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Educational, Scientific and
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ABSTRACTS

OF POSTER PRESENTATIONS

(in alphabetical order Author's Surname)

http://cdsagenda5.ictp.trieste.it/full_display.php?ida=a08163

Theoretical Studies of Interactions in Hfq protein from E.coli

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Protein-protein interactions are involved in many biological processes. These interactions form the basis of the quaternary structure of multimeric proteins, and represent one of the most complex levels of structural organization in biological molecules. The specificity of protein-protein interactions is of crucial importance for a specific biological function as to form a defined multimeric structure .

The Hfq protein was first discovered in Escherichia coli as a host factor for the Qβ phage RNA replication[1]. In last 15 years it was shown that Hfq protein is involved in many RNA processing events. A link between Hfq protein from E.coli and spliceosomal Sm proteins was indicated by remote sequence homology. The crystal structure of E.coli Hfq protein[2] showed that its monomer displays a charachteristic Sm-fold and forms a homo-hexamer in a ring-like morphology.

By molecular dynamics calculations we have attempted to explain the nature of interactions between the chains in crystal structure of Hfq protein (PDB code 1HK9). All the calculations were done with CHARMM force field and program[3].

Protonation state of each aminoacid was determened by solving poisson-boltzman equations with CHARMM, Karlsberg and H++ programs. Special care was taken in determinig the protonation state of active HIS 57 residue, and it was shown that it is neutral. This calculations have shown that each chain is positively charged (+3).

Analyzing the interaction energies between chains it was shown that there are repulsive electrostatic interactions between each two chains in the hexamer unit. This interactions are least repulsive between neighboring chains (chains that are in vdW contact). Van der Waals interactions between neigbouring chains are attractive. The calculation showed that vdW interactions plays an important role in chains polymerisation in Hfq protein.

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Influence of conformational polymorphism in the receptor-ligand complex formation

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Keywords: polymorphism, molecular modeling, molecular docking

Introduction

In a recent work [1] we shown the cysteine and serine proteases inhibitor capability of natural polyprenylated benzophenones (identify as guttiferone A, figure 1) isolated from *Garcinia brasiliensis*. In this work we have done a more detailed study about the effect of the benzophenone conformational polymorphism in the receptor-ligand complex formation. Specifically, we were interested in study the inhibitor effect of the planarity between bridge hydroxyl/carbonyl and 13,14-dihydroxyphenyl/phenyl groups presented in human cathepsine G [2].

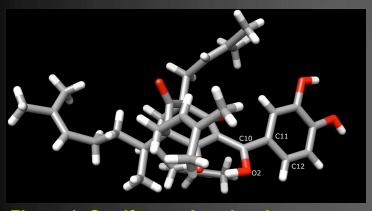


Figure 1. Guttiferone A molecule.

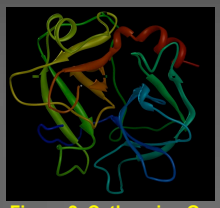
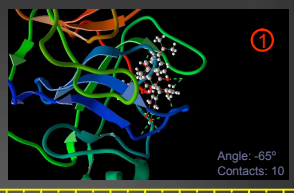


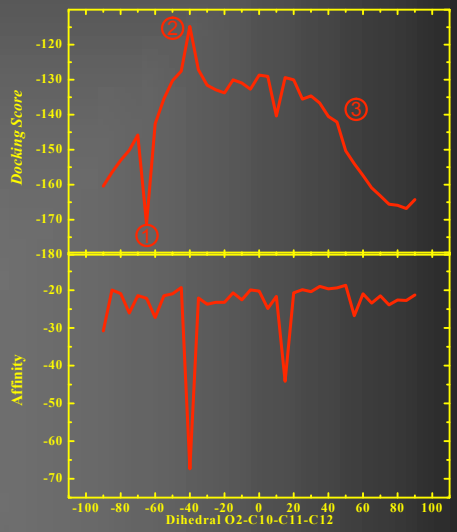
Figure 2. Cathepsine G

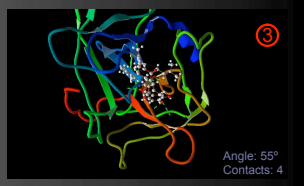
Methodology

In order to do the molecular docking simulation, we setup different ligands from the same molecule for which the torsional angle between atoms O2-C10-C11-C12 was vary in 5º starting from -180º to 180º degrees (see figure 1).

The simulations were carried out using different docking programs. We use GLIDE [3], Molegro [4] and GOLD [5]. As it is well know, these programs use a genetic algorithm (GA) for docking flexible ligands into protein binding sites. This means that each simulation run will use stochastic ligand conformations which in turn imply that the solutions obtained during one run differ from the solutions obtained in other runs. In order to perform the study described here, we make rigid dockings with all the created molecules.



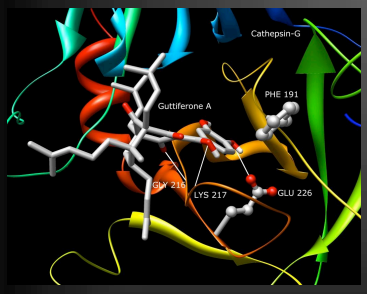




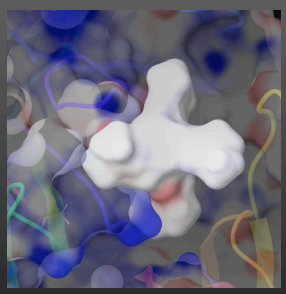
Results and Discussion

The result obtained from the best docking run is shown in figure 3. In the figure are shown the contacts between the guttiferone and the active site residues GLY216, GLU226 and LYS217.

Figure 3. Docking best pose



Ligand-protein interaction



Electrostatic potentials

Conclusions

Our results suggest that, using the genetic algorithm, there is not a clear relationship between the selected torsional angle and the possible inhibitor activity. Therefore we suggest that a more precise computational method (ab initio, DFT) should be used.

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FINEP

Investigation on conformational flexibility of KDR using REST (Replica Exchange with Solute Tempering) methodology

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VEGFRs (***V****ascular* ***E****ndothelial* ***G****rowth* ***F****actor* ***R****eceptors*) are receptors located on the surface of vascular endothelial cells. They are involved in angiogenesis and vasculogenesis and their inhibition has been proven to be attractive for a variety of therapeutic indications, particularly for cancer.[1]

We have focused our attention on human ***VEGFR-2*** which consists of four regions[2]: an extracellular domain that binds ***VEGF*** (***V****ascular* ***E****ndothelial* ***G****rowth* ***F****actor*), a single short transmembrane segment, a cytosolic domain possessing tyrosine kinase activity (also known as ***KDR*** domain) and a carboxy terminal domain. At present 19 structures of the ***KDR*** domain, solved by X-ray diffraction, are available in the Protein Data Bank (PDB)[3]. This domain is folded into two lobes connected by a large (about 30 residues) and highly flexible loop, referred to as the *activation loop*, whose conformation is postulated to regulate kinase activity. In most of the structures deposited in the PDB this part of the protein is not solved and in the only structure (pdb code: 2OH4[4]) that has the coordinates for the entire activation segment, high values of B-factors are associated with ***KDR*** *activation loop* atoms; this disorder is consistent with conformational mobility of this loop.

With the aim of using ***KDR*** structure in a ***S****tructure-****B****ased* ***D****rug* ***D****esign* (***SBDD***) approach, we have started the investigation on the conformational flexibility of the activation loop with an innovative ***R****eplica* ***E****xchange* ***M****ethod* (***REM***), the so-called ***R****eplica* ***E****xchange with* ***S****olute* ***T****empering* (***REST***), a computational technique which has been designed for the efficient sampling of biological systems in explicit water[5]. Both ***REM*** and ***REST*** use high temperatures to accelerate the crossing of local conformational barriers and enhance the sampling of the conformational space of biological systems. Unlike ***REM***, which applies the simulations to the whole system, in ***REST*** method the set of replicated Molecular Dynamics simulations in a defined range of temperatures are employed for the conformational sampling of a specific region of interest, the "central group". In such a way, the computational resources needed to calculate thermodynamic averages are significantly reduced. In the present work we have considered as the "central group" for ***REST*** calculations the ***KDR*** *activation loop*. ***REST*** simulations have been performed using **ORAC**[6], a program developed by the group of prof. Procacci at the Department of Chemistry of the University of Florence.

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ANTIFUNGAL ACTIVITY OF THE METHANOLIC EXTRACT AND PLUMBAGIN FROM *DIOSPYROS CANALICULATA*

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I- BACKGROUND

Plants have been used as a traditional medicine for the treatment of fungi diseases. Plants may provide drugs directly or template molecules on which to base further new structures by organic synthesis. In our search for antifungal agent from natural plants with potential for the treatment of opportunistic fungal infections, we investigate the antifungal activity of plumbagin and raw extract from *Diospyros canaliculata* on human pathogenic fungi.

II- MATERIAL AND METHODS

1- Plant extraction.

The stem bark of *Diospyros canaliculata* was air dried for 15 days and then pulverized. The resulting powder (1kg) was further macerated in methanol (1:1) for 48 hours; the solution obtained was evaporated to dryness under reduced pressure to give the crude extract (320 g).

2- Antifungal assay.

***Microorganisms*:**

Fungi isolates were obtained from clinical specimens and identified according to the colony morphology and microscopic features or using commercially available kits.

They included: *Aspergillus flavus*, *Penicillium sp*, *Cladosporium sp*, *Alternaria sp*, *Helmintosporum sp*, *and Fusarium sp*, *Trichophyton rubrum, Candida albicans*, *Candida glabrata, Candida kefyr and Geotrichum candidum*.

***Inoculum preparation*:**

stock suspensions were prepared by washing the surface of the slants with 2 mL of sterile saline (0.85%) and from 24 h cultures on SDA at 28 °C respectively for filamentous fungi and yeasts. All suspensions were adjusted spectrophotometrically and diluted as necessary to correspond to final concentrations of 1×10^6 cfu/mL for yeasts and 2×10^5 cfu/mL for filamentous fungi.

Determination of Diameters of inhibition zones :

This was done by the cup-plate agar diffusion method according to Murray *et al.* (1995) and Olurinola (1996) at concentrations of 200 mg/mL and 400 µg/mL respectively for raw extract and plumbagin.

***Minimum inhibitory concentration (MIC) determination*:**

MICs were performed by the visual broth macrodilution method. Fungal suspensions were diluted into RPMI-1640 medium to a concentration of approximately 0.5×10^5 CFU/mL for yeast and 2×10^4 CFU/mL for moulds. The final concentrations were 0.098–50 µg/mL (plumbagin) and 0.390–200 mg/mL (extracts).

***Isolation of the active compound*:**

The crude extract was subject to a bioguided fractionation by flash chromatography. Gradient elution was made using hexane-ethyl acetate with increasing polarity. The fraction eluted in hexane 100% crystallized and yielded after filtration 20mg of orange crystals identified as 5-hydroxy-2-methyl-1,4-naphtoquinone (plumbagin) from spectroscopic data.

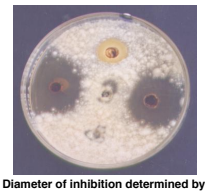
III- RESULTS

1- Diameters of inhibition zones.

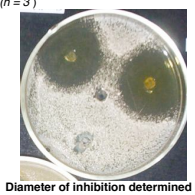
Tableau 1: Diameters of inhibition zones of extract, plumbagin and ketoconazole

	Diameters of inhibiting zones (mm)		
Fungi isolates	**Extract (mg/mL)**	**Plumbagin (µg/mL)**	**Ketoconazole (µg/mL)**
A. flavus	09,66±2,12	10,51 3,29	20,33 2,88
Penicillium sp.	10,06±2,36	15,84 1,87	20,00 2,00
Cladosporium sp.	10,23±4,45	15,52 2,32	18,00 1,73
Alternaria sp.	10,90±2,84	15,86 1,30	23,66 1,52
Helmintosporum sp.	10,73±3,19	14,62 1,07	18,66 1,52
Fusarium sp.	12,50±1,77	13,80 0,23	22,66 2,51
T. rubrum	11,93±2,76	13,15 1,06	18,06 2,00
C. albicans	13,10±3,26	12,86 1,62	17,33 2,51
C. glabrata	13,13±1,42	15,55 1,08	20,00 4,58
C. kefyr	12,76±1,92	12,68 1,99	20,66 1,52
G. candidum	11,76±2,07	17,20 0,10	22,33 2,5

Values are expressed as mean ± SD (n = 3)



Diameter of inhibition determined by the raw extract on ***Fusarium sp***



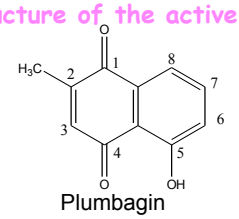
Diameter of inhibition determined by plumbagin on ***Geotichum candidum***

2- Minimum inhibitory concentration.

Tableau 2: Minimum inhibitory concentration of extract, plumbagin and ketoconazole.

	Minimum inhibitory concentration		
Fungi isolates	**Extract (mg/mL)**	**Plumbagin (µg/mL)**	**Ketoconazole (µg/mL)**
A. flavus	12,5	6,25	0,25
Penicillium sp.	25	6,25	2
Cladosporium sp.	12,5	3,12	0,5
Alternaria sp.	12,5	6,25	0,5
Helmintosporum sp.	12,5	3,12	0,25
Fusarium sp.	12,5	3,12	0,12
T. rubrum	25	6,25	025
C. albicans	6,25	3,12	0,25
C. glabrata	25	6,25	5
C. kefyr	12,5	3,12	0,5
G. candidum	25	6,25	2

Chemical structure of the active compound isolated.



Plumbagin

IV-CONCLUSION

The results indicate that, the methanolic raw extract from the stem bark of *Diospyros canaliculata and* plumbagin could be considered as a promising antifungal agents, compared with ketoconazole. Further investigations must be performed for pharmacological and toxicological studies on this compound to ensure the safety of use.

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Exploring 3D-QSAR Pharmacophore Mapping of Pyrimidine derivatives for CDK2/cyclin A inhibition Using HypoGen Technique

Abdulilah ECE and Fatma Sevin Düz

Abstract: A three-dimensional QSAR pharmacophore model for CDK2/cyclin A inhibition was developed from a training set of 17 compounds using Catalyst. To explore the reliable hypotheses, the Fischer's randomization test was employed to filter out the models with poor predicted qualities. The dominant pharmacophore features are Hydrogen Bond Acceptor, Hyydrogen Bond Donor and Hydrophobic. This model was used to search large libraries in order to find out new potent lead compounds. Finally, docking study of these compounds was done using CDK2/cyclin A x-ray structure with CDocker protocol in Discovery Studio 2.0.

An Expanded Hydrogen Bond Based Alphabet for Protein Structure Comparison

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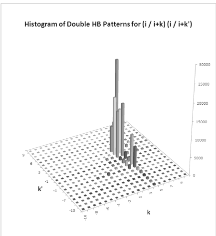
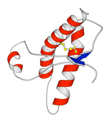
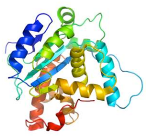
Introduction

Recent works state that using a protein local structure alphabet in combination with sequence alignment dynamical programming is an alternative way to perform structural alignments. The early structural alphabets were based on protein secondary structure, which is defined in terms of Hydrogen Bonding (HB), one of the main factors to protein folding stability. Those HB-based alphabets only consider Main-chain to Main-chain (MCMC) hydrogen bonds and cannot represent accurately protein 3D-structures. In this work, we analyze the correctness of the structural alignments generated by sequences of alphabets based on a comprehensive HB analysis.







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0004 86 QXTGXTEEEXXEXXXSXXEEEMXEEEXXXXXHEEQQQ 122
        . .. .|.. |  .  .|.. ...    .|||||
0005 53 XGRSEEEQQQLETEEEXXXEXXHXXXXXXXXXEEEQQQ  89
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Methods

- **HB patterns clustered from www.pdb.org** (Figure 1)
- **Tolerant geometric HB definition** using Vadar algorithm [1]
- **HB alphabets generation** from the most frequent HB patterns
- **Sequence comparison using DP** (Smith-Watermann)
- **Protein Structural Alphabets Comparison**
  - Entropy and Mutual Information
  - Jaccard Index of generated Alignments
  - ROC for classification performance – using 1NN and SVM
  - N-GRAM - using 1NN and N=1, 2 , 3 e 4

Results and Discussion

We analyzed the use of alphabets based on a comprehensive HB analysis, and we concluded that the yielded structural alignments are more suitable than the original HB-based alphabets and as accurate as the alignments obtained by Q16. We also verified the entropy and mutual information between the alphabets and verified that the characteristics of the HB-based alphabets are equivalent to the detailed ones, so it is likely that they have similar potential for fold recognition 7.

The structural classification accuracy using HB_A sequence alignments and Identity matrix as substitution-matrix suggests that the HB_A is robust and very conservative..

In addition, the structural classification performance of the alphabets were confirmed using an N-GRAM (1 to 4) representation with the inner product as a similarity measure.

HB_B Alphabet

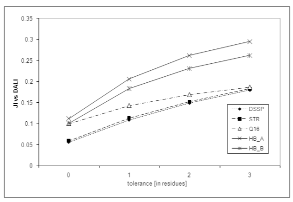
letter	Description	HB Type
A	Strand (or lonely residue) with a parallel strand partner	β-sheets
R	Strand with an antiparallel strand partner	β-sheets
N	Strand (or lonely residue) surrounded by two parallel strand partners	β-sheets
D	Strand (or lonely residue) surrounded by one parallel and one antiparallel partners	β-sheets
C	Strand surrounded by two antiparallel strand partners	β-sheets
Q	i/i+4	Any HB type
E	i/i+3	Any HB type
G	i/i+1	Any HB type
H	i/i+2	Any HB type
I	i/i+5	Any HB type
L	i/i-4	Any HB type
K	i/i-2	Any HB type
M	i/i-3	Any HB type
F	i/i-1	Any HB type
P	i/i-5	Any HB type
S	i/i+k, k<-5	Any HB type
T	i/i+k, k>5	Any HB type
X	No HB intramolecule	HB to solvent

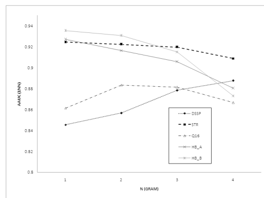
Entropy & Mutual Information

Alphabets	AA	DSSP	STR	Q16	HB_A	HB_B
DSSP	0.102	**2.409**				
STR	0.113	2.355	**2.818**			
Q16	0.178	0.890	0.897	**3.339**		
HB_A	0.158	0.948	1.398	0.649	**2.979**	
HB_B	0.125	0.982	1.432	0.644	2.089	**3.081**

Classification Performance for a Benchmark Dataset (SCOP40mini)

AAUC –1NN	AA	DSSP	STR	Q16	HB_A	HB_B
Total(46)	0.554	0.699	0.708	0.693	0.809	0.772
α (10)	0.320	0.587	0.597	0.617	0.621	0.613
β (12)	0.399	0.632	0.662	0.580	0.904	0.855
Mixed α-β(24)	0.729	0.778	0.777	0.782	0.839	0.792





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Synthesis and Anticonvulsant Activity of Amino Acid-Derived Sulfamides

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Epilepsy is not a specific disease, but a syndrome originated by different cerebral disorders of the central nervous system.[1] In developed countries, new cases are reported yearly in a number close to 40 to 70 per 100000 people. However, most of the epilepsy cases (90%) are found in developing regions, where the reported cases almost duplicate due to the higher risk of experiencing conditions that can lead to permanent brain damage.[2]

The past decades have witnessed many advances in the development of new strategies for the treatment of epilepsy, mainly focused in the prevention of seizures. The new antiepileptic drugs (AEDs) presently used provide adequate seizure control in a significant number of the patients.[3-6] Unfortunately, it is estimated that up to 30% of the affected people are still resistant to the available medication.[7] Furthermore, many AEDs have serious side effects, increasing their toxic actions when a lifelong medication is required.[8] As a result, intensive research efforts are being devoted to find new antiepileptic compounds with more selective activity and lower toxicity.[9]

Amino acids and their derivatives have been investigated for their anticonvulsant action, and some of them were found to be potent AEDs.[9] Several second-generation drugs, as well as drugs in development, have this functionality in their structures. We report herein the synthesis and anticonvulsant activity of a set of amino acid-derived sulfamides. In some compounds, the sulfamide is a nonhydrolyzable linker of two amino ester groups (compounds **1**-**3**, Figure 1), whereas in other structures it connects a lipophilic (alkyl/aryl) group with amino esters (**10**-**12** and **14**-**16**, Figure 1). The most promising compounds are **10**, **11**, and **16**, and for them the median effective dose ED_{50} values for the anticonvulsant activity in the MES test,[10] were determined.

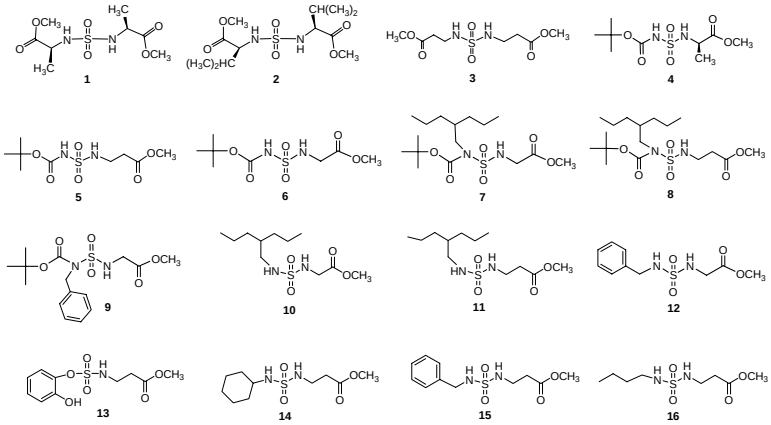

Figure 1.
Chemical structures of the compounds synthesized.

The results are encouraging, as compounds as active as classic anticonvulsant valpromide (ED_{50} 353 μmol/kg), zonizamide (ED_{50} 92 μmol/kg), or phenobarbital (ED_{50} 94 μmol/kg) have been attained.[11] A SAR analysis has allowed us to extrapolate new requirements for the activity, mainly related to the characteristics of a second substituent in the sulfamide function.

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Synthesis and biological evaluation against Cruzipain of new compounds based on 2D Virtual Screening

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Chagas disease is endemic throughout much of Central America, and South America where an estimated 8 to 11 million people are infected.The growing knowledge of the basic biology of *Trypanosome cruzi* facilitates the development of new, rationally developed approaches to Chagas disease-specific chemotherapy. The cathepsin L-like cysteine protease termed cruzipain, is responsible for the major proteolytic activity of all stages of the parasite life cycle and it is an interesting target for the development of potential therapeutics for the treatment of the disease.[1] Virtual screening (VS) methodology has proven to be very useful in the discovery and development of new drugs for neglected diseases.[2] The present work describes the selection and synthesis of novel potential cruzipain inhibitors based on computational tools. A Virtual Screening (VS) strategy was applied over on 537,503 chemical structures from ZINC 5 database to select new cruzipain inhibitors.[3] The methodology included a 2D QSAR approach based on application of linear discriminant analysis (LDA) and Multiple Linear Regression (MLR) on constitutional and topological descriptors from Dragon (Milano Chemometrics, 2003) , to derive five discriminant functions (DF1-5) and two QSAR models (Q1 and Q2) for the prediction of inhibitory activity on cruzipain. The models were internally validated through leave-group-out and randomization tests and externally validated with independent sets of compounds. Descriptor ranges methodology was used for Applicability Domain estimation.[4] Selected compounds were also ranked according to the number of neighbors in the training set with Tanimoto similarity coefficient (based on atom pairs) > 0.5, > 0.7 and > 0.8.[5] 14 compounds were selected by VS and synthesized or purchased and their inhibitory activity on cruzipain was assayed. The most active compound in this series was a quinoline, with an IC_{50} = 36 μM, this compound was also tested *in vivo* against the *trypomastigote* form of the parasite (CL Brener clone); survival of the parasites was 20% after 24 h, when the concentration of drug was kept at 100 μM. It is noteworthy that the structure represents a new scaffold not previously described as active; even though the level of interaction with the target is low or the compound is metabolized, and further structural modifications must be introduced to improve the activity.

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Rational Design of compounds against *T. Cruzi* tryparedoxine peroxidase: Looking for a new drug against Chagas disease.

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The Chagas disease constitutes an endemic public health problem in Latin America with several millions of people infected. It is caused by the protozoan parasite *Trypanosoma cruzi*, against which no vaccines are available at present. The protein Tryparedoxin peroxidase is synthesized by the parasite and helps to render it more resistant to the organic defenses. The protein belongs to the family of typical peroxiredoxins, and exists as homodimers (a2) or homodecamers (a2)5. The crystal structure has already been reported for both, the *T. Cruzi* and the human *protein*.
In this work we present two strategies for the identification of anti Chagas compounds that bind on a specific active site of the Tryparedoxin peroxidase. The specific site was defined as the non-conserved residues on the *T. Cruzi* peroxiredoxin against the *human* protein. This site resulted in the Met46, Glu81 and Lys108 residues in the *T. Cruzi* protein, which are changed by Val77, Gln80 and Val107 in the *human* protein.
The first strategy consist on a multistep virtual screening of large freely available drug-like libraries (ZINC database), and the second consist in the *de novo* design of a peptidomimetic molecule based on a set of molecular dynamics (MD) simulations of oligopetides inserted in the enzyme's active site. The multistep virtual screening was performed using FRED and Autodock programs for rigid and flexible docking respectively on the *T. cruzi* and *human* peroxiredoxins. Several compounds were found to be potential candidates using this strategy. The best molecules were chosen as those that have strong affinity for the specific binding site of the *T. Cruzi* protein while the affinity for the human protein is very much decreased. Additionally, using an alternative strategy based on MD simulation, a tetra peptide was designed in such a way that, by construction, fit within the active site of *T. Cruzi* protein.
Finally, the best compounds and the tetra peptide will be tested on biochemical assays to assess theirs inhibitory capabilities.

Targeting flavin-dependent thymidylate synthase: in silico studies

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Until recently TS was thought to be the only enzyme responsible for the synthesis of dTMP and both the TS and the DHFR have been widely used as targets for compounds that inhibit cellular proliferation[1]. However comparative genomics and experimental works has revealed a large number of microbial species that lack genes encoding either of these enzymes. The data demonstrate that two major pathways for dTMP formation operate in microbial world. A large family of previously uncharacterized ThyX proteins corresponds to a novel class of flavin-dependent thymidylate synthase (FDTS), present in up to 30% of completed microbial genome sequences[2,3]. ThyX shares no sequence or structural homology to the classical TS and they differ in their reductive mechanism: TS uses mTHF both as reductant and methyl donor, while the FDTS enzymes employ mTHF only as donor of the carbon unit maintaining the folate in its reduced form (THF) at the end of the catalytic cycle. This enzyme requires flavin adenine dinucleotide (FADH) as reductant cofactor for its function. Since the two enzymes differ markedly and many of the organisms that contain the alternative enzyme are severe human, animal and plant pathogens, drugs targeting ThyX may be specific to those pathogens with little, if any, toxic effect to humans[4].

The final aim of the project is the inhibition of ThyX enzyme.

First of all an *in silico* model for the virtual screening of large libraries of compounds, for the identification of *M. tuberculosis* enzyme's inhibitors, was to develop. The right "conditions" and parameters to perform the docking for a structure-based virtual screening of a library were set up. DOCK6.2, AutoDock4.0.1 and Goldv3.2 were evaluated running different trials in order to reproduce substrate and cofactor's conformations observed in the x-rays. Secondly the set up docking algorithm was used to dock our *in-house* compound libraries. After the docking the binding modes of the best compounds were analyzed and some were suggested for the experimental inhibition's assays. The enzyme was purified in our lab and activity assay of the former compounds is still a work in progress.

Perspective works are (i) the study and thus the improvement of the selectivity of the compounds and their specificity versus the ThyX respect to the classical hTS; (ii) the virtual screening of a larger database, as for example the NCI database, to find new hit molecules; (iii) the building of the homology model of *H.pylori* ThyX, whose crystal structure is not available.

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Identification of Novel Inhibitors for *Mycobacterium tuberculosis (Mtb)* enoyl acyl carrier protein (ACP) reductase (InhA) by virtual screening

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The Global epidemic of tuberculosis (TB) continues, and it remains a significant threat to public health. There is an urgent need for the development of new anti-tubercular agents that will combat the increasing resistant strains of *Mtb.* InhA is involved in the synthesis of mycolic acids via the fatty acid synthase II complex. Mycolic acids are an essential part of the mycobacterium cell envelope, and an important component of the bacterial defence mechanism. InhA is known as the target for Isoniazid, which requires activation by *Mtb* catalase-peroxidase (KatG)[1] before binding to InhA.

Our main focus is to search for compounds that will lead to the development of antitubercular drugs, which target InhA but do not require activation. InhA remains a good candidate for drug design because (i) the mycobacteria utilises the product of its catalysis to create mycolic acids essential for their survival; (ii) most of the mutations linked to resistance of Isoniazid are associated with KatG[2]; (iii) InhA has specificity for long chain fatty acids differentiating it from other enoyl-ACP reductases; (iv) it is the only enoyl-ACP reductase found in *Mtb*.

To this end, we have carried out modelling studies on the protein[3] using SYBYL and virtual screening of a library of compounds[3] against our target using GOLD. Using this approach, GOLD has been validated and potential inhibitors have been identified (Fig.1) considering fitness scores, interactions with target, visual inspection etc. We will present details of docking studies with these compounds, and how they might interact in the active site of InhA as well as their preliminary biological evaluation.

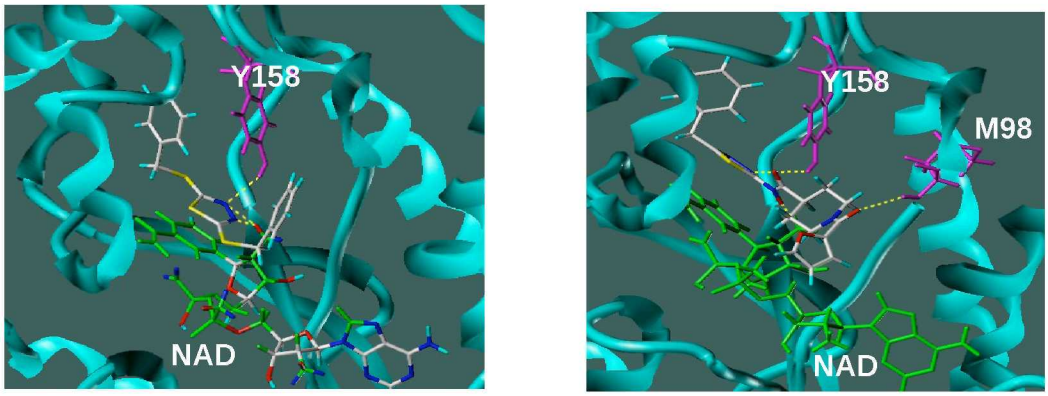

Fig 1. Example of identified potential inhibitors making hydrogen bond contact to InhA. (Created in SYBYL)

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Preparation of ^{99m}Tc-Ciprofeloxacin

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Infectious disease is still a common problem in developing countries. Access to rapid and accurate method help us to better management of infection disease. Techniques involving radiolabeling leukocytes can pinpoint the site of inflammation. Since some perivious labeling techniques have failed to distinguish between bacterial-mediated infection and non-bacterial inflammation new labeling focuse don antibiotic labeling 99mTc-cip was prepared by mixing ciprofloxacine and formamidine sulphonic acid as A reducing agent with pertechnetate solution. The the mix solution was passed through the silica cephadex column . The radiochemical purity and stability of the 99mTc-CIP were determined by using ITLC (SG) and Whatman No.3 chromatographic strips with acetone and saline as solvent. The labeled CIP and colloids remain at the origin while pertechnetate migrates at solvent front (Rf=1). Radiolabelled efficiency of 99mTc-CIP was approximately 95%.

ALMIGHTY: Tool for Analysis of DNA and Protein Sequences

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Abstract:

In the field of bioinformatics, there is a long list of tools available for sequence analysis, each performing a single function. To perform different types of analysis of a sequence, one need to use different tools, which is time consuming? ALMIGHTY aims at providing a one stop solution for all types of sequence analysis requirements for the biologist. It is a multi-functionality tool so that a biologist can install and use only ALMIGHTY for most of his analysis requirements. It is an offline tool (desktop application), so one does not need to depend on the internet connection\speed to perform his analysis. The tool is developed with a graphical user interface in PERL. The Perl/TK module has been used to develop the front-end application and different bioperl packages have been used to perform respective functions available under various heads. We can use Almighty to analyze the composition of sequences, format them for further use, transform them to other types of sequences, align them etc.

Structure prediction of a β_3- Adrenergic G-Protein coupled receptor

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The G-protein coupled receptors have a major role in transmembrane signaling in most eukaryotes and many are important drug targets. β -adrenergic receptors (βARs) belong to most widely studied Class A of the large family of seven transmembrane receptors or G-protein coupled receptor (GPCR) super family. The βARs bind catecholamines and transduce intracellular signals of appropriate magnitude and specificity through tightly regulated mechanisms of receptor activation, desensitization, and internalization. On the basis of their different sensitivity to a series of agonists and antagonists, and their different tissue distribution they are classified into three types β1, β2, and β3. They seem to possess distinct intracellular signaling and functional properties. β3 adrenergic receptor has received considerable attention as a potential therapeutic target for obesity and adult-onset diabetes on the basis of studies that have used rodent models of these diseases.

The crystal structure of only β2-adrenergic receptor in human is known. The human β3 adrenergic receptor which is a sequence of 408 amino acids was downloaded from UNIPROT database. The human β3 receptor is 50% and 45% identical to human β1 and β2 receptors respectively. The structure prediction was carried out using various tools- I-TASSER, Phyre, HHPRED, Modeller, Robetta which were ranked highest among the CASP8 (8th Community Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction), The structure prediction was done using algorithms for homology modeling, threading and ab-initio methods. The models generated were analyzed based on their 3D- structures, energy, Ramachandran plot and superimposition techniques.

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Study on the interactions and recognition between HIV-1 Integrase and its ligands

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1. The drug resistance and the binding mode of HIV-1 Integrase with L-Chicoric acid (LCA) inhibitor

The binding mode of the core domain of the wild type integrase (IN) core domain and its G140S mutant with LCA inhibitor were investigated by using multiple conformation molecular docking and MD simulation. Based on the binding modes, the drug resistance mechanism was explored for the G140S mutant of IN with LCA. The results indicate that the binding site of the G140S mutant of the IN core domain with LCA is different from that of the core domain of the wild type IN, which leads to the partial loss of inhibition potency of LCA. The flexibility of the IN functional loop region and the interactions between Mg^{2+} ion and the three key residues (i.e. Asp64, Asp116, Glu152) stimulate the biological operation of IN. The drug resistance also lies in several interaction changes, such as the repulsion between LCA and E152 in the G140S mutant core domain, the weakening of K159 binding with LCA and Y143 pointing to the pocket of the G140S mutant.

2. The recognition and inhibition molecular mechanism of HIV-1 Integrase by EBR28 peptide inhibitor

Recently synthesized 12-mer peptide EBR28, which can strongly bind to Integrase (IN), is one of the most potential small peptide leading compounds inhibiting IN binding with viral DNA. However, the binding mode between EBR28 peptide with HIV-1 IN and the inhibition mechanism remain uncertain. The binding modes of EBR28 with HIV-1 IN monomer core domain (IN_1) and dimmer core domain (IN_2) were investigated by using molecular docking and MD simulation methods. The results indicate that EBR28 binds to the interfaces of the IN_1 and IN_2 systems mainly through the hydrophobic interactions with the β3, α1 and α5 regions of the proteins. Based on this binding mode, the binding free energies for IN_1 with a series of EBR28

mutated peptides were calculated with the MM/GBSA model, and the correlation between the calculated and experimental binding free energies is obvious (r = 0.88). Thus, the validity of the binding mode of IN_1 with EBR28 was confirmed. The inhibition mechanism of EBR28 was explored by the essential dynamics (ED) analysis, energy decomposition and the mobility of EBR28 in the two docked complexes. The proposed inhibition mechanism is represented that EBR28 binds to the interface of IN_1 to form the IN_1_EBR28 complex and prevents the formation of IN dimmer, and finally leads to the partial loss of binding potency for IN with viral DNA.

3. The conformational changes and binding mode of HIV-1 Integrase with viral DNA

The specific binding mode between intergrase (IN) and its substrate 27 bp segments of viral DNA was obtained via the molecular docking method. The results show that the key residues for IN dimer binding with viral DNA are Lys14, Arg20, Lys156, Lys159, Lys160, Lys186, Lys188, Arg199 residues in the chain b and Lys219, Trp243, Lys244, Arg262, Arg263 residues in the chain A. The explanation for the minimum length of 15 bp viral DNA to activate IN was given based on the docked complex structure. Through the binding energy analysis, it is found that non-polar interactions are the principal factor favoring the binding of IN with DNA; whereas, the stable association of viral DNA with the key residues are mainly driven by polar interactions.

Based on the binding mode obtained by molecular docking, the change of motive mode, correlative movement and viral DNA conformational change were explored with MD simulation and statistical methods. Then, solvent effect during the association IN dimer with viral DNA was analyzed briefly. The result shows that viral DNA can be divided into five regions (i.e. non-binding region, high-affinity region 1, weak-affinity region, high-affinity region 2 and reaction region) according to the binding ability. After the association of viral DNA with IN dimer, there are some significant changes in both the motive mode and the cooperative movement for each system. Compared with viral DNA before binding with IN dimer, some big conformational changes occurred for the bases in the binding region other than the non-binding region for viral DNA complexed with IN. The obvious deviation from standard B-DNA in the viral DNA main chain of the complex and the broading of the

minor groove in the binding site are both the fundamentals for the recognition basis of viral DNA with IN dimer. Through analyzing the hydrogen bonds formed by water in the interface between IN dimer and viral DNA, it is found that water molecules play an important role in the recognition between IN and viral DNA.

Key words: Drug design; molecular modeling; HIV-1 integrase; viral DNA

Prediction of the most favorable configuration in protein-membrane interactions based on computational calculations

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To estimate the importance of the non-specific electrostatic energy in the ACBP-membrane interaction, we computationally modeled the interaction of HgACBP with both anionic and neutral membranes. Acyl-CoA binding proteins (ACBPs) are highly conserved 10 kDa cytosolic proteins that bind medium- and long-chain acyl-CoA esters. They act as intracellular carriers of acyl-CoA and play a role in acyl-CoA metabolism, gene regulation, acyl-CoA-mediated cell signaling, transport-mediated lipid synthesis, membrane trafficking and also, ACBPs were indicated as a possible inhibitor of diazepam binding to the GABA-A receptor . To compute the Free Electrostatic Energy of Binding (dE), we used the Finite Difference Poisson Boltzmann Equation (FDPB) method as implemented in APBS. In the most energetically favorable orientation, ACBP brings charged residues Lys18 and Lys50 and hydrophobic residues Met46 and Leu47 into membrane surface proximity. This conformation suggests that these four ACBP amino acids are most likely to play a leading role in the ACBP-membrane interaction and ligand intake. Thus, we propose that long range electrostatic forces are the first step in the interaction mechanism between ACBP and membranes.

2nd Conference on Drug Development for the Third World: From Computational Molecular Biology to Experimental Approaches

Inferring drug targets from Gene-Networks

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Predicting the mechanism of action of drugs can greatly facilitate rational drug design. Predictions, however, are difficult to obtain, both because drugs can induce broad cascades of perturbations beyond the primary target, and because a single chemical species could have multiple targets, dynamically interfering with different pathways, enzymes and metabolites. To address these issues, it would be desirable to develop a systems biology approach aimed at identifying the genes that are mediating the response to a drug's perturbations at a genome scale, and in a time-dependent manner. A related important question is whether the understanding and prediction of individual drug responses could help identify pairs of drugs whose simultaneous action synergistically improves killing efficiency. Such combination therapies may play a major role in fighting the increasing emergence of resistant strains. From the computational perspective, while several methods for the identification of drug targets rely on binary control/untreated experimental data, it is still unclear how to best utilize data from time-series experiments that monitor the stimulus-induced transient response.

Here, we present the method of Dynamic Network Enrichment (DNE), which aims to reverse engineer the regulatory mechanisms observed in gene expression time courses through integration with steady state profiles. A gene network model is first trained on a large compendium of gene expression measurements taken under steady state conditions. A temporal gene expression profile is then used on the top of the previous network. A non-equilibrium description reveals the causality of specific gene-gene interactions. Genes showing an incoherent behavior relative to the initial model are chosen as the best candidates for direct targets of external perturbations. The underlying network and the significantly perturbed gene candidates are identified through a rigorous statistical analysis. By appropriately combing inferred responses to individual drugs we finally suggest a possible method for predicting the effects of drugs combination.

We tested our approach by using previously published time-course gene expression profiles of *E. coli* in response to a sub-lethal dose of a variety of antibiotics associated with different mechanisms of action. In applying DNE to these expression profiles, we identified many known antibiotic targets among the most significantly perturbed genes. Moreover, our novel DNE predictions, amenable to experimental testing, suggest a prominent role of previously unsuspected genes in the antibiotic response, as well as novel epistatic interactions between drug pairs.