LECTURE

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Design of New Chemical Entities as Therapeutic Agents

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IN spite of the fact that the biological system is highly complicated phenomenon and new drug discovery is time consuming and enormously expensive, several new chemical entities (NCEs) for human therapeutic use are being introduced in the world market. In 1995, thirty seven NCEs were introduced while in 1991, 1992, 1993 and 1994, this figure was 36, 37, 43 and 44 respectively. Most of these mainly launched by developed countries are believed to have resulted upon a minor improvement of chemical "lead structure" giving rise to so-called 'me too' products.

Broadly, there are two main sources for new drug discovery. These are 1) Natural products and 2) old/existing drugs.

Historically, natural products isolated from plants have continued to provide a source for new drugs. These products serve as chemicals "leads" whose structures are modified to obtain more specifically active drugs. Recent examples of drugs from natural source are anti-cancer drugs: vinblastine and vincristine from *Vinca rosea* and taxol from *Taxus brevifolia*. Another simple way for the discovery of new molecule is to start with old/existing drugs. The chemical structures of these are cleverly modified to obtain superior activity and selectivity. For example, the meticulous structure modification of nalidixic acid resulted in the discovery of fluroquinolones as the fourth generation broad-spectrum antibacterial drugs. Similarly many products belonging to 1,4-dihydropyridines, H^+/K^+ - ATPase inhibitors, H_2-receptor antagonists are some interesting examples of structure modification.

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RATIONAL APPROACH

With the advent of greater understanding of physiological mechanisms, it has become possible for a rational approach to obtain new lead molecules/drugs. The advances in science and technology such as chemistry, biology, biophysics, X-ray crystallography, multi-dimensional NMR spectroscopy and computers have played significant role in drug discovery. The knowledge of macromolecule-ligand interactions, database search of legands from a library of chemical compounds and various models of quantitative structure-activity relationships, molecular modeling and combinatorial chemistry have increased the opportunities for new lead generation.

Computer-aided drug design (CADD) methods are of particular interest in the early stages of drug discovery. They provide a variety of information (i) to examine, evaluate and compare complex molecular structures (ii) to modify structures and assess geometric and energetic consequences of such modifications (iii) to perform conformational analysis (iv) to build macromolecules (v) to dock small molecules into macromolecules (vi) to map pharmacophore of ligands (vii) to analyze relationship between chemical structure and biological activity and (viii) to predict activity of compounds/analogues before their synthesis.

METHODS

There are three major methods for drug discovery using the computer.

These are:

1) Quantitative Structure-Activity Relationship (QSAR) 2) Molecular Modeling and 3) Database Searching.

QSAR, a method introduced in 1962, is a useful tool to establish quantitatively the relationship between various physico-chemical properties and biological activities of compounds. Among the various models, the Hansch approach has gained tremendous importance over the years and many successful prediction of activities for new analogs have been reported. Basically, QSAR is applied for optimization of activity in a given series of compounds. The latest advances of QSAR are 3-D CoMFA, APEX-3D and automated HQSAR. There are number of successful applications of these and other QSAR methods. Few are shown here. (Fig. 1).

Molecular modeling has become a well established research area during the last decade due to advances in computer hardware and software. Molecular modeling systems are powerful tools for building, visualizing, analyzing and storing models of complex molecular structures that can help interpret structure-activity relationships. There are two major modeling strategies: direct and indirect design which are currently used in the conception of new drugs. In the direct design approach the three-dimensional features of known receptor sites are considered which are obtained from the X-ray crystallography and in the indirect approach, the design is based on the comparative analysis of the structural features of known active and inactive molecules that are complementary with a hypothetical receptor site. Both these methods help to understand drug-receptor interactions, molecular dynamics and ligand docking, analyse molecular surface similarity, molecular shape and fitting and calculate 3-D electrostatic and

SUCCESSFUL APPLICATION OF CADD

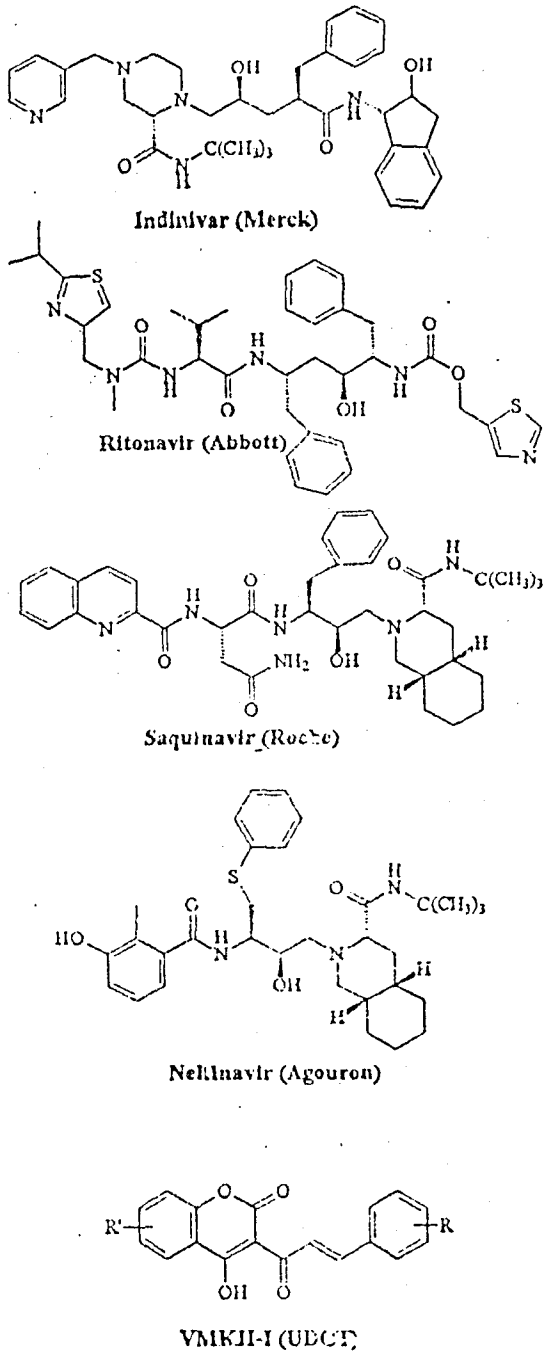


Fig. 1

X-RAY CRYSTAL STRUCTURES OF INTEREST IN DRUG DISCOVERY:

* AIDS Targets :
  HIV - PR, HIV-RT, Human CD4
* Bacterial Targets:
  DHFR, β-lactamase, DNA-gyrase, Cystein-PR (Malarial)
* Inflammation Targets:
  IL-1β, PLA2, ICE, COX-II
* Fungal Targets:
  Cytochrome P-450, Squalene Epoxidase
* Degenerative diseases of lung
  Human Leukocyte Elastase (HLE)
* Cardiovascular Targets:
  Renin
* Alzheimer's Disease:
  AChE, Serum amyloid-P Component
* Cancer Targets:
  Matrix Metallo PR, Farnesyl Phosphate Transferase

Fig. 2

lipophilic potentials. The X-ray crystal structure of macromolecules and their complexes with ligands may be obtained from Brookhaven Protein Data Bank (Fig 2). Number of scientific journals which focus on these strategies are: Journal of Medicinal Chemistry, Journal of Computer Aided Molecular Design, Journal of Molecular Recognition, Bioorganic and Medicinal Chemistry and Journal of Chemical Information and Computer Sciences.

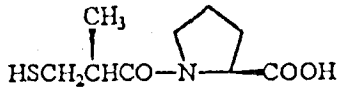
Software such as QUANTA CHARMm, INSIGHT II, CERIUS-2, CATALYST (Molecular Simulations Inc., USA) SYBYL (Tripos Associates,USA) and CHEM-X (Chemical Design Co., UK) are some of the most conveniently used programmes. These are menu driven and run very conveniently on the Silicon Graphics computer hardware (INDIGO, INDY etc.) Our division of Pharmaceutical Sciences and Technology in the Department of Chemical Technology of University of Bombay has these facilities for the basic research in new drug discovery.

At this point one may tempt to raise a question: "is there a drug that has come out successfully by the application of computers?". Certainly yes, but through the combined efforts of organic; medicinal computational chemists, pharmacologists, toxicologists, biopharamceutical and pharmacokinetic and clinical scientists and others. Unfortunately there is lack of interaction among these scientists in India.

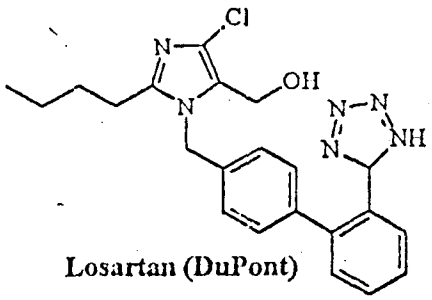
Following presentation covers some important applications of QSAR and Molecular Modeling using computers for the discovery of new chemical entities as therapeutic agents.

1. Discovery of Captopril

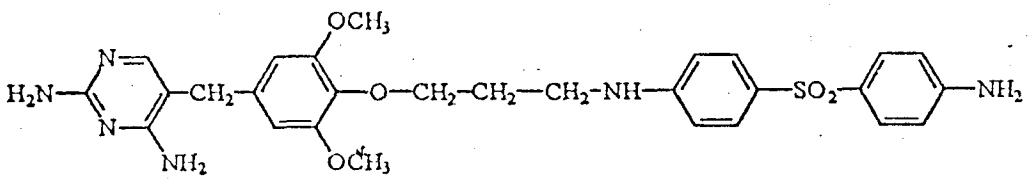
During 1974-75, the scientists of Squibb company in USA began efforts directed towards the design of low-molecular weight Angiotensin-Converting Enzyme (ACE) inhibitors. To address this challenge, they constructed a conceptual working model for the ACE active site by using the X-ray crystallography of carboxypeptidase A as a guide. From the model it was postulated that the replacement of carbonyl group of glutaryl and succinyl groups by a group having a higher affinity for zinc should result in a more potent inhibitor. Indeed, the substitution of a sulfhydryl group for this carbonyl gave captopril which showed a 1000-fold activity and good oral bioavailability. Models of this type which were studied in more detail by the use of computer graphics yielded Merck and Roche, analogs of captopril and later enalaprilat and cilazapril as antihypretensive drugs. The discovery of recent anti-hypertensive drug, losartan has also been through the building of hypothetical model of Angiotensin- II receptor (Fig.3). Correct prediction of new analogues have been reported by CoMFA application.



Captopril (Squibb)



Losartan (DuPont)



K-130 (UDCT - CDRI)

Fig. 3

2). Discovery of fluoroquinolones

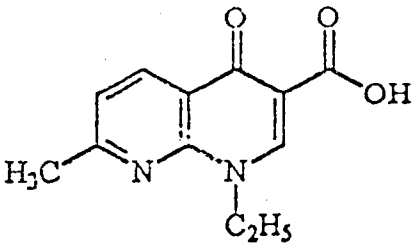
QSAR and Molecular modeling methods applied to several quinolones for antibacterial activity suggested that substituent effects at various positions were additive and the combination of favorable substituents gave excellent new compounds having expected activity. A steric parameter (L= 4.2) for a substituent at N1- position and a lipophilic parameter (logP= -1.0) for substituents at C7 -position afford optimal potency (Fig. 4). There are about 30 different fluoroquinolone drugs in clinical practice in the world. The X-ray crystallography of DNA-gyrase complexed with a quinolone compound should be more useful to find better antibacterial agents.

3. Discovery of ketorolac

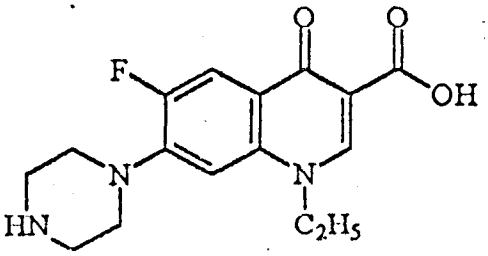
A series of benzoylpyrrolopyrroles was studied by QSAR for optimizing the analgesic activity. The variation of 4-phenyl substituents yielded better active compounds. Further study by QSAR suggested that 4-vinyl group would be more favourable than unsubstituted analong for activity (Fig.5).

4. Drugs as enzyme inhibitors

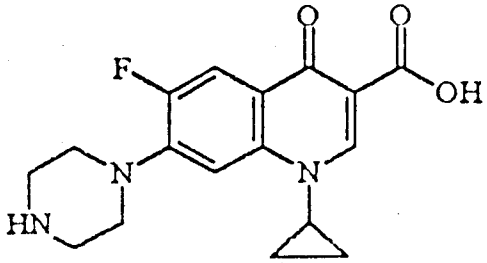
a) Development of anti-mycobacterial drug : A new highly active inhibitor of bacterial dihydrofolate reductase (DHFR) was developed by computer graphics. The compound, K-130 exhibited especially high



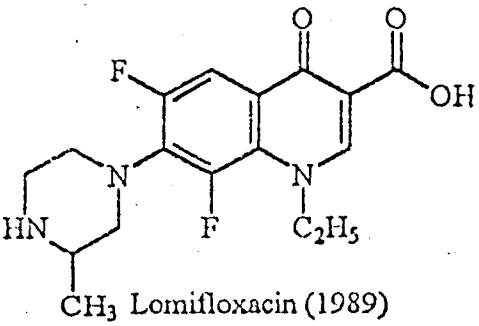
Nalidixic acid



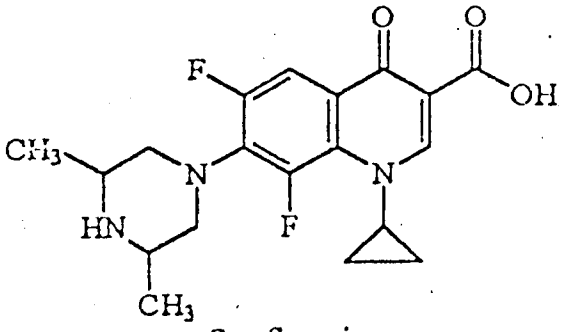
Norfloxacin (1984)
Kyonn



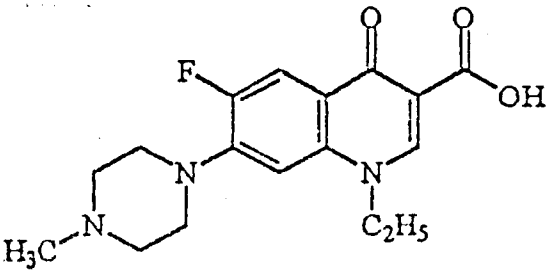
Ciprofloxacin (1985)
Darichi Seiyaku



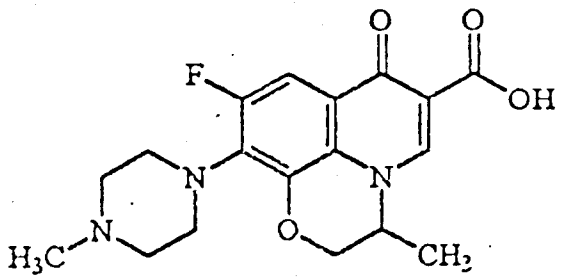
Lomifloxacin (1989)



Sparfloxacin
Dainippin



Pefloxacin



Ofloxacin

Fig. 4 : QUINOLONE ANTIBACTERIAL DRUGS

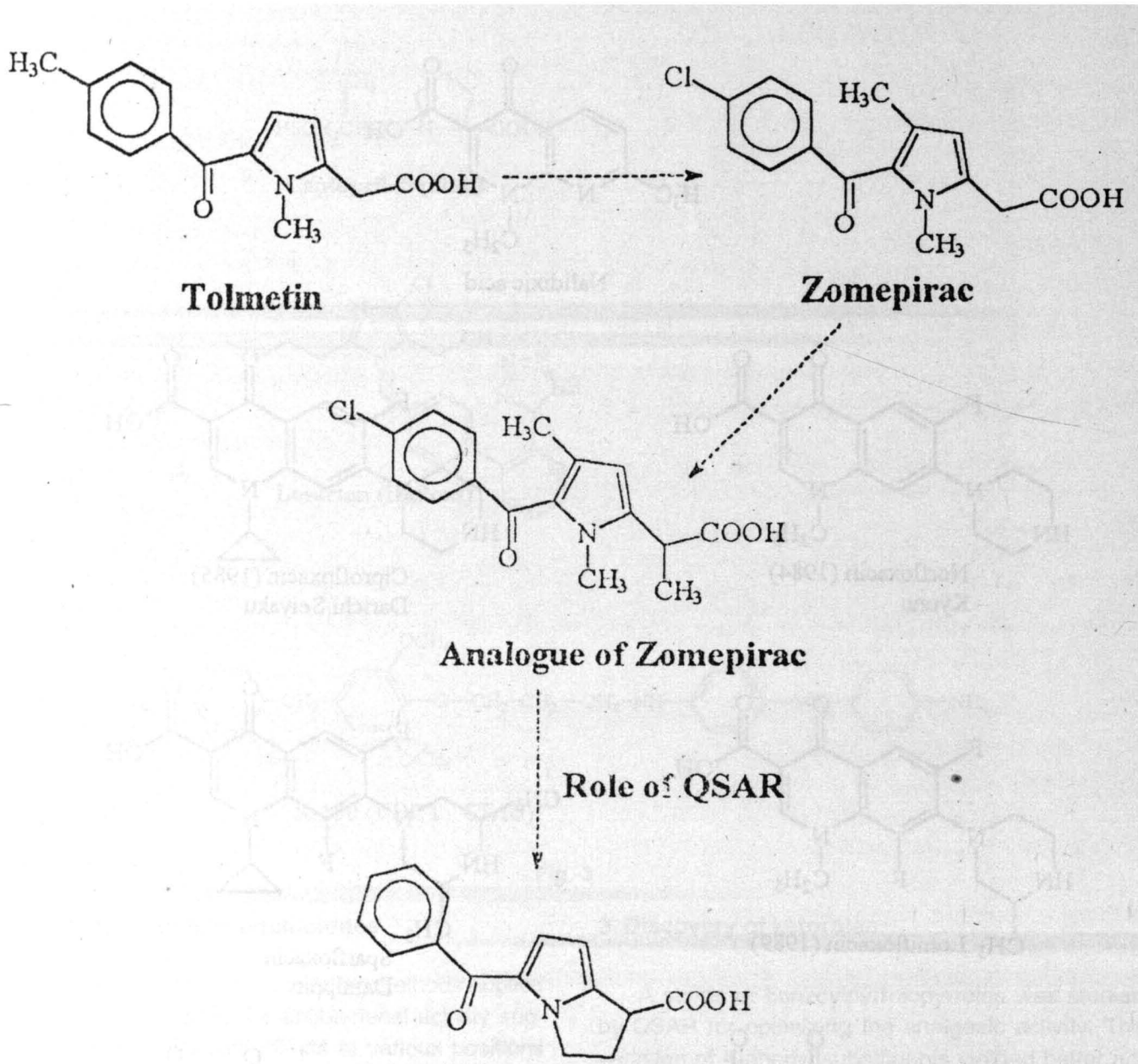


Fig. 5 :DISCOVERY OF KETOROLAC

activity against mycobacteria. This is an example of structure-based drug design since the X-ray crystal structure on the DHFR complexed with methotrexate in the active site was available. Further pharmacological and toxicological studies led the compound to clinical trials (Fig. 3).

b) Development of anti-HIV drugs: Human immunodeficiency virus 1 (HIV-1) protease is a dimer of 99- residue proteins which is vitally important to polyprotein processing in the life cycle of the Acquired Immunodeficiency Syndrome (AIDS) virus. This enzyme is currently the only significant macromolecular component of the AIDS virus for which detailed X-ray crystal structure information complexed with an

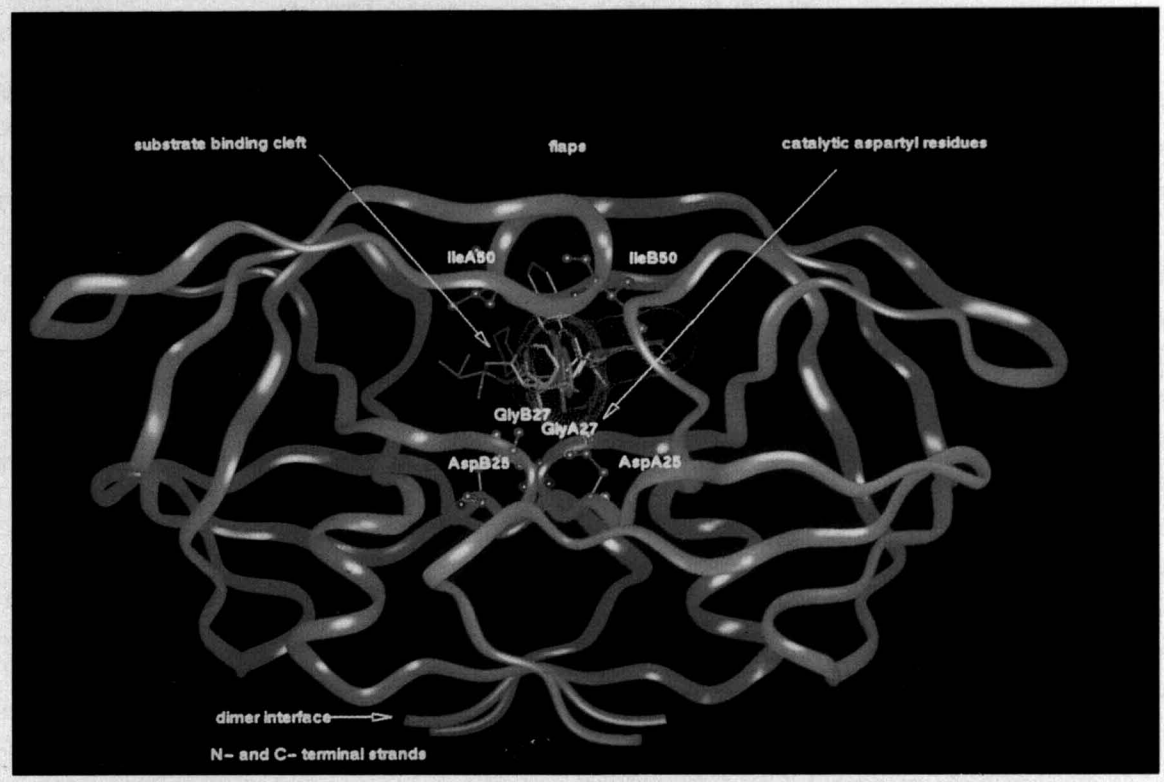


Plate 1: Docking of a coumarin analogue (VMKH-1) into the active site of HIV-1 PR enzyme

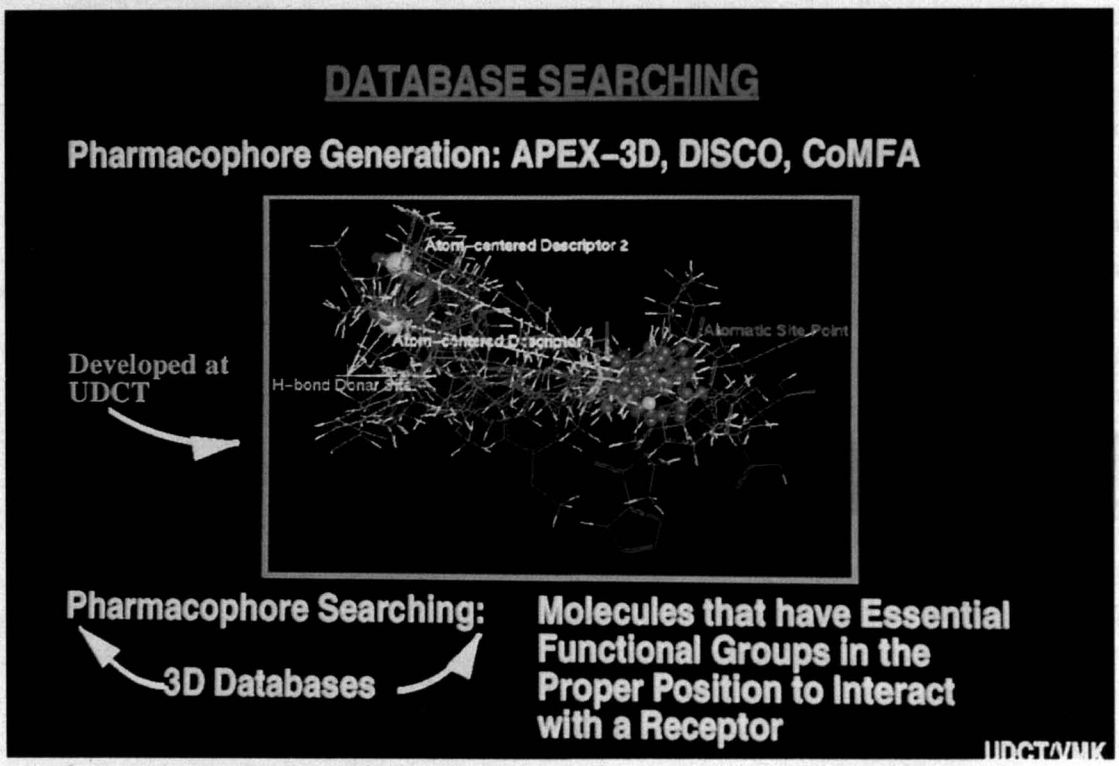
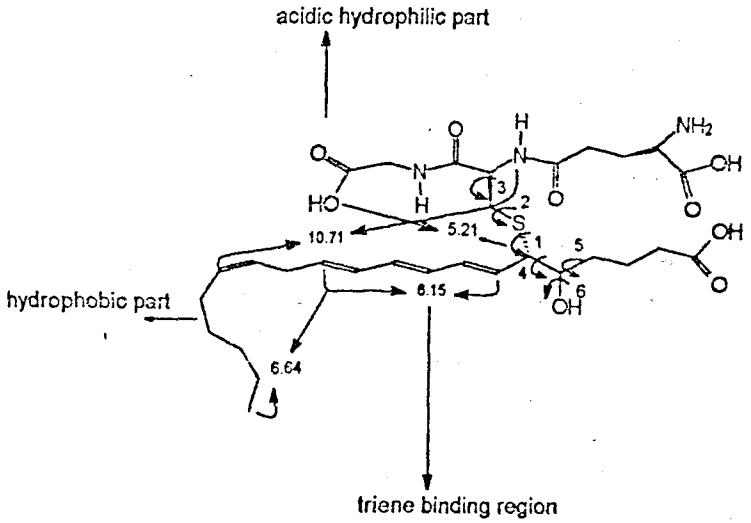


Plate 2: Pharmacophore modeling and database searching of peptide leukotriene receptor antagonists

Fig. 6: Receptor binding model for LTC_4



inhibitor in the active site is available and is a target of considerable pharmaceutical interest in the quest for AIDS therapies. The disease AIDS is spreading alarmingly and is of great health concern worldwide. Non-peptidic and good bioavailable drugs are required at the present time.

Computer modeling has been instrumental in the design of many of the HIV-1 protease drugs. These are: ritonavir (Abbott), indinavir (Merck), saquinavir (Roche) and nelfinavir (Agouron). However, these drugs are peptidic and have been reported with unfavorable pharmacokinetic properties. Using the crystal structure of HIV-1 protease complexed with U-75875 as starting point, we designed and synthesized a simple coumarin compound and tested *in vitro* for enzyme inhibition Plate-1. Out of very few compounds thus designed and synthesized, all showed very promising activity *in vitro*. Thus, a "lead" compound was generated and its further optimization yielded many more active analogs which are in extensive further biological screening.

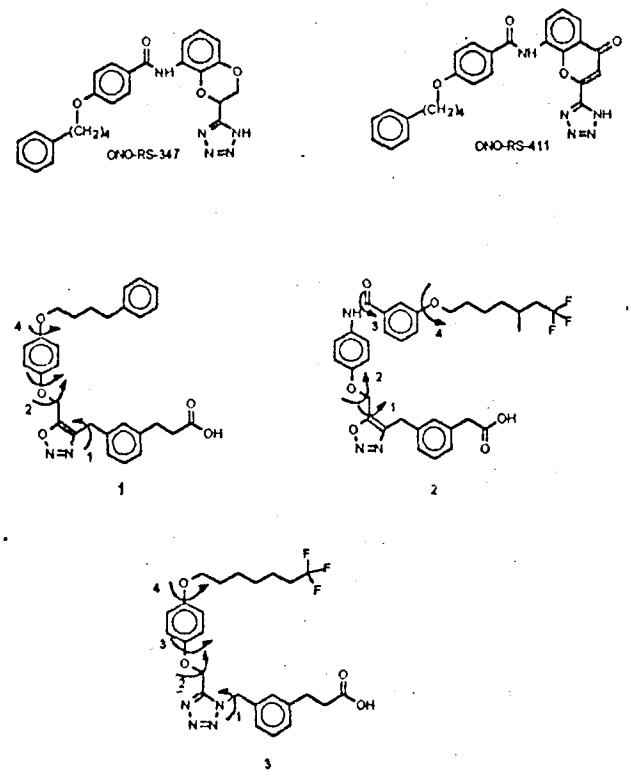


Fig. 7: DESIGN OF LTC_4 RECEPTOR ANTAGONIST

5. Development of LTC_4- antagonists

The leukotriens C, D and E are reported as potent mediators of allergic, inflammatory and other pathological events. Leukotrinene C_4 has been shown to cause bronchoconstriction, increased microvascular permeability etc. Since no structural information is available about the receptor active site, an indirect approach was adopted to understand important interactions necessary for binding (Fig. 6). Based on the natural agonist on the structure-activity relationship of known LTC_4 antagonists, we proposed conceptual receptor binding model and few new molecules were suggested for synthesis and biological testing (Fig. 7).

There are innumerable examples of successful applications of QSAR and Molecular modeling for the design and discovery of new drugs for a variety of diseases. However, it is impossible to cover all those in this presentation. Interested persons may refer abundantly available literature on the application on these techniques.

3-D DATABASE SEARCHING

Three-dimensional searching of large databases of chemical structures has recently gained attention for its ability to discover new leads in the drug development. This approach allows one to achieve rapid identification of compounds that possess the pattern(s) of atoms constituting the putative pharmacophore(s). A pharmacophore in a molecule refers to the three-dimensional arrangement of atoms or functional groups that is necessary for the compound to bind to a specific enzyme or receptor. 3-D database pharmacophore searching thus attempts to identify molecules possessing a particular pharmacophore in their structure.

National Cancer Institute (NCI) has built a searchable 3-D database of ca.408 000 structures of both synthetic and natural tested against cancer. Databases have also been built by Maybridge, Chapman & Hall, Current drugs, Cambridge Crystallographic Data, Chemical Abstracts Service, Derwent Drug Index, MACCS-3D and Aldrich.

The searching is performed through molecular modeling softwares through which the pharmacophore of the molecule is identified. We, in UDCT, have thus proposed a spatial pharmacophore for active conformations of some peptide leukotriene receptor antagonists (plate. 2), for cytochrome-P450 azole antifungals (Fig. 8). The pharmacophore developed for these compounds through APEX-3D and LUDI programs available within INSIGHT II and through UNITY within SYBYL was searched in a database of NCI and Maybridge. 165 new hit molecules were identified. Ten compounds were tested for antifungal activity. Encouraging results were obtained. Our method is similar to the NCI database searching where fifteen novel non-peptide HIV-1 protease inhibitors were identified by flexible 3-D database pharmacophore searching. Thus database searching is a powerful tool to quickly target those compounds in a library of hundreds of thousands of compounds that fit the pharmacophore and that make qualified to candidates to test for desired property.

COMBINATORIAL CHEMISTRY

Recent trends in the search for novel pharmacological agents have been on the preparation of "chemical libraries" as potential sources of new leads for drug discovery. Chemical libraries are collections of different molecules prepared either synthetically or biosynthetically and screened for biological activity. Combinatorial chemistry is a type of synthetic strategy which leads to large chemical libraries. It is defined as the systematic and repetitive, covalent connection of a set of different "building blocks" of varying structures to each other to yield a large array of diverse molecular entities. Combinatorial chemistry, teamed with computational technology, yields diverse libraries of chemical compounds-an important step in targeting compounds or collections of compounds with the most potential.

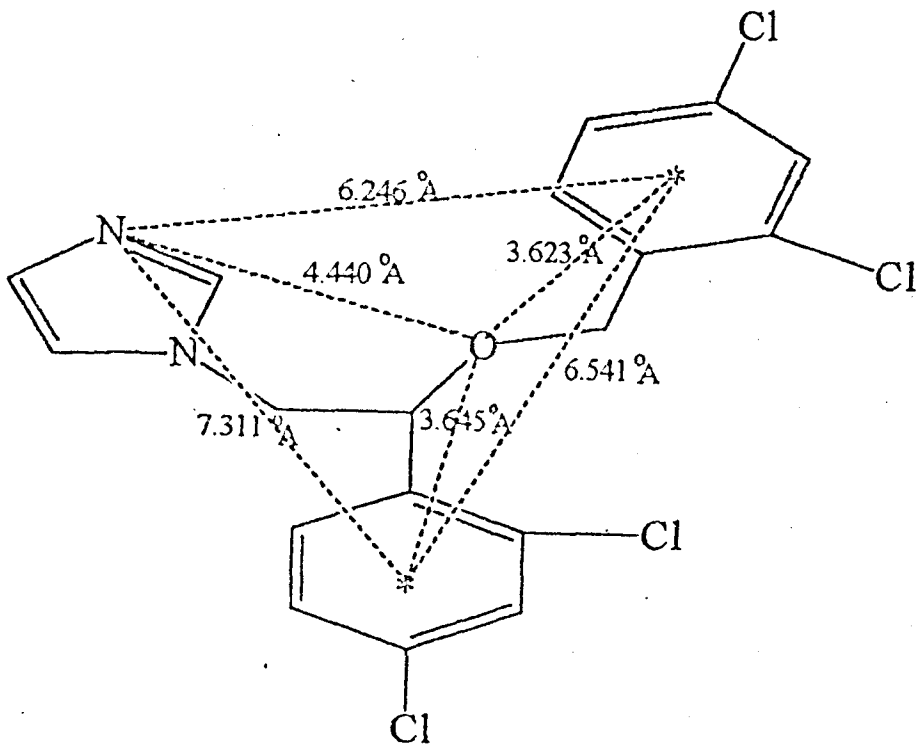


FIG. 8: PROPOSED PHARMACOPHORE FOR AZOLE ANTIFUNGALS

CONCLUSION

In today's competitive environment, maximizing resources is a key component to successful and timely discovery. Making the most of our resources means eliminating redundancies and charting a research course that is most likely to provide a pathway to new product leads. Pioneering technologies, used together in a systematic way, can deliver significant time saving advantages.

Advances made in the scientific and technological arena have contributed to new applications of computational tools for chemists and biologists. Finally, from the context of present scenario prevailing in Indian Pharmaceutical Industries i.e. the bulk drug business diminishing due to dumping, rapid and short living new drug entries in the market and implementation of GATT threat, the future has become a great challenge to all those involved in the area of pharmaceutical sciences and technology. Multidisciplinary approaches need to be employed for new drug research. Time only will tell how difficult are the days ahead. Experience it.

ACKNOWLEDGEMENT

I take this opportunity to thank the members of the committee for the honor bestowed upon me to deliver this lecture at the 48th Indian Pharmaceutical Congress Association being held here. I thank all my research students who work very hard on CADD projects.