IN-SILICO DISCOVERY OF NONANTIBIOTIC DRUG , "(5-[(3-CARBOXY-5-CYANO-PHENYL)-(3-CARBOXY-PHENYL)-METHYL]-4-ETHYL-2-METHYLENE-CYCLOHEXA-3,6-DIENE-1,3-DICARBOXYLIC ACID 3-ETHYL ESTER 1-METHYL ESTER) " *FOR PLAGUE DISEASE.*

Dissertation

Submitted to Orissa University of Agriculture & Technology, Bhubaneswar in partial fulfillment of the requirement for the award of degree of

MASTER OF SCIENCE IN BIOINFORMATICS

By

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CENTRE FOR POST GRADUATE STUDIES

ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

Bhubaneswar – 751003

2009

Dedicated to

My beloved parents and friends

ORISSA UNIVERSITY OF AGRICULTURE & TECHNOLOGY
DEPARTMENT OF BIOINFORMATICS
CENTRE FOR POST GRADUATE STUDIES
BHUBANESWAR, ORISSA

Dr. P.N. Jagadev
Head of the Department

CERTIFICATE-I

This is to certify that the thesis entitled "**IN-SILICO DISCOVERY *OF NONANTIBIOTIC DRUG*** , "(5-[(3-CARBOXY-5-CYANO-PHENYL)-(3-CARBOXY-PHENYL)-METHYL]-4-ETHYL-2-METHYLENE-CYCLOHEXA-3,6-DIENE-1,3-DICARBOXYLIC ACID 3-ETHYL ESTER 1-METHYL ESTER) " ***FOR PLAGUE DISEASE.*** submitted for the award of degree of **MASTER OF SCIENCE** of **BIOINFORMATICS** of the Orissa University of Agriculture and Technology, Bhubaneswar is a faithful record of *bona fide* and original research work carried out by **Soma Dash (Adm.No 11BI/07)** under my guidance & supervision . No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been fully acknowledged.

16/7/09

Place - Bhubaneswar
Date- 16.07.09

(Dr. P. N. Jagadev)
chairman
Advisory committee

CERTIFICATE-II

This is to certify that the thesis entitled "IN-SILICO DISCOVERY *OF NONANTIBIOTIC DRUG* , "(5-[(3-CARBOXY-5-CYANO-PHENYL)-(3-CARBOXY-PHENYL)-METHYL]-4-ETHYL-2-METHYLENE-CYCLOHEXA-3,6-DIENE-1,3-DICARBOXYLIC ACID 3-ETHYL ESTER 1-METHYL ESTER) *" FOR PLAGUE DISEASE.*

submitted by Soma Dash to the Orissa University of Agriculture &Technology, Bhubaneswar in the partial fulfillment of the requirements for the award of the **Degree of Master of Science in Bioinformatics** has been approved or disapproved by the students advisory committee after an oral examination of the same in collaboration with external examiner.

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Soma Dash

Place: Bhubaneswar **Regards**

Date: 16.07.09 **SOMA DASH**

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Admission No.	:	11 BI/07
Title of Thesis	:	In-Silico Discovery of Non – Antibiotic drug, "(5-[(3-carboxy-5 -cyano-phenyl)-(3-carboxy-phenyl)-methyl]-4-ethyl-2-Methylene -cyclohexa-3, 6-diene-1, 3-dicarboxylic acid 3-ethyl ester 1-methyl ester)" for plague disease.
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Name of Advisor	:	Dr. P.N.Jagadev

ABSTRACT

Plague is an infectious disease caused by bacteria Yersinia Pestis . Doxycyclin is one of the main drug for Plague. Doxycycline , is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis. From recent literature, Aurintricarboxylic Acid is one of the main ligand which shows anti-bacterial activity.

Our aim is to find new drug which is NOT an antibiotic drug. This is synthesized chemically, not from an organism.

We created hypothetical molecules and observed that , our molecules (5-[(3-carboxy-5-cyano-phenyl)-(3-carboxy-phenyl)-methyl]-4-ethyl-2-methylene-cyclohexa-3,6-diene-1,3-dicarboxylic acid 3-ethyl ester 1-methyl ester) is showing Ic50 values (MDL_QSAR value), and showing interaction with protein target. We used chemical drawing, energy minimization, QSAR, Docking studies in this thesis work.

Dr. P.N.Jagadev
Advisor

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ABBREBATIONS

- **3blu:** pdb id of Tyrosine-protein phosphatise yopH.
- **ADME properties:** Absorption, distribution, metabolism, and excretion are the pharmacokinetic parameters of drug like compounds.
- **MOPAC:** Molecular Orbital PACkage.
- **MDL QSAR:** Model Quantitative structural activity relationship study.
- **PASS:** Prediction of Activity Spectra for Substance
- **MM2:** Molecular mechanics2
- **Descriptors**: Descriptors are the independent variables in a QSAR study. They provide information of molecules that can be correlated to their biological affinity in a QSAR or QSPR (Quantitative structural property relationship) study.
- **Training set:** Training set is defined as the set of compounds in a dataset used to develop a QSAR model.
- **Test set (Predictive set):** Test sets are the compounds used to externally validate the predictive property of a developed QSAR model.
- **Dependent variables:** These are the molecular properties of the compounds. These are also called as descriptors in QSAR analysis.
- **Predicted values:** These are the biological activity predictions of the QSAR model.
- **Actual (Experimental) values:** These are the experimentally reported biological affinity data used in developing the structure biological correlation analysis.
- **Residuals:** Residuals are the difference between the experimental values and the predicted biological activity values in a QSAR model.
- **P**: Inhibition potential (IC50 ,LD50, ED50, Ki).
- **Biological Affinity - IC50 :** The amount of compound (concentration) that can produce a response in 50 % of the population.

INTRODUCTION

INTRODUCTION

Drug designing is the approach of finding a new drug molecule with desired response and minimal side effect with demonstrably preferred over existing therapy.

1.1 Insilico drug designing

Today, the process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, *de novo* design, *in vitro*, *insilico* ADMET screening and structure-based drug design.Insilico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding/ active sites, generate candidate molecules, check for their drug likeness and dock these molecules with the target.[1]

1.2 Why Insilico drug designing is significant?

As structures of more and more protein targets become available through crystallography, NMR and bioinformatics methods, there is an increasing demand for computational tools that can identify and analyze active sites and suggest potential drug molecules that can bind to these sites specifically.[1,7]

1.3 Insilico ADEM-T

Ligand molecule to be a drug should have "drug likeness". A molecule to be drug should have the ADEM-T properties i.e

Absorption- Drug must easily absorbed through membranes into the body.

Distribution- It has to be distributed throughout whole body.

Metabolism- Drug molecule must metabolised inside body otherwise it is useless.

Excretion-toxicity- After its work is over the unnecessary drug residue must secreted out automatically or if it left inside it must have minimum side effect (toxicity).

Target - A target can be an enzyme, DNA or protein in body which cause a disease.

Drug - Is a molecule that interact with a target and produce some pharmacological effect.

1.4 Rational drug designing

Rational drug design begins with a knowledge of specific chemical responses in the body.The structure of the drug molecule that can specifically interact with the bio-molecules can be modelled using computational tools. These tools can allow a drug molecule to be constructed within the bio-molecule using knowledge of its structure and the nature of its active site.The crystal structure of a ligand bound to a protein provides a detailed insight into the interactions made between the protein and the ligand. Structure designed can be used to identify where the ligand can be changed to modulate the physicochemical and ADME properties of the compound, by showing which parts of the compound are important to affinity and which parts can be altered without affecting the binding. The equilibrium between target and ligand is governed by the free energy of the complex compared to the free energy of the individual target and ligand.

A target can be an enzyme, DNA or protein in body which cause a disease.

It is a molecule that interacts with a target and produces some pharmacological effect. [1]

1.5 Energy Minimization-

Energy minimization (energy optimization) methods are common techniques to compute the equilibrium configuration of molecules. The basic idea is that a stable state of a molecular system should correspond to a local minimum of their potential energy. we can only obtain a final state of system that corresponds to a local minimum of potential energy. Different methods used in energy minimisation are MOPAC, MM2 and Mechanics.

1.6 Docking-

A method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex, is Docking. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs.

Types of docking:

- Protein-protein–

  Protein docking is the determination of the molecular structure of complex formed by two or more proteins. The three dimensional structure provides many insights on protein – protein interactions, allowing more rational approaches toward drug development and the treatment of disease.

- Protein-ligand -

  Protein ligand docking is to predict the position and orientation of a ligand when it is bound to a protein receptor or enzyme. One way that drugs can work is competitive inhibition; binding to proteins more strongly than their natural binding partners and thereby interrupting whatever process the protein mediates.

- Rigid receptor-

  If the bond angles, bond length and torsion angles of the components are not modified at any stage of complex generation, it is known as rigid body docking.

- Flexible receptor-

  In this model the three dimensional structures of the ligand and the receptor complement each other in the same way that a lock complement a key.[7]

1.7. QSAR

Quantitative structure-activity relationship (QSAR) is the process by which chemical structure is quantitatively correlated with a well defined process, such as biological activity or chemical reactivity. For example, biological activity can be expressed quantitatively as in the concentration of a substance required to give a certain biological response .Additionally, when physicochemical properties or structures are expressed by numbers, one can form a mathematical relationship, or quantitative structure-activity relationship, between the two. [16].

1.7 Plague

Plague is a zoonotic primarily carried by rodents (most notably rats) and spread to humans via fleas.The Plague of Justinian in A.D. 541–542 is the first known attack on record, and marks the first firmly recorded pattern of bubonic plague. During World War II it was used as biological weapon.

There three most common forms of plague are:

- Bubonic plague is an infection of the lymph nodes.
- Pneumonic plague is an infection of the lungs.
- Septicemic plague is an infection of the blood.

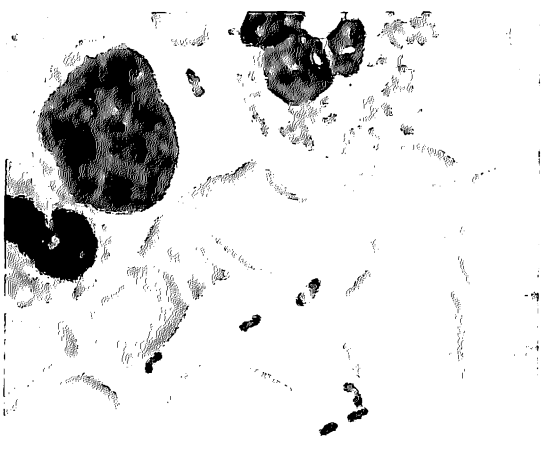


Fig-1
Peripheral Blood Smear from Patient with Septicemic Plague

Fig-2
Patients with Naturally Occurring Plague

People can get the plague when they are bitten by a flea that carries the plague bacteria from an infected rodent.

In rare cases, you may get the disease when handling an infected animal.

It is an infectious disease caused by bacteria Yersinia Pestis.

Occurance

Infection in a human occurs when a person is bitten by a flea that has been infected by biting a rodent that itself has been infected by the bite of a flea carrying the disease.

The bacteria multiply inside the flea, sticking together to form a plug that blocks its stomach and causes it to begin to starve.

The flea then bites a host and continues to feed, even though it cannot quell its hunger, and consequently the flea vomits blood tainted with the bacteria back into the bite wound.

Transmission

When someone with the plague coughs, microscopic droplets carrying the infection move through the air.

Anyone who breathes in these particles can catch the disease.

Risk factors for plague include a recent flea bite and exposure to rodents, especially rabbits, squirrels, or prairie dogs, or scratches or bites from infected domestic cats.

Treatment

Antibiotics are used to treat plague. Oxygen, intravenousfluids, and respiratory support are usually also prescribed. Vladimir Havkin, a doctor of Russian-Jewish origin who worked in India, was the first to invent and test a bubonic plague vaccine, on January 10, 1897.

The traditional treatments are:

- Streptomycin 30 mg/kg IM twice daily for 7 days.
- Chloramphenicol25–30 mg/kg single dose, followed by 12.5–15 mg/kg four times daily.
- Tetracycline2 g single dose, followed by 500 mg four times daily for 7–10 days (not suitable for children).
- More recently, Gentamicin2.5 mg/kg IVor IM twice daily for 7 days.
- Doxycycline 100 mg (adults) or 2.2 mg/kg (children) orally twice daily have also been shown to be effective.[6]

1.8 Yersinia pestis

Scientific classification

Kingdom: Eubacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Yersinia*

Species: ***Y. pestis***

Binomial name

Yersinia pestis

(Lehmann & Neumann, 1896)

van Loghem 1944

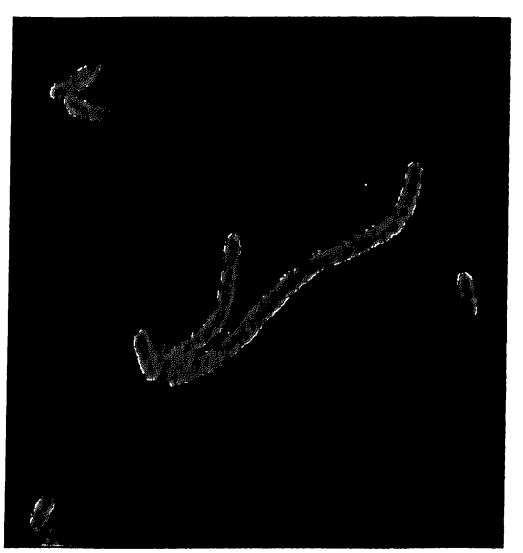


Fig-3
Bacteria – *Yersinia Pestis*

Y. pestis is a nonmotile gram-negative bacillus, a lactose nonfermenter, urease and indole negative member of Enterobacteria. *Yersinia* are causative agents in human diseases ranging from gastrointestinal syndromes to Bubonical Plague. such as *Yersinia Pestis* that causes plague.Yersinia pestis can reproduce inside cell so evenif phagocytosed they can still survive. *Y. pestis* spreads through the lymphatics of the infected human until it reaches a lymph node, where it stimulates severe haemorrhagic inflammation that causes the lymph nodes to expand.[1]

1.9 Protein

YopH is an essential virulence factor whose protein-tyrosine phosphatase (PTP) activity is required for *Yersinia* pathogenicity. Consequently there is considerable interest in developing potent and selective YopH inhibitors as novel anti-plague agents. YopH is a protein tyrosine phosphatase that contributes to the ability of *Yersinia pestis* to evade immune system cells. This tyrosine phosphatase YopH is produced by pathogenic Yersinia species. YopH is translocated into host cells via a type III secretion system. N-terminal domain of YopH acts as a substrate-binding domain.[1]

1.10 Importance of nonantibiotic on antibiotic

Antibiotics have been the cure to many diseases (by killing or stopping the growth of bacteria). Antibiotics have also played a major role in the pharmaceutical industry.
But Antibiotics have some very undesirable properties, such as:

1. They have side effects that can sometimes prove to be more difficult to manage than the ailment they are meant to cure.
2. They destroy friendly bacteria along with disease-causing bacteria. The body needs friendly bacteria for a number of processes like detoxification, easy elimination of wastes and cleansing of the blood and the liver.
3. Over-use of antibiotics lead to their becoming ineffective due to bacterial mutation. Simply stated, it means that certain bacteria get used to a particular antibiotic and start using it for their own benefit. Penicillin and tetracycline are classic examples of how antibiotics become redundant for killing some bacterium.

So now a day a nonantibiotic is much more necessary than an antibiotic which is purely a chemical product. To examine or develope the drug we need not to use a bioproduct. For nonantibiotic drug discovery maximum work can be done by computer.[1]

OBJECTIVE

The main objectives of work are:

- To discover a drug molecule for Plague which is not a traditional antibiotic drug molecule but a non-antibiotic one.
- To construct a number of analogous ligand molecules from a known and effective drug molecule from which the appropriate one ligand is screened out which will help to block the protein of the bacteria causing Plague disease.
- *To Insilico* designing of a ligand molecule that will react with protein having least side effect and with pharmacological effect using softwares. This is developed totally from chemicals not from any bio-molecule.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1. Targeted bacteria:

Yersinia are causative agents in human diseases ranging from gastrointestinal syndromes to Bubonical Plague. such as Yersinia Pestis that causes plague. Infection was induced by inhalation of aerosolized Yersinia Pestis. Y. Pestis is a nonmotile gram-negative bacillus, a lactose nonfermenter, urease and indole negative member of Enterobacteria. YopH is an virulence factor whose protein-Tyrosine phosphatise(PTP)activity is required for Yersinia pathogenicity. *Y. pestis* spreads through the lymphatics of the infected human until it reaches a lymph node, where it stimulates severe haemorrhagic inflammation (the root "haem" means "blood" and "haemorrhage" means to bleed) that causes the lymph nodes to expand.The expansion of lymph nodes is the cause of the characteristic "bubo" associated with the disease.Yersinia pestis can reproduce inside cell so evenif phagocytosed they can still survive.

2.2. Targeted Protein

The tyrosine phosphatase YopH is an essential virulence factor produced by pathogenic Yersinia species.

MOLECULE	Tyrosine-protein phosphatise yopH
CHAIN	A
PDB ID	3BLU
EXPERIMENTAL METHOD	X-ray diffraction
RESOLUTION	2.00 A
LENGTH	305

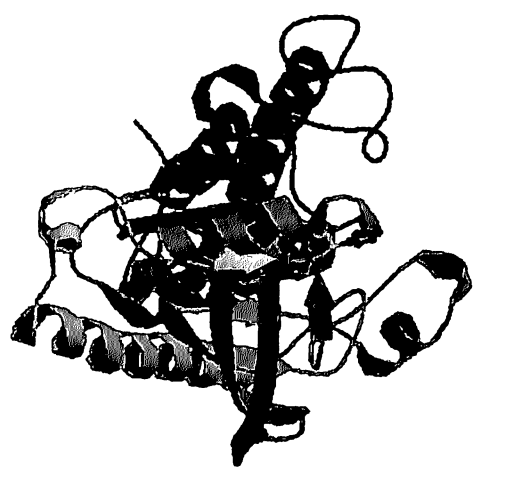


Table-1

2.3 The traditional drug

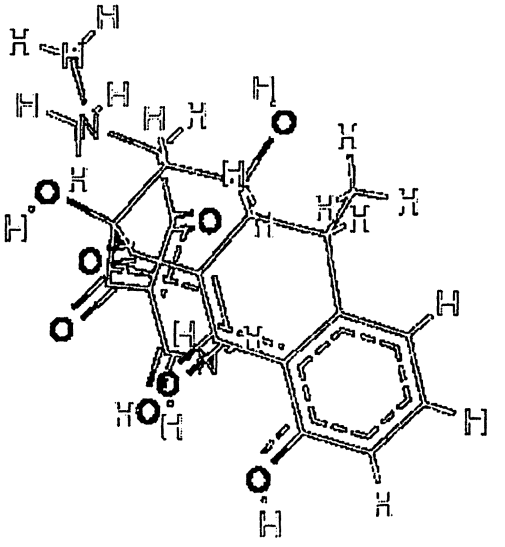
1. Doxycycline

Chemical formula $C_{22}H_{24}N_2O$

Target 30S ribosomal protein S4
30S ribosomal protein S9
50S ribosomal protein L10
16S rRNA

Mechanism of action Doxycycline , is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis.

Chemical structure:



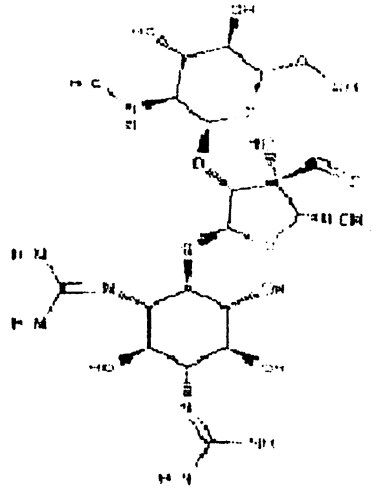
2. Streptomycin

Chemical formula $C_{21}H_{39}N_7O_{12}$

Target 30S ribosomal protein S12
16S rRNA

IUPAC name 2-[(1S,2R,3R,4S,5R,6R)-5-(diaminomethylideneamino)-2-[(2R,3R,4R,5S)-3-[(2S,3S,4S,5R,6S)-4,5-dihydroxy-6-(hydroxymethyl)-3-methylaminooxan-2-yl]oxy-4-formyl-4-hydroxy-5-methyloxolan-2-yl]oxy-3,4,6-trihydroxycyclohexyl]

Chemical Structure



3.Gentamicin

Chemical formula $C_{21}H_{43}N_5O_7$

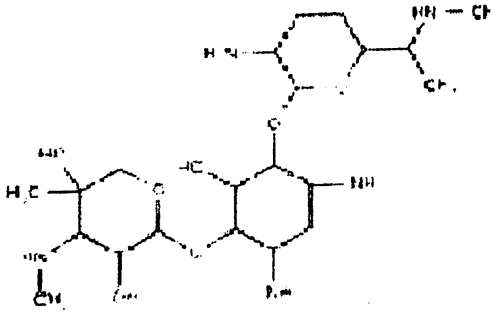
IUPAC name 2-[4,6-diamino-3-[3-amino-6-(1-methylaminoethyl)oxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-5-methyl-4- methyl aminooxane-3,5-diol

Target 30S ribosomal protein S12

16S rRNA

Low-density lipoprotein receptor-related protein 2

Chemical structure :



2.4. Vaccination

The US-licensed formaldehyde-killed whole bacilli vaccine was discontinued by its manufacturers in 1999 and is no longer available. Plans for future licensure and production are unclear. This killed vaccine demonstrated efficacy in preventing or ameliorating bubonic disease, but it does not prevent or ameliorate the development of primary pneumonic plague. It was used in special circumstances for individuals deemed to be at high risk of developing plague, such as military personnel working in plague endemic areas, microbiologists working with *Y pestis* in the laboratory, or researchers working with plague infected rats or fleas. Research is ongoing in the pursuit of a vaccine that protects against primary pneumonic plague.

2.5. Importance of antibiotic

The term antibiotic (from the Greek word *anti*, against and Greek *bios*, life) was coined by Selman Waksman in 1942 to describe any substance produced by a micro-organism that is antagonistic to the growth of other micro-organisms in high dilution.Antibiotics are one of the most frequently prescribed medications in modern times.

- Antibiotics have been the cure to many diseases (by killing or stopping the growth of bacteria) such as bacterial meningitis, neurosyphilis, endocarditis, burn wounds, skin infections, respiratory and urinary tract infections, pneumonia, anthrax, STDs, Lyme disease, bronchitis, diarrhoeal diseases, abdominal infections, severe acne, gastrointestinal tract infections, blood poisoning, TB, ear infections - the list goes on.
- Antibiotics have played a major role in the pharmaceutical industry. Before WW2, the pharmaceutical industry was a small enterprise in the chemical industry.
- After antibiotics (such as penicillin) were first discovered, the pharmaceutical industry began to grow rapidly into the huge businesses we see today. Companies such as Squibb, Merck, Lederle, and Eli Lilly owe their success to the discovery of antibiotics.
- There are three main uses of antibiotics in livestock production. Antibiotics can be used as a therapeutic (managing and curing clinical diseases), as a growth promoter (small antibiotic additives to feed can cause animals to grow faster) and as a prophylactic (preventing disease). In agricultural industry the uses of antibiotics are much the same except that it does not help growth.
- Therefore antibiotics are necessary to society - used properly or not. The commercial importance of antibiotics is not only for treating infections in humans but also for food production and keeping animals and plants disease-free.

- A growing body of informed opinion now believes that repeated courses of antibiotics can so disturb a person's internal ecology that they can trigger ME or even cancer.
- A big worry is that repeated courses of antibiotics appear seriously to disturb immune system function. WE also don't know the long term effects on the current generation of children, who receive many courses of antibiotics before they even reach their teens.
- Repeated courses of antibiotics only encourage the development of supergerms in the body, which will resist treatment from the antibiotics so that, when it is really needed, the drug won't work.

Antibiotics have some very undesirable properties, such as:

- They have side effects that can sometimes prove to be more difficult to manage than the ailment they are meant to cure.
- They destroy friendly bacteria along with disease-causing bacteria. The body needs friendly bacteria for a number of processes like detoxification, easy elimination of wastes and cleansing of the blood and the liver.
- Over-use of antibiotics lead to their becoming ineffective due to bacterial mutation. Simply stated, it means that certain bacteria get used to a particular antibiotic and start using it for their own benefit. Penicillin and tetracycline are classic examples of how antibiotics become redundant for killing some bacterium.

Nonantibiotic

Some of the chemical compounds are under research to give a drug to show druglikeness. Nonantibiotics are more prefferable because no biomolecule in necessery to develope it. It is totally dependent on chemicals.

Derivatives of sulfonamide are such cheical compounds-

Table 1. **Sulfonamide Nonantibiotic Drugs.**

Acetazolamide	Cyclopenthiazide	Glyburide	Probenecid
Acetohexamide	Dapsone	Glymidine	Quinethazone
Bendroflumethiazide	Diazoxide	Hydrochlorothiazide	Sulfasalazine
Benzthiazide	Dichlorphenamide	Hydroflumethiazide	Sulthiame
Bumetanide	Furosemide	Indapamide	Tolazamide
Chlorothiazide	Glibornuride	Mefruside	Tolbutamide
Chlorpropamide	Gliclazide	Methyclothiazide	Torsemide
Chlorthalidone	Glimepiride	Metolazone	Xipamide
Clopamide	Glipizide	Piretanide	
Clorexolone	Gliquidone	Polythiazide	

2.6. Antibiotic Treatment on Experimental Pneumonic Plague in Mice

William R. Byrne,Susan L.Welks,M.louise Pitt, Kelly J.Davis,Joan W.Ezzell,Arthur M.Frielander.

Recieved on 21 August 1997 & Acceptd on 19th December 1997

Unitedstates Army Medical Research Institute of Infectious Diseases.Maryland

A mouse model was developed to evaluate the efficacy of antibiotic treatment of pneumonic plague; streptomycin was compared to antibiotics with which there is little or no clinical experience.Infection was induced by inhalation of aerosolized *Yersinia pestis* organisms. Antibiotics were administered by intraperitoneal injection very 6 hours for 5 days, at doses that produced levels of drug in serum comparable to those observed inhumans treated for other serious infections. These studies compared in vitro to in vivo activity and evaluated the efficacy of antibiotics started at different times after exposure. Early treatment (started 24 h after challenge, when 0 of 10 mice tested had positive blood

cultures) with netilmicin, ciprofloxacin, ofloxacin, ceftriaxone, ceftazidime, aztreonam, ampicillin, and rifampin (but not cefazolin, cefotetan, or ceftizoxime) demonstrated efficacy comparable to streptomycin. Late treatment (started 42 h after exposure, when five of five mice tested had positive blood cultures) with netilmicin, ciprofloxacin, ofloxacin, and a high dose (20 mg/kg of body weight every 6 h) of gentamicin produced survival rates comparable to that with streptomycin, while all of the beta-lactam antibiotics (cefazolin, cefotetan, ceftriaxone, ceftazidime, aztreonam, and ampicillin) and rifampin were significantly inferior to streptomycin. In fact, all groups of mice treated late with beta-lactam antibiotics experienced accelerated mortality rates compared to normal-saline-treated control mice. These studies indicate that netilmicin, gentamicin, ciprofloxacin, and ofloxacin may be alternatives for the treatment of pneumonic plague in humans. However, the beta-lactam antibiotics are not recommended, based upon poor efficacy in this mouse model of pneumonic plague, particularly when pneumonic plague may be associated with bacteremia.

2.7. Impact on Resistance Selection & Mutant Growth Fitness on the Relative Efficacies of Streptomycin & Levofloxacin For Plague Therapy.

Arnold Louie, Mark R.Deziel, George L.Drusano

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Ordway Research Insititute, New York.

Yersinia pestis, the bacterium that causes plague, is a potential agent of biowarfare and bioterrorism. The aminoglycoside antibiotic streptomycin is the gold standard for treatment. However, this recommendation is based on scant animal and clinical data. We used an in vitro pharmacodynamic infection model to compare the efficacies of 10-day regimens of streptomycin versus the fluoroquinolone antibiotic levofloxacin for the treatment of *Y. pestis* infection and to evaluate for emergence of resistance. The human serum concentration-time profiles for standard clinical regimens of 1 g of streptomycin given every 12 h and 500 mg of levofloxacin givenevery 24 h were simulated. The growth fitness of drug-resistant mutants was examined in neutropenic and immunocompetent mouse thigh infection models. In the in vitro infection system, untreated bacteria grew from 107 to 1010 CFU/ml. Streptomycin therapy caused a 105 CFU/ml reduction in the number of bacteria over 24 h, followed by regrowth with streptomycin-resistant mutants. Levofloxacin resulted in a 107 CFU/ml reduction in the number of bacteria within 12 h, ultimately sterilizing the culture without resistance selection. In both the normal

and neutropenic mouse infection models, streptomycin-resistant and wild-type strains were equally fit.However, 90% of levofloxacin-resistant isolates, cultured from the control in vitro infection arm, did not proliferate in the mouse models. Thus, the fluoroquinolone antibiotic levofloxacin was superior to streptomycinin our in vitro infection model. The majority of levofloxacin-resistant mutants werelessfitthanstreptomycinresistantandwildtype *Y.pestis*.

2.8. Aurintricarboxylic Acid Blocks in Vitro & in Vivo Activity of YopH An Esssential virulence Factor of Yersinia pestis the Agent Of Plague.

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Yersinia are causative agents in human diseases ranging from gastrointestinal syndromes to Bubonic Plague.There is increasing risk of misuse of infectious agents,such as *Yersinia pestis*, as weapons of terror as well as instruments of warfare for mass destruction. YopH is anessential virulence factor whose protein-tyrosine phosphatise (PTP) activity is required for *Yersinia* pathogenicity.Consequently, there is considerable interest in developing potent and selective YopH inhibitors as novel anti-plague agents. We have screened a library of 720 structurally diverse commercially available carboxylicacids and identified 26 YopH inhibitors with IC50 values below 100 _M. The most potent and specific YopH inhibitor is aurintricarboxylic acid (ATA), which exhibits a *Ki* value of 5 nM for YopH and displays 6–120-fold selectivity in favor of YopH against a panel of mammalian PTPs. To determine whether ATA can block the activity of YopH in a cellular context, we have examined the effect of ATA on T-cell signaling in human Jurkat cells transfected with YopH. We show that YopH severely decreases the T-cell receptor-induced cellular tyrosine phosphorylation, ERK1/2 activity, and interleukin-2 transcriptional activity. We demonstrate that ATA can effectively block the inhibitory activity of YopH and restore normal T-cell function. These results provide a proof-of-concept for the hypothesis that small molecule inhibitors that selectively target YopH may be therapeutically useful. In addition, it is expected that potent and selective YopH inhibitors, such as ATA, should be useful reagents to delineate YopH's cellular targets in plague and other pathogenic conditions caused by *Yersinia* infection.

MATERIALS & METHOD

MATERIALS

Web site used-

- For searching of protein : PDB
- For drug information : Drug bank
- For searching of active site of protein : Qsite Finder
- For searching of related articles for disease : High wire Press

Software/tools used-

- construction and minimizing energy of ligands : ISIS Draw, Chemdraw Ultra, Chem 3d Ultra
- Methods for insilico drug designing : Pass, MDL qsar, DS viewer
- For docking : Autodock

3.1.PDB: The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. (See also crystallographic database). The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are released into the public domain, and can be accessed at no charge on the internet.
For PDB format files, use, e.g., http://www.pdb.org/pdb/files/4hhb.pdb.g

3.2.EXPDB The structure database used by SWISS-MODEL is derived from theProtein Data Bank (PDB). In order to allow rapid retrieval of the relevent structral information, each protein chain of the database was placed in an individual file. The ExPDB codes are constructed according to the following rule:

PDBCODE+ChainID

3.3.DRUGBANK: The Drugbank database available at the University of Alberta is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, pathway) information. The database contains nearly 4800 drug entries including >1480 FDA-approved small molecule drugs, 128 FDA-approved biotech (protein/peptide) drugs, >71 nutraceuticals and >3200 experimental drugs.Additionally, more than 2500 protein (i.e. drug target, non-redundant) sequences are linked to these drug entries. It is maintained by David Wishart and Craig Knox.

3.4.HIGHWIRE PRESS:

HighWire Press is a division of the Stanford University Libraries that produces the online versions of high-impact, peer-reviewed journals and other scholarly content. Recipient of the 2003 Association for Learned and Professional Society Publishers (ALPSP) Award for "Service to Not-for-Profit Publishing", HighWire collaborates with scholarly societies, university presses and publishers to host a large body of clinical and research literature. Over 70 of the 200 most- frequently- cited journals in science are hosted on HighWire.

3.5.Q-SITE FINDER:

Q-SiteFinder is a new method of ligand binding site prediction. It works by binding hydrophobic (CH3) probes to the protein, and finding clusters of probes with the most favourable binding energy. These clusters are placed in rank order of the likelihood of being a binding site according to the sum total binding energies for each cluster.

3.6.ISIS DRAW

ISIS/Draw was a chemical structure 2D drawing program for Windows, published by MDL Information Systems. It is available free of charge for academic and personal use. ISIS/Draw uses its own proprietary file format, with the extension *.skc, and also supports standard chemical file formats such as MDL molfile, Rxnfile, and TGfile. ISIS/Draw is a chemical drawing program somewhat similar to ChemDraw. It has some 3D rotation features and can interface with Rasmol for 3D visualization and rendering. ISIS/Draw also includes structure and reaction validation features and can calculate elementary properties such as formula and molecular weight.

3.7.CHEMDRAW ULTRA8.0

ChemDraw is a molecule editor developed by the cheminformatics company CambridgeSoft. ChemDraw is, along with Chem3D and ChemFinder, part of the ChemOffice suite of programs and is available for Macintosh and Microsoft Windows.

Chemical structure to name conversion

Chemical name to structure conversion

3.8. CHEM 3D ULTRA 8.0

Chem3D provides affordable building, visualisation and computation tools on the desktop. Chem3D can be used to display many types of molecular surfaces and molecular orbitals.chem. 3d ultra contains enery minimization tools like MOPAC, MM2, Mechanics MOPAC- In computational chemistry, MOPAC is a popular computer program designed to implement Semi-empirical quantum chemistry algorithms Its name is derived from *Molecular Orbital PACkage.* The author of MOPAC, James Stewart, released

in 2006 a public domain version of MOPAC7 entirely written in Fortran 90 called MOPAC7.1.

3.9. PASS pass is a tool mainly used to findout the toxicity and possible Pharmacological Effect.but beyond this it also gives possible activities, possible molecular mechanismPossible Side effects. We can also change the properties by reconstructing ligand structure.

3.10. MDL QSAR

MDL QSAR helps researches establish reliable quantitative structure-activity and Structure property relationship in drug desiging. Predictive models like this one built using MDL QSAR can be valuable tools in early decision making. One Touch method-Gives the result for all the test molecule without having any descriptor.Advanced method- Descriptor , it will compare.

3.11. DS Viewer pro6.0 trial:

Discovery studio (DS) products, which include MODELER, Ligand Fit, DS Visualizer etc., are very useful for computational biologists and Bioinformaticists. For most practical applications, including rendering protein structure files obtained from the Protein Data Bank (PDB), DS Viewer 5.0 is very handy and comes with attractive options for the visual display of molecules. To begin with, DS Viewer can read a large number of input file formats including pdb.This is the tool used to save the protein and the ligand molecules which has to be docked with the protein in pdb format. These are saved in MGLtools .

3.12.AUTO DOCK:

AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, a receptor of known 3D structure such as substrates or drug candidates bind to a receptor of known 3D structures. AutoDock actually consists of two main programs.

AutoDock performs the docking of the ligand to a set of grids describing the target Protein , autoGrid pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to guide organic synthetic chemists design better binders.

METHOD

Collection of Data

Collection of Literature for datas

The datas related to the protein target such as their metabolism, regulation and the involvement in the diseases are collected from the reviews and the research articles, journals from *Science Direct, High wire Press, Nature* and *Springer link* websites.

Retrieval of protein

- Structure of desired protein is retrieved from protein data bank.
- By using the URL www.rcsb.org the site is opened.
- Then protein name YopH is provided in field then site search is clicked.
- Many structure appeared from which accurate one is downloaded by clicking on the arrow mark provided at the top (as in fig-1 below).
- The pdb file of protein structure is saved at required site and open by notepad or crimson editor.

◎ **Resolution:less than 2 bcoz coputation time is less and to avoid grid related errors and smaller the resolution more clear the visualisation of structure.**

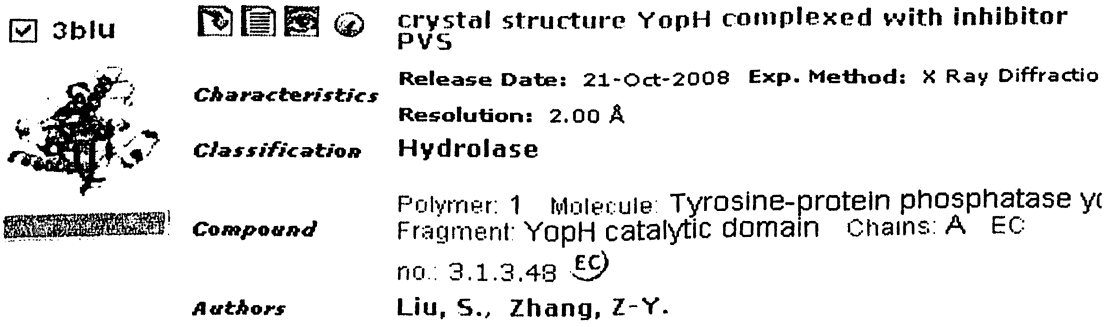


Fig-4
Pdb page of protein tyrosin phosphates YopH

Retrieval of drug /ligand molecule from literature-

- The article searched from *High Wire Press.*
- The homepage of High Wire Press is opened by using its url Or from Google directly.
- Then Journal of Biochemistry is choosen where year-2003, volume-378, page-41734 is given.
- And the article is found "Aurintricarboxylic Acid Blocks *in vivo & invitro* activity of YopH, an essential virulent factor of Yersinia Pestis" ,the agent of plague.
- Here the key ligand for our drug designing is found with some more YopH inhibitors with their IC50 value.

Active site-

- Active site of the protein is needed so it is retrieved from Q-Site finder using its *url* Or by typing Q-site finder in the space available at Google homepage and click for *Search.*
- From the number of amino acid the more repeating one is choosen as active site of Protein Tyrosine Phosphatase YopH.

Development of Ligand molecules

Construction of analogues of known molecules

i.ISIS draw-

- First of all MDL ISIS Draw2.5 software is loaded and opened with double click on it.
- In the page there are options for bonds symboled as c-c by clicking on all kinds of bonds appears, atoms(C,O,N,H.....etc) symboled as *'A' arrow* (←) symbol is for select all option likely using other options the unknown structures are drawn.
- The name of structure is derived from the last option 'Auto Nom' in vertical column.
- From the known 26 structure of article unknown 51 structures are drawn.

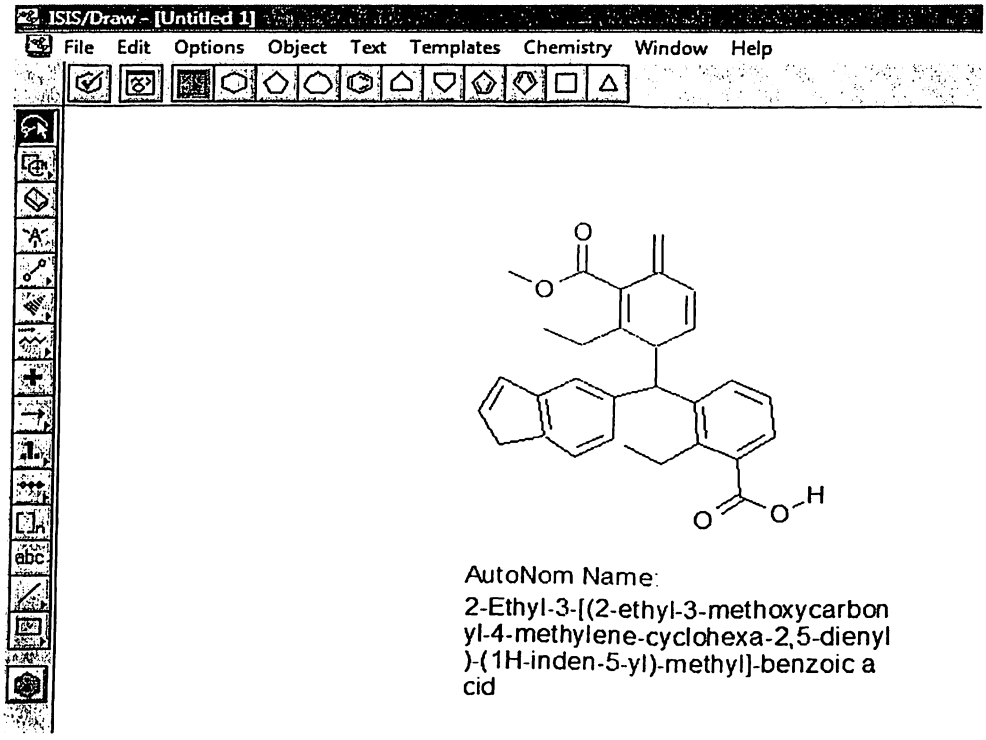


Fig-5

[Unknown structure drawn from Aurintricarboxylic acid using ISIS Draw] Home page of ISIS Draw

iii.PASS

After all the known and unknown structure drawn ,are tested in PASS to know whether the structure have any side effect or not.

- As PASS is opened file option is clicked to retrieve the structure then by choosing the options at the right side the effects are shown.
- To change the effects again go for ISIS draw and change structure then PAS is done.
- The PASS is overed if structure has no toxicity and Effect above 0.7.

It is more appropriate if Drug Likeness is also above 0.7 .

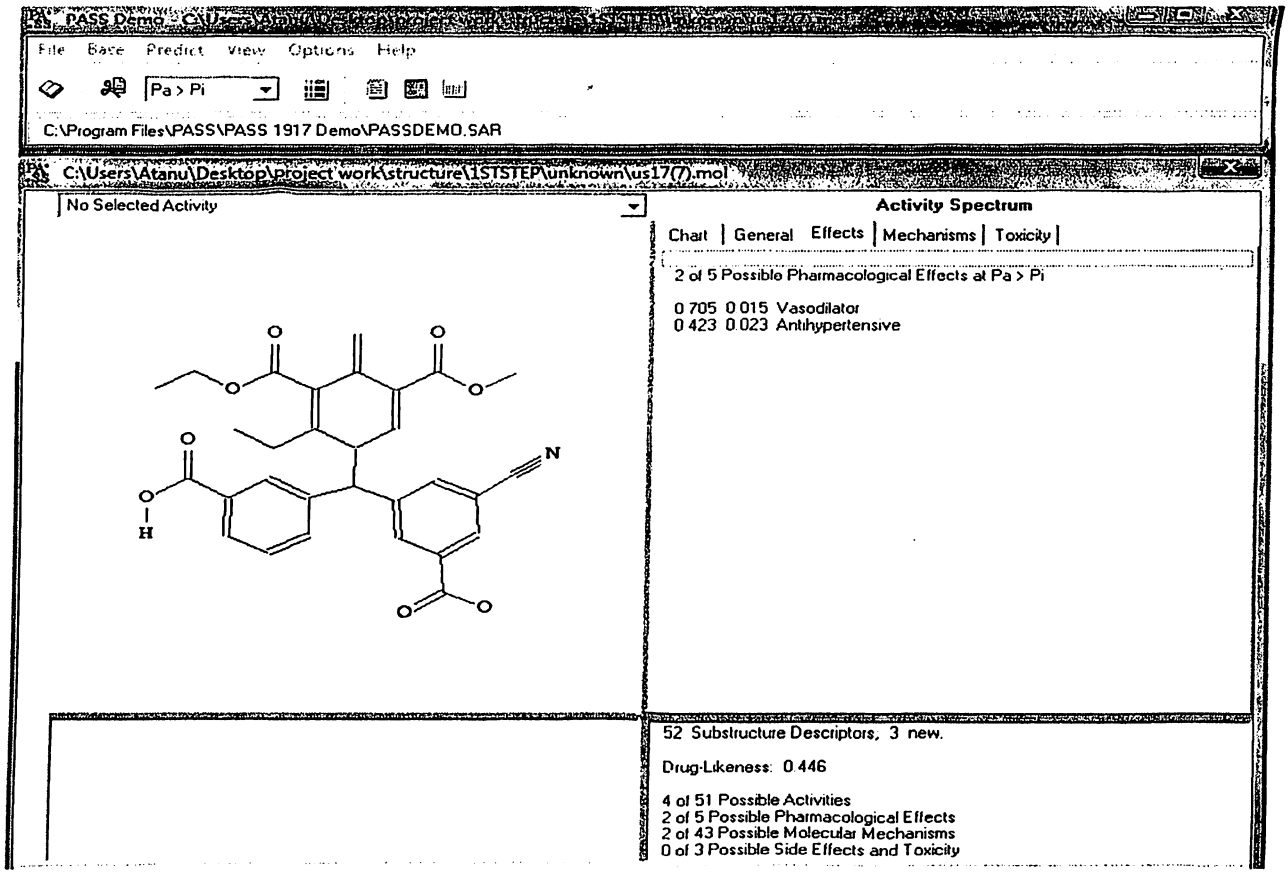


Fig-6
Home page of PASS tool

iii.Chem 3D Ultra-

- As a stable structure should have minimum energy so after PASS it is done by Chem 3D.
- Chem 3D is opened and from the three options *Enable hardware acceleration for this session only* is clicked.
- To browse structure File ->open
- MM2 ->minimize energy ->Dynamics->target tempreture made 273-> Run.
- MOPAC ->minimize energy ->properties ->select all except polarisability ->Run.

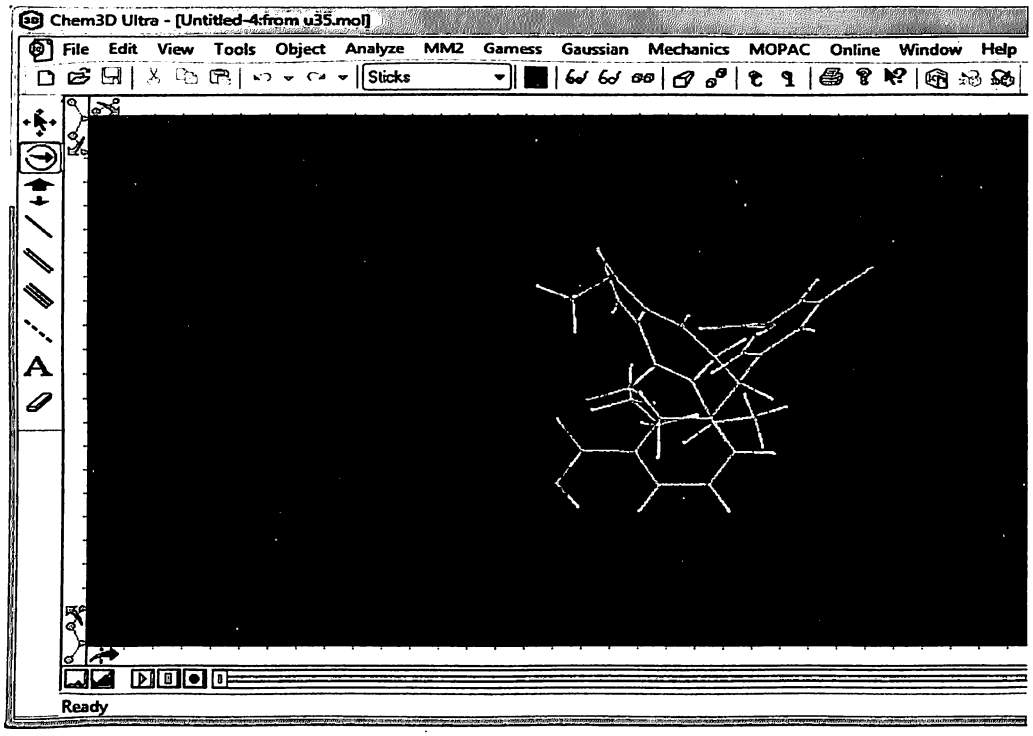


Fig-7
Home page of chem3d ultra for
Energy minimisation of molecule

- As shown in fig 7 if Ready is appreaed means structure is minimized,but if Error comes then
  MOPAC ->Energy minimization ->job type->minimum RMS gradient convert 0.1 to 0 .0001->properties ->select any four ->Run.

MDL QSAR-

One Touched:

- After energy minimization the 24 known and 51 unknown molecules are saved within six folders in MDL folder present inside c:/programefiles/MDL/qsar/examples.
- Then MDL qsar software is opened .There at the left side QSAR is found, on which with right click a folder is made with same name as inside MDL for known structures.
- File is opened and import known structures filewise or by batch from example folder inside MDL.
- After all the structures are appeared on table click on Regression analysis->create model ->onetouch->ok.

- As shown in result page fig-10 if there are distract molecules from straight line ,then to increase R-square value

  double click on molecule->Regression Analysis->model handling.
- Then again a folder is created by rightclick on QSAR in previous page for unknown structures , same name as the folder inside MDL folder .
- In this folder structures are browse as

  File ->import ->batch ->open->ok.
- The Calculation Preferences page will open where the appropriate Model name is selected, required Columns added by right click on page.
- As all structures are inserted in table Screen ->calculate.
- Likely other four folders are browsed and screened in same way.

The equation is created using the pMED values of the 20 known structures.

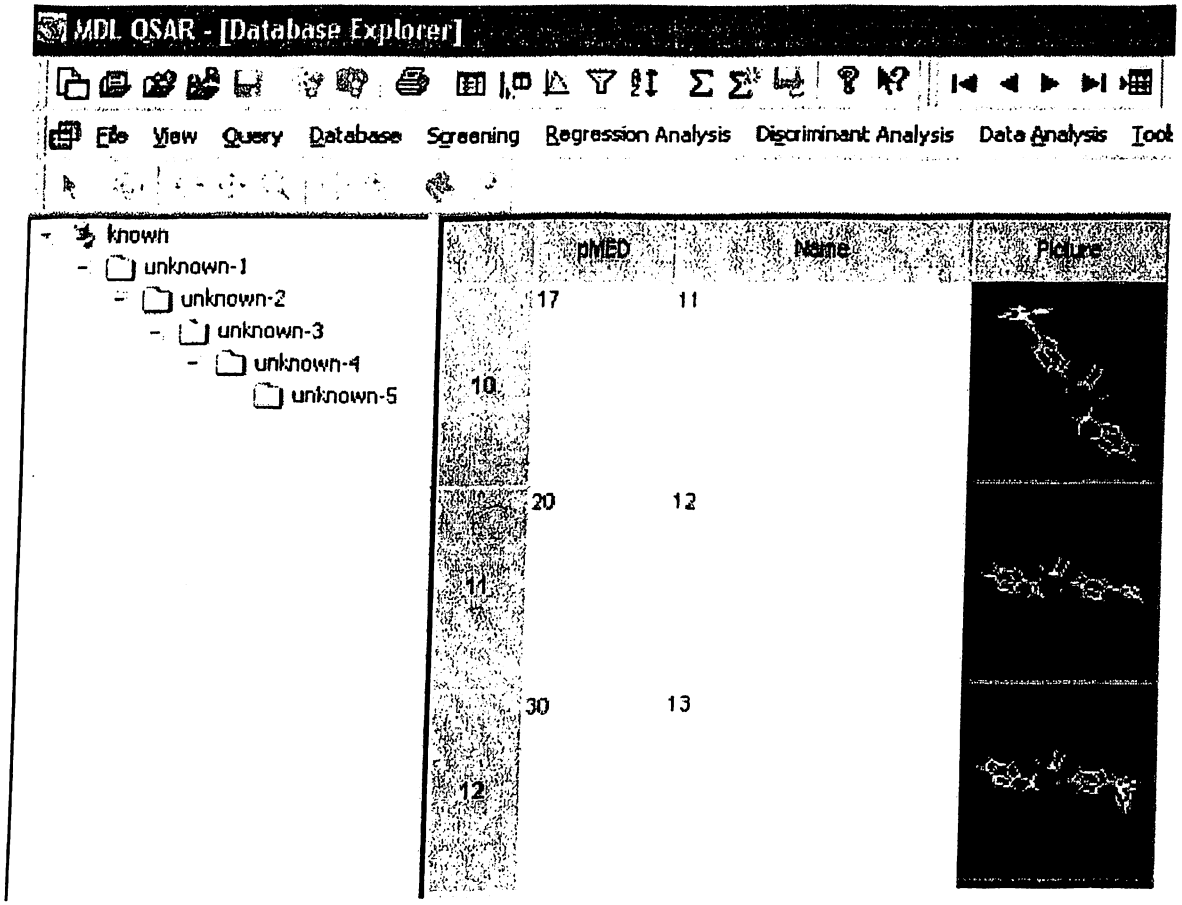


Fig-8
Home page of MDL qsar

Auto Dock-

- Before going for autodock the protein and ligand are opened in DSviewer and save as pdb format in MGL tools inside the c:/ programefiles/mgltools.
- Auto dock software is opened then the steps are as follows for docking:

Steps-For protein:

- File ->Read molecule -> protein is choosen.
- Colour -> By atoms type -> by line ->ok
- Select ->select from string ->type HOH* in residue box and * in Atom box ->add
  ->ok
- Edit ->delete -> delete atom set ->continue.
  (it is needed to remove heteroatoms like H_2O in order to avoid double ligands)
- Edit -> hydrogens ->add ->ok
- File -> save as ->save write pdb ->ok.

For ligand:

- Ligand -> input -> open.
- Ligand ->torsion tree -> choose root
  ->detect root
  ->show root expansion
  ->show/hide root marker
  ->choose torsion
  ->set number of torsion

Is choose torsions the bonds which cannot be rotated are colour red.set no of torsion is to allow to set total no of active bonds to fewest atoms.(these steps are for calculating the change in free energy caused by the loss of torsional Degree)

- Ligand ->output ->saves as PDBQT.
- Flexible residue -> input -> chooses macromolecule->protein ->yes.
- Select ->select from string ->write the active site in residue box.
- Flexible residue ->choose torsion in currently selected residue
- Flexible residue ->output -> save flexible pdbqt (save it as proteinflex.pdbqt)
- Flexible residue ->output ->save rigid pdbqt.
- Edit ->delete ->delete molecule->protein is deleted.

(Grid is build to rotate the ligand freely when it is in its most fully extended conformation)

- Grid ->macromolecule ->open ->prtrigid.pdbqt->yes
- Grid ->set map atoms ->choose ligand
- Grid ->grid box->adjust the width of box to select all ->file close saving current.
- Grid ->output ->save GPF
- Run ->run autogrid ->browse (C:/cygwin/user/local/bin/autogrid) ->lunch.
- Docking ->macromolecule ->set rigid file name(choose rigid molecule)
- Docking ->ligand ->choose ligand ->accept
- Docking ->macromolecule->set flexible residue(choose flex molecule)
- Dock->search parameters ->genetic algorithm->make it short and click accept.
- Dock ->docking parameter->accepts.
- Dock ->output ->Lamarckian GA ->save as ligand.dpf(docking parameter file tells autodock which map file to use the ligand to more ,no of torsions
- Run ->run autodock-> browse(c:/cygwin/user/local/bin/autodock) ->launch.

Autogrid4 and dock 4 are used because these steps must run in the directions where the macromolecule, ligand and pf are found.

The PVM molecule protein and ligand are deleted by right click .

- Analyze ->docking ->open
- Analyze ->macromolecule->open
- Analyze ->conformation ->play
- Analyze ->conformation ->load

After loading the result page appeared as follows

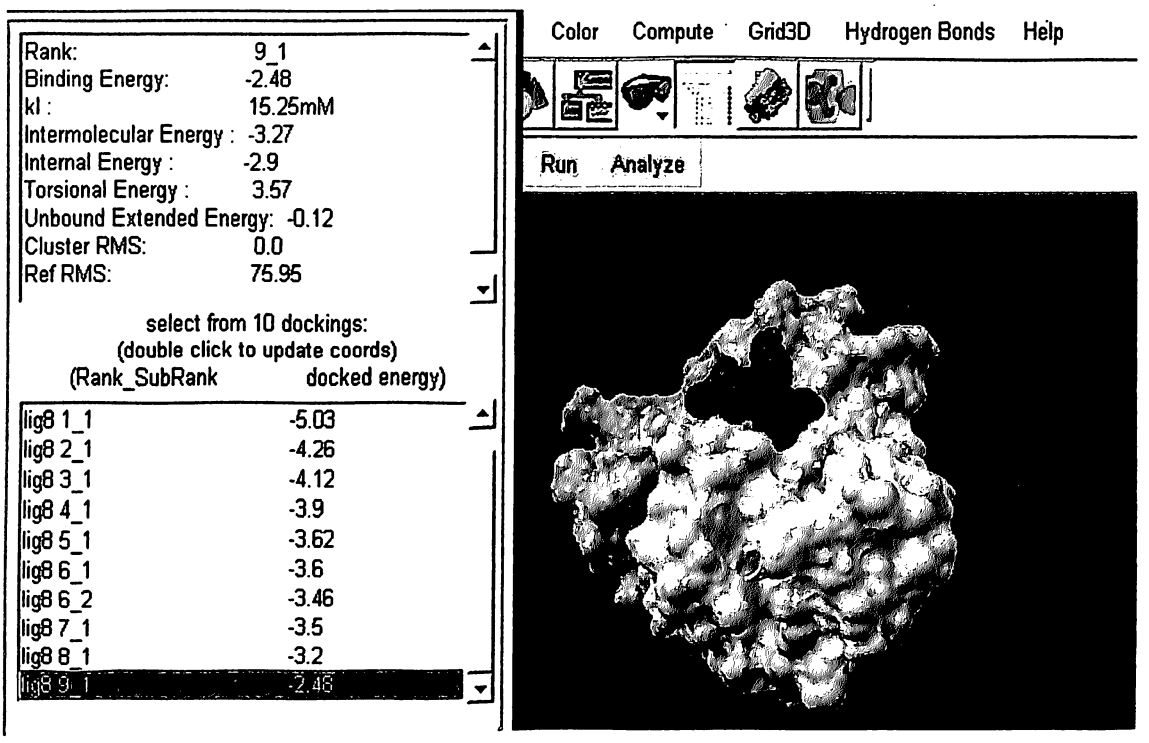


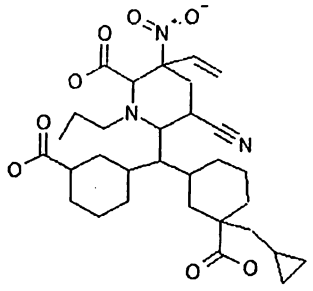
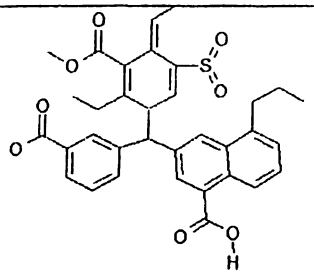
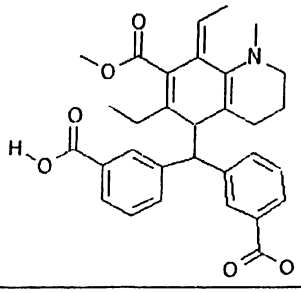
Fig-9
The page appeared after completion of Autodocking

RESULT

RESULT

PASS result

From the PASS tool we got eight ligand molecules which have no toxic effect rather having positive pharmacological effects which satisfy Drug likeness. Those molecules are drawn below:

Ligand	Ligand structure	Toxicity	Effect value-pharmacologycal properties
U28/Lig1		0	0.717 0.014 Vasodilator 0.209 0.109 Antihypertensive
U29/lig2		0	0.649 0.008 Antihypertensive 0.628 0.024 Vasodilator
U30/lig3		0	0.642 0.022 Vasodilator 0.390 0.029 Antihypertensive

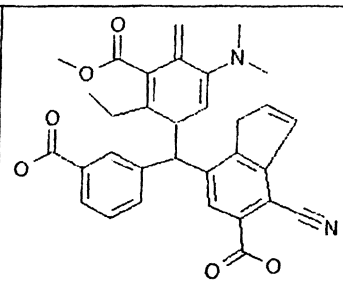
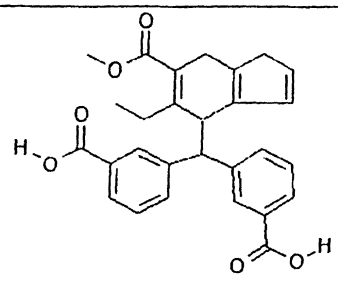
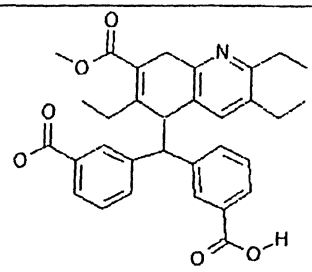
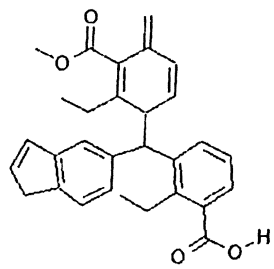
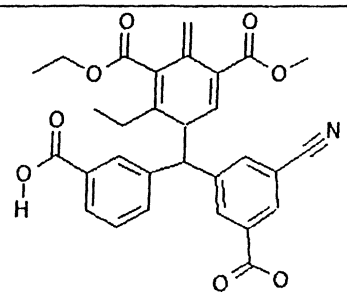
U31/lig4		0	0.660 0.020 Vasodilator 0.295 0.058 Antihypertensive
U32/lig5		0	0.702 0.015 Vasodilator 0.346 0.041 Antihypertensive 0.189 0.176 Uric acid excretion stimulant
U33/lig6		0	0.741 0.012 Vasodilator 0.525 0.013 Antihypertensive
U34/lig7		0	0.680 0.017 Vasodilator 0.323 0.048 Antihypertensive
U35/lig8		0	0.705 0.015 Vasodilator 0.423 0.023 Antihypertensive

Table-4

Chemical structure ,toxicity,pharmacological effect and value of the eight useful ligand

Qsar results

The result page of MDL qsar shows the calculated pMED values,graph and equation of all known molecules:

Results | Means & Correlations | Model Details

	Molecules	pMED	pMED(calc)
1	1	8.4	6.26693
2	2	11	10.1411
3	3	43	18.3734
4	4	67	35.4551
5	5	33	33
6	6	60	15.968
7	7	23	6.60558
8	9	17	16.3393
9	10	11	18.905
10	11	17	21.4853
11	12	20	19.8865
12	13	30	17.5288
13	14	17	16.7353
14	15	18	16.1393
15	16	2.1	3.51495
16	17	0.01	5.91192
17	18	17	9.17263
18	19	2.6	1.30916
19	20	23	-12.5839
20	21	82	21.4853
21	22	4.6	-14.2568
22	23	21	18.7562
23	25	8.5	15.7213
24	26	12	9.62412

Table-5

Calculated pMED value of 24 ligand molecule

The pMED value equation and R-squared value is also appeared in QSAR result page.

Algorithm < Ordinary Multiple Regression >. Regression
pMED = 703.3*xch9 - 3.953*xp7 + 22.246
Regression Quality :
Multiple R-Squared = 0.9214 Standard error of estimation = 2.803 F-statistic = 58.64 P-value = 2.994E-006

Fig-10

The equation evolved in QSAR regression analysis and r-square value
The graph shown below is the result of comparison of pMED values of unknown ligands with known molecules:

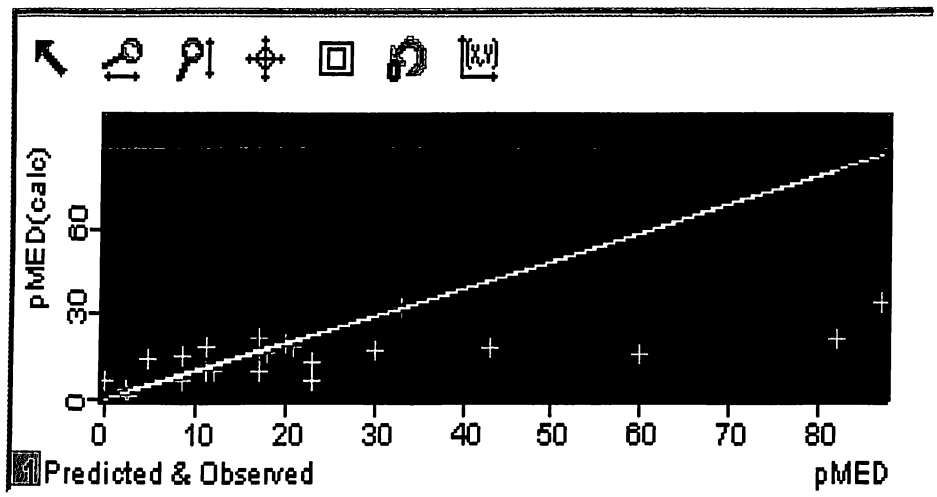


Fig-11

graph showing quality of the model generated with observed pmed values against predicted pmed values. The blue star marks are 26 known ligand molecules.

- This ligand is also showing effect in PASS as shown above table.
- The result of MDL qsar also include some execl sheets showing the comparision between pMED value known and unknown molecules.
- After all structures are screened compared excel sheets are analysed to find out which molecule is more nearer to Aurintricarboxylic acid.

	A	B
1	Name	pMED_skıl
2	U1	4.6089
3	U2	30.105
4	U3	4.0136
5	U4	-6.9678
6	U5	5.5219
7	U6	6.626
8	U7	16.847
9	U8	32.086
10	U9	24.374
11	U10	35.178

	A	B
1	Name	pMED_skı
2	U11	13.699
3	U12	10.471
4	U13	3.805
5	U14	2.894
6	U15	6.4133
7	U16	-0.24715
8	U17	-0.25259
9	U18	12.552
10	U19	13.721
11	U20	20.178

	A	B
1	Name	pMED_ski
2	U21	15.537
3	U22	19.277
4	U23	17.183
5	U25	14.03
6	U26	16.285
7	U27	14.82
8	U28	14.82
9	U29	3.5149
10	U30	-0.95576

	A	B
1	Name	pMED_ski
2	u31	-1.7796
3	u32	8.3351
4	u33	12.435
5	u34	-2.89
6	u35	0.4313
7	u36	20.284
8	u37	-4.8305
9	u38	8.7748
10	u39	-2.8559

	A	B
1	Name	pMED_ski
2	U40	16.186
3	U41	25.105
4	U42	18.968
5	U43	17.163
6	U44	16.955
7	U45	12.767
8	U46	18.02
9	U47	12.661
10	U48	8.0473
11	U49	9.6241
12	U50	11.077

Above five tables are showing the compared ic50 value of all 51 ligand molecule

- The MDL qsar done and result gave a single ligand, U35 (as shown in Table-2) having pMED/IC50 value 0.43 closer to IC50 value of Aurintricarboxylic Acid i.e. 0.010 as shown in Training set table-1.
- Now the appropriate ligand molecule is docked with target protein.
- So lig-8 is taken to docked with target protein 3blu(protein tyrosine phosphataseyopH) retrieved from PDB (rcsb)site.

Auto Dock

- After auto dock it is found that ligand-8 is showing the appropriate fitness function 0.43.
- The energy shown is also in –ve so minimum energy is used in 10 conformation.
- If different conformation are choose the binding of ligand at different site can be visualize.

Docking results :

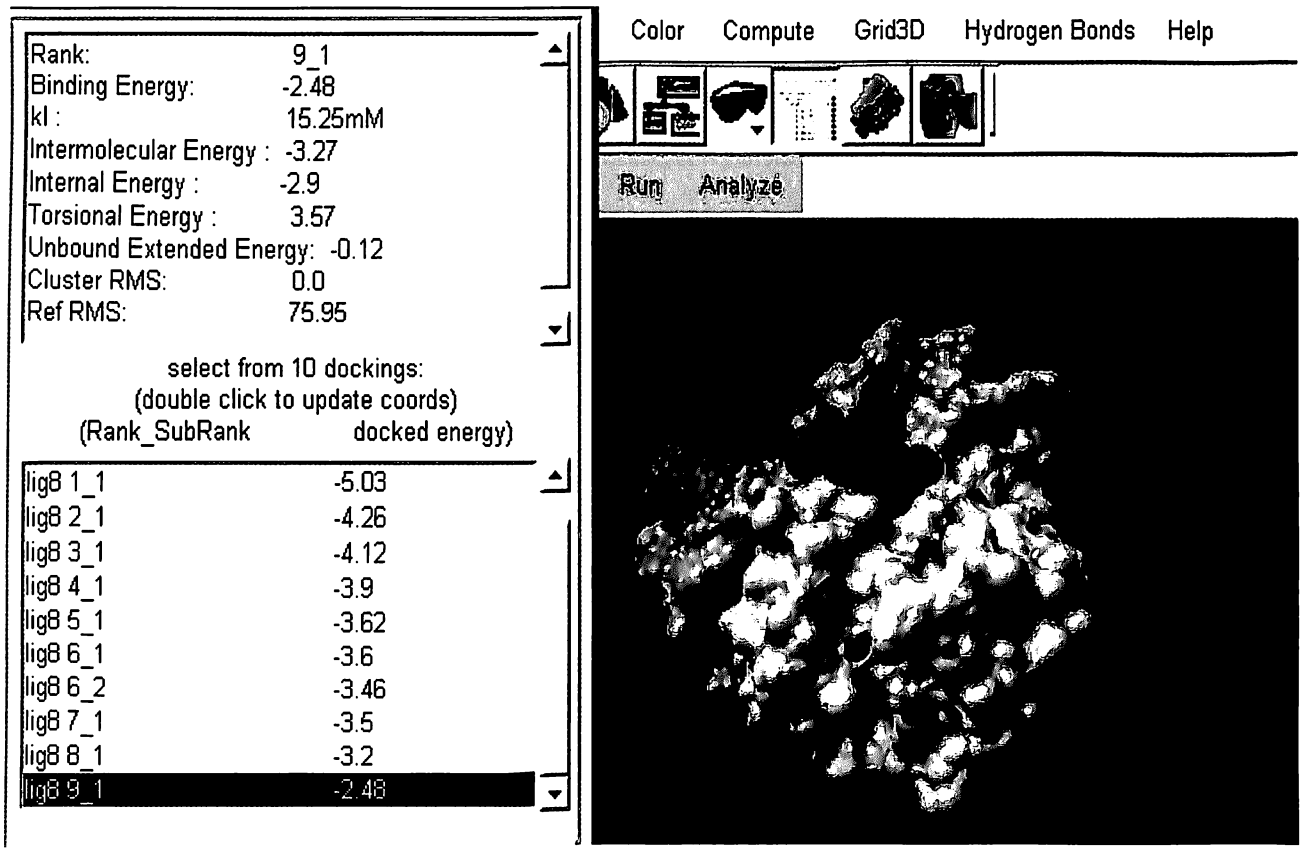


Fig-12

Table(leftside) showing the energy of molecule while docked & cavity(active site) of protein(white) where ligand(blue) is docked(right).

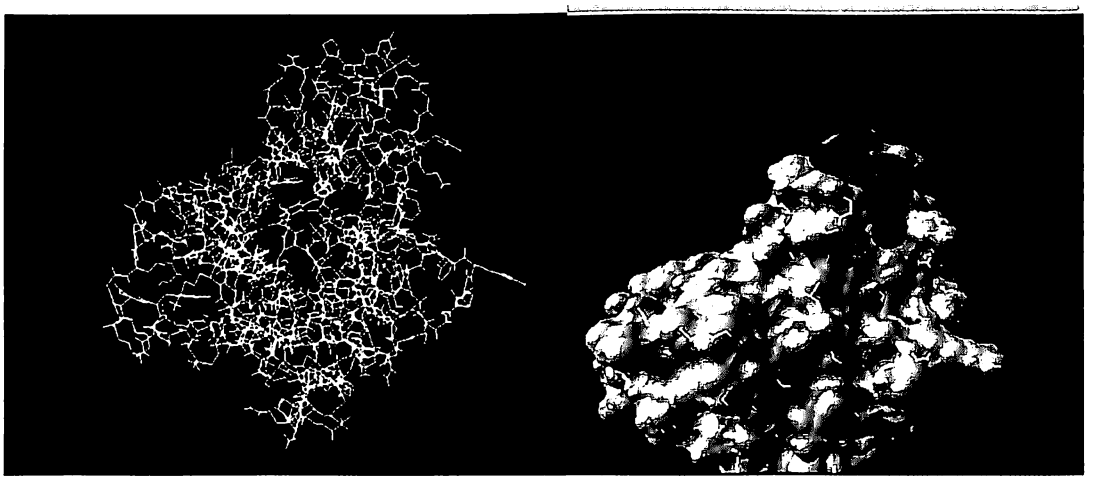


Fig-13

The ligand in blue is docked in the cavity (active site) of the protein.

fig-14

secondary structure of protein showing ligand docked at alpha helix(pink).

DISSCUSION

DISCUSSION

Till now researches and experiments on drug designing were mostly resulting an antibiotic. However, few potent and selective YopH inhibitors are described. Some of the YopH inhibitors that have been identified are molecules from Carboxylic acid library .There are 720 structurally diverse commercially available carboxylic acids and identified 26 YopH inhibitors as shown in table-1. The most potent and specific YopH inhibitor is Aurintricarboxylic acid (ATA), which exhibits a *Ki* value of 5 nM for YopH and displays 6–120-fold selectivity in favor of YopH against a panel of mammalian PTPs.

But now its time to move on so this experiment gives a result of totally chemically developed molecule of drug not from any bio molecule i.e. a nonantibiotic one. So new nonantibiotic drug molecule are developed taking this Aurintricarboxylic acid as parent structure. From the above sequentially steps fighty unknown ligand molecules are constructed which are then screened to get the best one. In the PASS result in table-3 it is found that seven ligands showing positive effect so they were again send for QSAR studies.

QSAR studies the regression analysis produced a QSAR model based on input data and molecular descriptors. Here the input data is 26 known molecules and 46 descriptors were used. The graph (fig 11) showed very good agreement between the observed and predicted pMED values of the 26 molecules. The r-square value (fig.10) suggests that the model generated is stable. On basis of the above model pMED values for the 50 constructed unknown structures were calculated. The calculated pMED values were displayed in form of five excel sheets, each taking 10 molecules at a time. As –ve pMED values cannot be taken so maximum of the molecules were rejected. When compared

with the pmed values of the 50 unknown molecules only one (U35) molecules gave values nearest to the parent pMED value. Partial Least Squares Regression algorithm is used to predict the results.

Autodock is widely used because it provides more reliable result. The ligand 8 is docked with the target protein with active site residue ARG380. The result showed ten confirmations in which the ligand was docked to the protein. Out of these ten the 1st conformation showed the best docking result with best minimum energy of -5.03. Minimum energy describes the stability of the interaction and it should come in negative. But it may come in positive sometime because energy of a confirmation depends on the binding type (lock & key or induced fit type).

Fig 12 reports the –ve values of the 10 confirmations. Fig 12 clearly showed the ligand binds to the protein in its given active site or cavity. Further fig 13 & 14 predicts that the ligand (large blue colour molecule) interacts with the alpha helix of the protein in its secondary structure. This information supports the prediction that this 8 no.ligand have binding affinity and inhibitory action against protein tyrosin phosphatase YopH. As the type of docking is more towards periphery than centre thus this could be confer as peripheral docking.

SUMMARY

SUMMARY

We collected Aurintricarboxylic Acid analogues molecules which have experimental data specillay for Ic50 values.yopH is our protein target which will interact with Aurintricarboxylic Acid .We drawn Known molecules, and hypothetical molecules using ISIS Draw and Chemoffice. Later converted to 3D strctures and minimization of energy using various force fields such as mechanics , mopac,mm2.PASS is used for finding toxicity, pharmacophore properties. This is used for filtration of hypothetical molecules, and we collected best 8 molecule showing zero toxicity and pharmacological properties above 0.7 i.e closer to druglikeness.

Molecule 6-[(3-carboxy-cyclohexyl)-(3-carboxy-3-cyclopropylmethyl-cyclohexyl)-methyl]-5-cyano-3-nitro-1-propyl-3-vinyl-piperidine-2-carboxylic acid having 0.717 values which is itself a very positive result.

Molecule 5-[(3-carboxy-5-cyano-phenyl)-(3-carboxy-phenyl)-methyl]-4-ethyl-2-methylene-cyclohexa-3,6-diene-1,3-dicarboxylic acid 3-ethyl ester 1-methyl ester having 0.705 value and so on.

These eight molecules were then send for energy minimization. we trained experimentally known molecule with MDL-QSAR , and the equation we got is with in allowed range of statistics. This equation is used for prediction of IC50 values, and we got top 1 molecule ligand 8 having pMED value 0.43.

Equation : pMED=703.3* X ch9-3.953* X p7 + 22.246

top1 molecule :"(5-[(3-carboxy-5-cyano-phenyl)-(3-carboxy-phenyl)-methyl]-4-ethyl-2-methylene-cyclohexa-3,6-diene-1,3-dicarboxylic acid 3-ethyl ester 1-methyl ester) "

This molecule then processed for Docking. This molecule interacted with protein YopH tyrosin phosphatase target molecule this is done by using Autodock tools /software.

Finally, a new ligand which can binds with plague protein , a nonantibiotic molecules

""(5-[(3-carboxy-5-cyano-phenyl)-(3-carboxy-phenyl)-methyl]-4-ethyl-2-methylene-cyclohexa-3,6-diene-1,3-dicarboxylic acid 3-ethyl ester 1-methyl ester) " is identified.

This work can be extended for pharmacophore models, structural biology studies, and wet lab work for further confirmation.

APPENDICES

Appendix -1

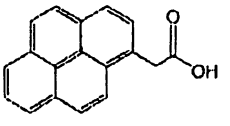
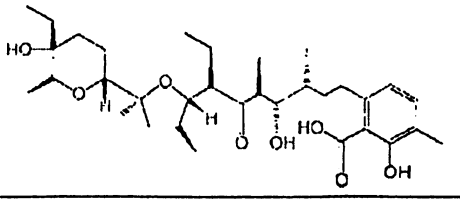
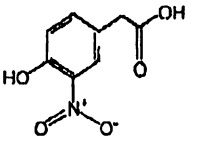
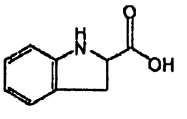
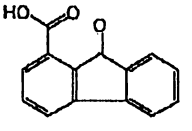
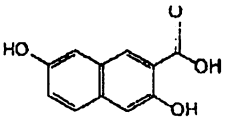
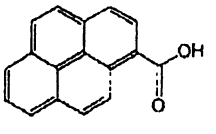
Training set for 26 known structure

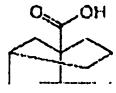
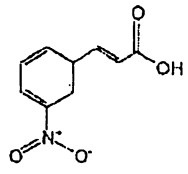
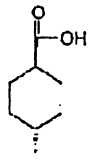
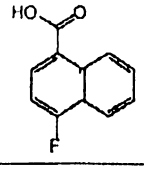
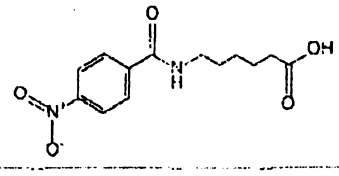
[Article collected from JOURNAL OF BIOCHEMISTRY, VOLUME-278,PAGE-41734,YEAR-2003]

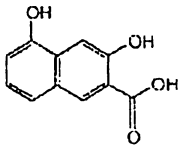
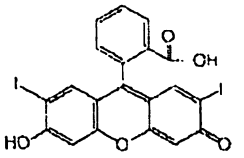
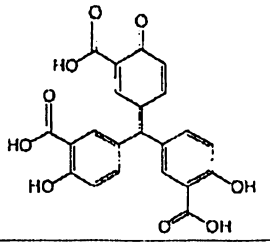
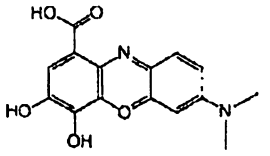
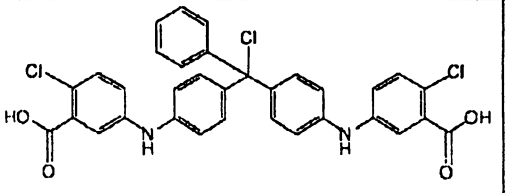
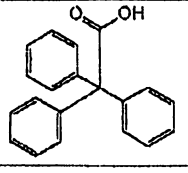
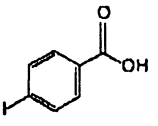
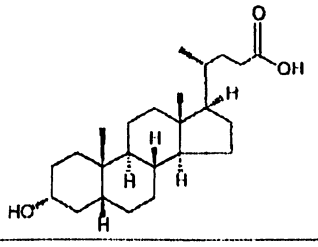
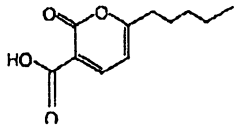
TABLE I

Structures and IC_{50} values of carboxylic acid inhibitors of YopH

The IC_{50} value was measured at a *p*NPP concentration fixed at the experimentally determined K_m for YopH

Compound		IC_{50} for YopH
Name	Structure	(μM)
1-Pyreneacetic acid		8.4±1.0
Lasalocid		11±2.7
4-Hydroxy-3-nitrophenylacetic acid		43±10
(S)-(-)-Indoline-2 carboxylic acid		87±7.4
9-Fluorenone-1-carboxylic acid		33±6.1
3,7-Dihydroxy-2-naphthoic acid		60±11
1-Pyrenecarboxylic acid		23±5.4

Ferrocenecarboxylic acid		23±2.1
3-Noradamantanecarboxylic acid		17±3.4
3-Nitrocinnamic acid		11±1.9
Trans-4-methyl-1-cyclohexanecarboxylic acid		17±5.8
2-Fluoro-6-(trifluoromethyl)benzoic acid		20±4.4
4-Fluoro-1-naphthoic acid		30±6.4
N-(4-Nitrobenzoyl)-6-aminocaproic acid		17±3.6

3,5-Dihydroxy-2-naphthoic acid		18±3.4
Diiodofluorescein		2.1±0.87
Aurintricarboxylic acid		0.010±0.002
Gallocyanine		17±3.0
4,4'-Bis(3-carboxy-4-chloroanilino)trityl chloride		2.6±0.44
Triphenylacetic acid		23±8.3
4-Iodobenzoic acid		82±9.8
Lithocholic acid		4.6±0.84
2-Oxo-6-pentyl-2H-pyran-3-carboxylic acid		21±5.7

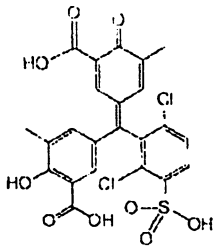
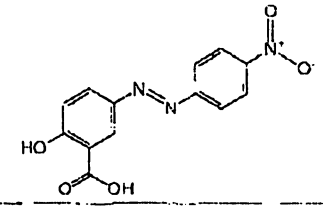
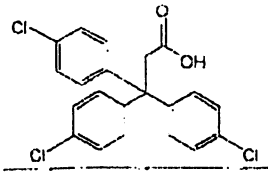
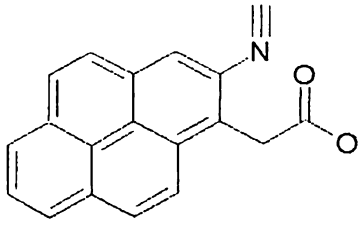
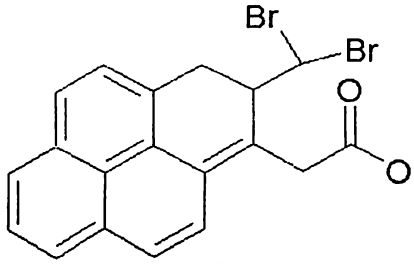
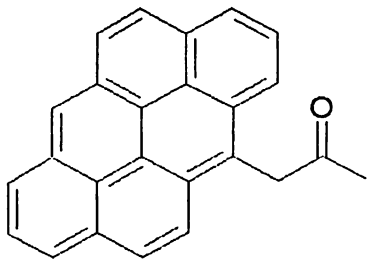
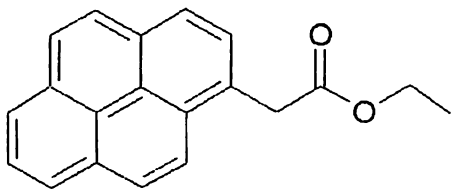
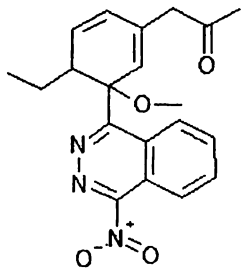
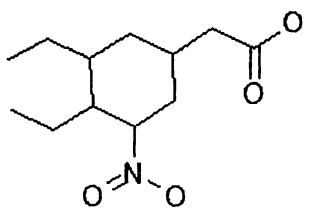
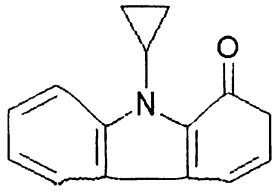
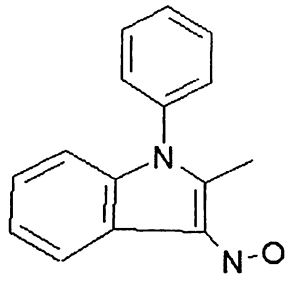
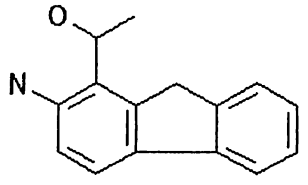
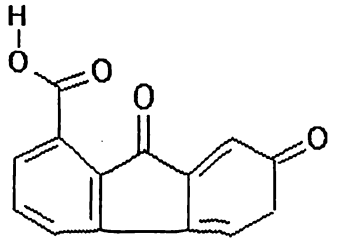
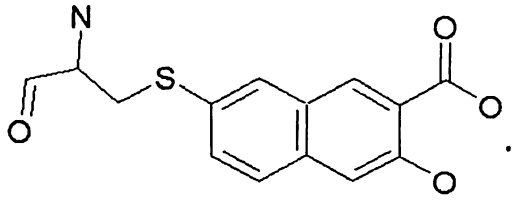
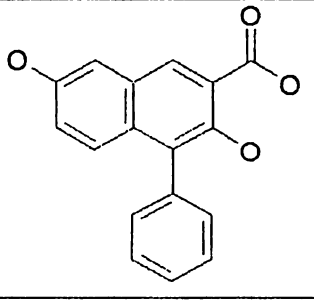
Chrome Azurol S		2.1±0.39
Mordant Orange 1		8.5±2.2
3,3,3-Tris(4-chlorophenyl) propionic acid		12±2.3

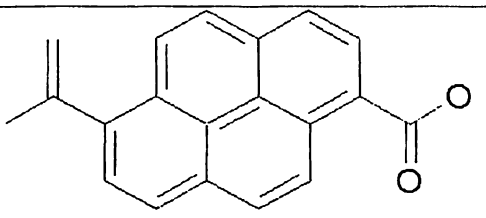
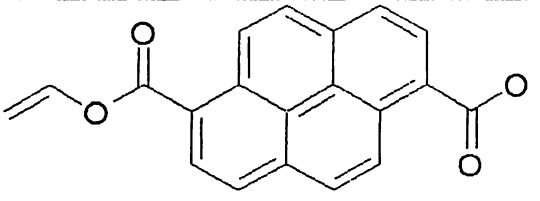
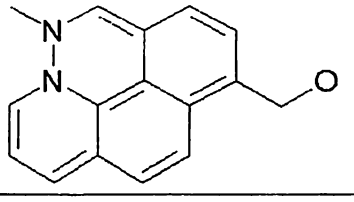
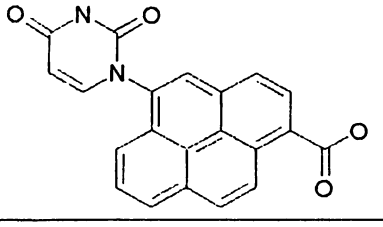
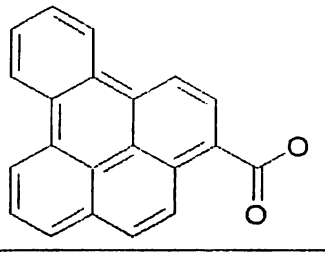
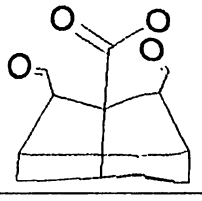
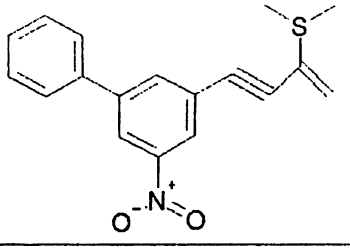
Table-2

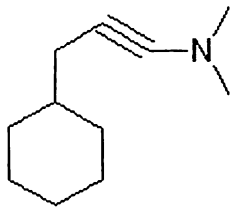
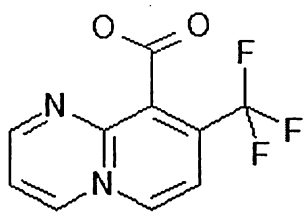
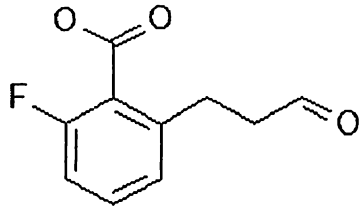
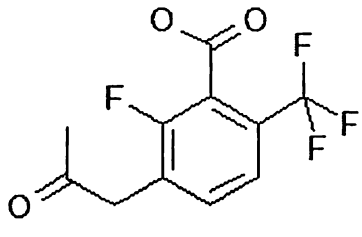
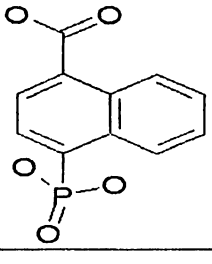
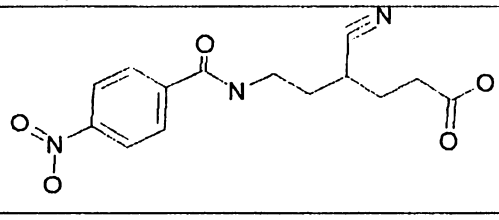
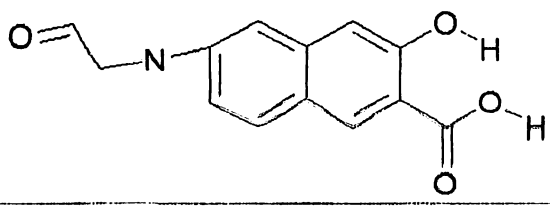
Appendix-2

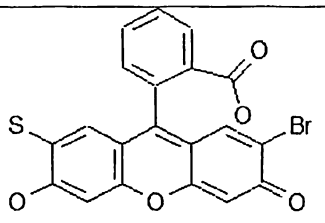
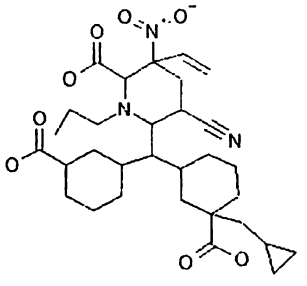
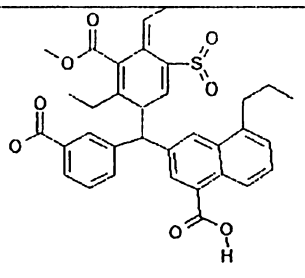
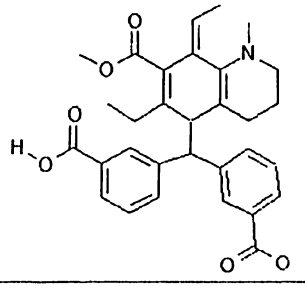
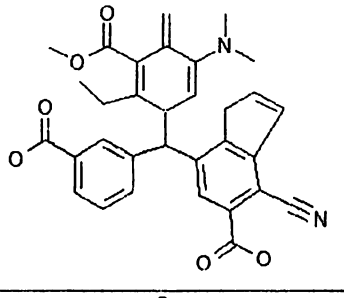
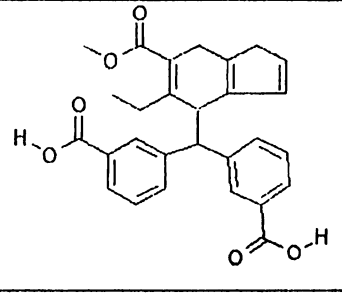
Hypothetical analogues unknown structures are drawn as follows

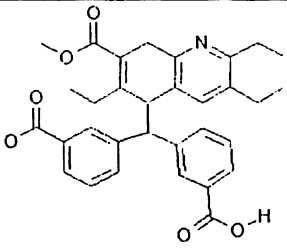
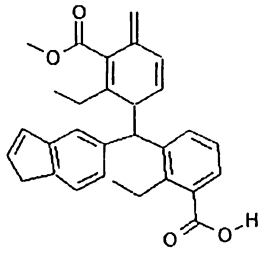
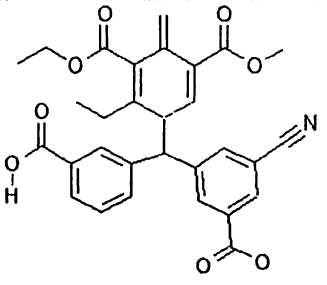
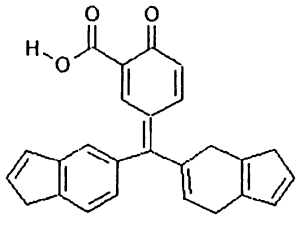
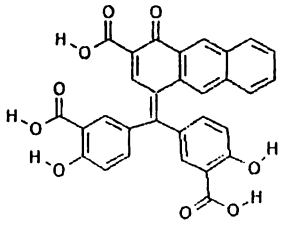
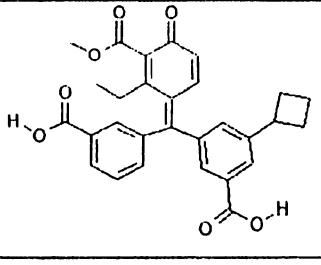
J1		(2-Isocyano-pyren-1-yl)-acetic acid
J2		(2-Dibromomethyl-2,3-dihydro-pyren-1-yl)-acetic acid
J3		1-Dibenzo[def,mno]chrysen-6-yl-propan-2-one
J4		Pyren-1-yl-acetic acid ethyl ester
J5		1-[4-Ethyl-3-methoxy-3-(4-nitro-phthalazin-1-yl)-cyclohexa-1,5-dienyl]-propan-2-one
J6		(3,4-Diethyl-5-nitro-cyclohexyl)-acetic acid

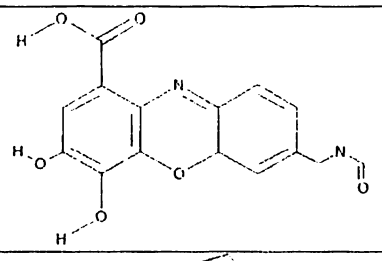
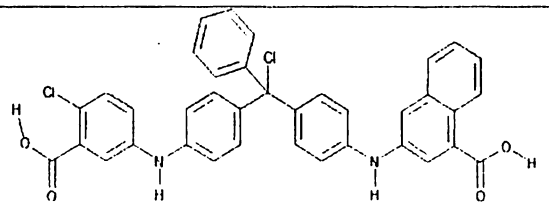
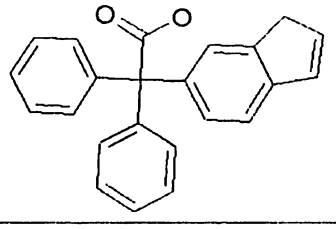
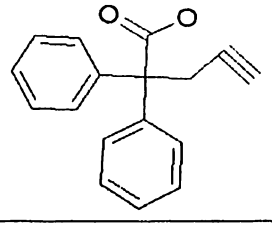
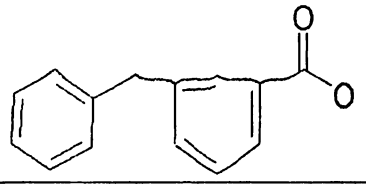
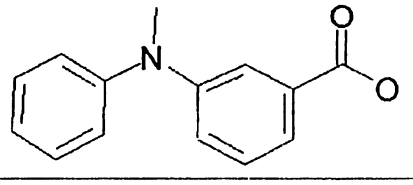
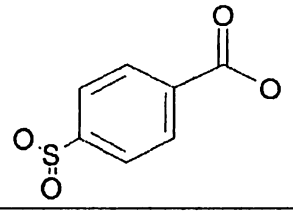
7		9-Cyclopropyl-2,9-dihydro-carbazol-1-one
8		N-(2-Methyl-1-phenyl-1H-indol-3-yl) -hydroxylamine
9		1-(2-Amino-9H-fluoren-1-yl)-ethanol
10		7,9-Dioxo-7,9-dihydro-6H-fluorene-1 -carboxylic acid
11		7-(2-Amino-3-oxo-propylsulfanyl)-3-hydroxy-naphthalene-2-carboxylic ac id
12		3,7-Dihydroxy-4-phenyl-naphthalene-2-carboxylic acid

J13		6-Isopropenyl-pyrene-1-carboxylic a cid
J14		Pyrene-1,6-dicarboxylic acid monovi nyl ester
J15		(4-Methyl-4H-3a,4-diaza-pyren-8-yl) -methanol
J16		5-(2,4-Dioxo-3,4-dihydro-2H-pyrimid in-1-yl)-pyrene-1-carboxylic acid
J17		Benzo[e]pyrene-3-carboxylic acid
J18		3,4-Diformyl-hexahydro-2,5-methano-pentalene-3a-carboxylic acid
J19		5-[3-(Dimethyl-lambda*4*-sulfanyl)-but-3-en-1-ynyl]-3-nitro-biphenyl

U20		(3-Cyclohexyl-prop-1-ynyl)-dimethyl -amine
U21		8-Trifluoromethyl-pyrido[1,2-a]pyri midine-9-carboxylic acid
U22		2-Fluoro-6-(3-oxo-propyl)-benzoic a cid
U23		2-Fluoro-3-(2-oxo-propyl)-6-trifluo romethyl-benzoic acid
U24		4-Phosphono-naphthalene-1-carboxyli c acid
U25		4-Cyano-6-(4-nitro-benzoylamino)-he xanoic acid
U26		3-Hydroxy-6-(2-oxo-ethylamino)-naph thalene-2-carboxylic acid

U27		2-(2-Bromo-6-hydroxy-7-mercapto-3-oxo-3H-xanthen-9-yl)-benzoic acid
U28/Lig1		6-[(3-Carboxy-cyclohexyl)-(3-carboxy-3-cyclopropylmethyl-cyclohexyl)-methyl]-5-cyano-3-nitro-1-propyl-3-vinyl-piperidine-2-carboxylic acid
U29/Lig2		
U30/Lig3		5-[Bis-(3-carboxy-phenyl)-methyl]-6-ethyl-8-ethylidene-1-methyl-1,2,3,4,5,8-hexahydro-quinoline-7-carboxylic acid methyl ester
U31/Lig4		7-[(3-Carboxy-phenyl)-(5-dimethylamino-2-ethyl-3-methoxycarbonyl-4-methylene-cyclohexa-2,5-dienyl)-methyl]-4-cyano-1H-indene-5-carboxylic acid
U32/Lig5		7-[Bis-(3-carboxy-phenyl)-methyl]-6-ethyl-4,7-dihydro-3H-indene-5-carboxylic acid methyl ester

U33/Lig6		5-[Bis-(3-carboxy-phenyl)-methyl]-2 ,3,6-triethyl-5,8-dihydro-quinoline -7-carboxylic acid methyl ester
U34/Lig7		2-Ethyl-3-[(2-ethyl-3-methoxycarbon yl-4-methylene-cyclohexa-2,5-dienyl)-(1H-inden-5-yl)-methyl]-benzoic a cid
U35/Lig8		5-[(3-Carboxy-5-cyano-phenyl)-(3-ca rboxy-phenyl)-methyl]-4-ethyl-2-met hylene-cyclohexa-3,6-diene-1,3-dica rboxylic acid 3-ethyl ester 1-methy l ester
U36		3-[1-(4,7-Dihydro-3H-inden-5-yl)-1- (1H-inden-5-yl)-meth-(E)-ylidene]-6 -oxo-cyclohexa-1,4-dienecarboxylic acid
U37		4-[Bis-(3-carboxy-4-hydroxy-phenyl) -methylene]-1-oxo-1,4-dihydro-anthr acene-2-carboxylic acid
U38		3-{(3-Carboxy-phenyl)-[2-ethyl-3-me thoxycarbonyl-4-oxo-cyclohexa-2,5-d ien-(E)-ylidene]-methyl}-5-cyclobut yl-benzoic acid

U39		7-Formylaminomethyl-3,4-dihydroxy-5 aH-phenoxazine-1-carboxylic acid
U40		3-(4-{[4-(3-Carboxy-4-chloro-phenyl amino)-phenyl]-chloro-phenyl-methyl }-phenylamino)-naphthalene-1-carbox ylic acid
U41		(3H-Inden-5-yl)-diphenyl-acetic aci d
U42		2,2-Diphenyl-pent-4-ynoic acid
U43		3-Benzyl-benzoic acid
U44		3-(Methyl-phenyl-amino)-benzoic aci d
U45		4-Sulfino-benzoic acid

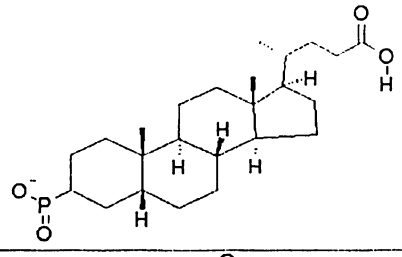
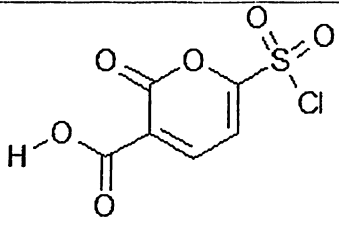
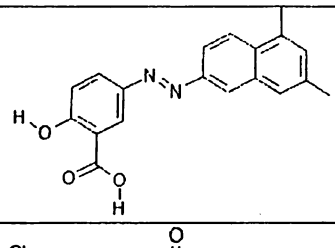
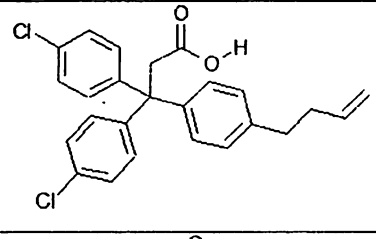
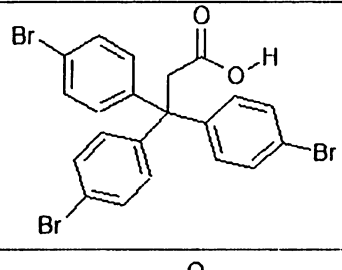
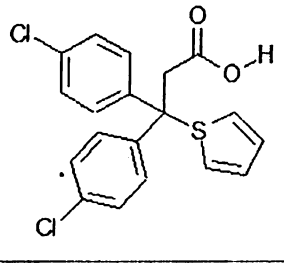
U46		(5R,8R,9S,10S,13R,14S,17R)-17-((R)-3-Carboxy-1-methyl-propyl)-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthrene-3-phosphinic acid anion
U47		6-Chlorosulfonyl-2-oxo-2H-pyran-3-carboxylic acid
U48		5-(5,7-Dimethyl-naphthalen-2-ylazo)-2-hydroxy-benzoic acid
U49		3-(4-But-3-enyl-phenyl)-3,3-bis-(4-chloro-phenyl)-propionic acid
U50		3,3,3-Tris-(4-bromo-phenyl)-propionic acid
U51		3,3-Bis-(4-chloro-phenyl)-3-(1H-1lambda*4*-thiophen-1-yl)-propionic acid

Table-3
Chemical structure and IUPAC name of Hypothetical ligands, analogous to Aurintricarboxylic acid