on estrogen receptor interaction and possible binding orientation of estrogens and antiestrogens led to a composite model that delineates five subsites on the estrogen receptor, some of which are important for estrogenic activity while others are more suitable for antioestrogenic activity.

(c) Compounds capable of making chloroquine-resistant malarial parasite susceptible to chloroquine have been discovered.

(d) A new proaggregatory factor of platelets has been isolated from mouse plasma. The factor is of low molecular weight, heat-resistant and calcium ion-dependent for its activity.

(e) Site of action of clonidine has been demonstrated to be localized in the ventral surface of medulla. Cholinergic link in its action was demonstrated with the help of localized application of drugs, microiontophoretic studies on single neuron and receptor binding studies.

(f) Role of monoamines has been elucidated in the interaction of nonconventional cardiovascular areas like central nucleus of amygdala, A5 cell group of medulla, caudate nucleus, nucleus tractus solitarius, paraventricular nucleus of hypothalamus and dorsal raphe nucleus. The excitatory role of adrenergic mechanism and the presence of delta opioid receptors predominantly in the vasomotor areas of medulla have been demonstrated.

(g) Neurotransmitter binding sites of *Setaria cervi*, a bovine filarial, have been characterized by high-affinity radioligand binding assay. The binding sites of $^3$H-GABA are atypical and different from mammalian GABAergic receptors. $^3$H-spiroperidol exhibits high and low affinity binding with *S. cervi* membrane, the sites appear to be dopaminergic and serotonergic respectively. The typical nature of neurotransmitter receptor in filarial parasites suggests their probable use as targets for drug design.

(h) Biogenetic studies have been undertaken on several isocoumarin, aporphine and protoberberine alkaloids in relevant plant tissues.

**Future developments**

The R&D programmes of the Institute are being constantly reviewed. Several major facilities are planned to be added during the next few years. These include: (i) Medium voltage electron microscopy for detailed study of cell organelles in their natural habitat and of macromolecules, (ii) parasite bank for procurement, maintenance and supply of protozoal and helminth parasites and vectors for parasite antigens/biochemical studies; (iii) protein engineering facilities for application in development of new vaccine and site-specific drug delivery systems and study of drug-receptor interactions; (iv) cell culture facility for maintenance of cell culture lines and hybridoma, and (v) primate behaviour facility developed on existing primate centre to study the effect of psychopharmacological agents, dependence producing compounds and for development of primate models for neurological disorders and (vi) computer modelling and computer graphics for design of new molecules.

B. N. Dhowan is Director and Zaka Inam is Scientist, Central Drug Research Institute, Lucknow 226 001.