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CHEMOINFORMATIC APPROACH TO IDENTIFY BEST OF ISATIN –3 IMINE AND 4-AMINO-4, 5 DIHYDRO-1H-1, 2, 4 TRIAZOLE-5-ONESDERIVATIVES FOR CYTOCHROME P450 IN*ASPERGILLUS FLAVUS*

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ABSTRACT

Chemoinformatics is the use of computer and informational techniques, applied to a range of problems in the field of chemistry. These *in silico* techniques are used for designing antifungal potent drug in pharmaceutical companies, it is also known as target based drug designing or structure based drug designing. From many interrelated heterogeneous resources data are integrated that can help in understanding complex biological processes and help to improve drug discovery.In this work chemo-informatics is applied to obtain the effective antifungal agent amongst derivatives of Isatin –3 imine and 4-amino-4,5 dihydro-1H-1,2,4 triazole-5 ones against *Aspergillus flavus*. The work has been out using NCBI {Database}, cactus server for protein format conversion {Database}, Swiss model{Server} for automated comparative modeling of three dimensional protein structure, MVD {Software} for docking purpose.The results obtained demonstrate electronic, steric and lipophilic properties of the substituents and stimulates the biological activity of Isatin –3 imine and 4-amino-4,5 dihydro-1H-1,2,4 triazole-5 ones against *Aspergillus flavus.*

Keywords: Virtual screening, 5-(3 Chloro-1-benzothien-2 yl)-4-phenyl-4H—1,2,4 triazole-3-thiol, *insilico*

INTRODUCTION

Chemoinformatics is the mixing of those information resources to transform data into information and information into knowledge for the intended purpose of making better decisions faster in the area of drug lead identification and optimization. Many five membered heterocyclic compound such as pyrroles, imidazole, oxazole, itraconazol act as effective antimicrobial agents **[1]**. However, the triazoles have antibacterial, antimycobacterial, antimycotic ,antifungal and antidepressant properties such as N-acylated aminoacids are known for their hepatoprotective and antimicrobial effects **[2]**. Triazole with Isatin moity which is exposed to a weak base **[3]** mainly react at three different sites aromatic substitution at C-5, alkylation at N-, and carbonylation at C-3. Isatin has been recently found to exhibit biological activity in mammals. Isatin also is a synthetically versatile substrate that can be used to prepare a large variety of heterocyclic compounds, such as indoles and quinolines, and as a raw material for drug synthesis **[4]**.

Triazole has broad range of application in the treatment of both superficial and systemic fungal infections and it also shows greater affinity for fungal Cytochrome P-450 enzymes for ex. Fluconazole for treatment of Histoplasmosis **[5]**. The commonly used imidazole and triazole-class antifungal drugs work by inhibition of the fungal Cytochrome P-450 14- α demethylase. This interrupts the conversion of lanosterol to ergosterol, a component of the fungal cell membrane. The fungal infections still remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents. Immunocompromised patients are particularly at risk of developing these infections, with *Aspergillus* sp. that are resistant to antifungal agents, making treatment options a concern **[6]**.

The cell wall of pathogens containing mannoproteins, chitins, and α and beta-glucans play an important role in protection, cell morphology, cell rigidity, metabolism, ion exchange and filtration, antigenic expression, primary interaction with the host and resistance to host cell-mediated immune function **[7]**. Thus, novel targets have been explored in an attempt to overcome the problems derived from the resistivity of traditional targets. *In silico* techniques, which have the power to cut down unavoidable steps, pains and labour, have been used to identify antimicrobial agent **[8]**.

The objective of this study was to identify best of isatin –3 imine and 4-amino 4, 5

dihydro-1H-1,2,4 triazole-5-ones derivatives and to locate the novel drug target for cytochrome p450 in *Aspergillus flavus.*

MATERIALS AND METHODS

Materials

Databases, softwares and online servers used during the study were as follows:

PDB {http://www.pdb.org}

NCBI {Database: www.ncbi.nlm.nih.gov}

Cactus server for protein format conversion {Database}

Swiss model {Server :- http:/swissmodel.expasy.org}

Molegro Virtual Docker {Software}

Pharma algorithm {Database}

METHODOLOGY:

Sequence retrieval

Homology modeling

Generation of ligand library

Virtual screening of the ligand library for minimum energy calculation

In silico adme/tox analysis of drug like molecules

RESULTS AND DISCUSSION

1. **Sequence Retrieval**

   For *insilico* analysis of 14-α sterol demethylase in *A.flavus* sequence was retrieved from NCBI. The protein retrieved in FASTA Format is as follows-

   14-α sterol demethylase [*Aspergillus flavus*]

   WFPFIGSTISYGMDPYRFFFNCR
   EKYGDIFTFYLLGKKTTVYLGT
   KGNDFILNGKLROVCAEEVYSP
   LTTPVFGRHVVYDCNAK

2. **Homology Modeling**

   Homology based modeling to predict a structure from its sequence with an accuracy of enzymes, was done by Swiss Model server **[9]**. **(Figure 1 a and b)**.

3. **Generation of Library of Triazoles**

   Ligand library was generated with the help of cactus server (CADD groups) by changing the substituents and their positions in the ring. Library is generated in mol 2 format. 3D structure and properties of the derivatives were tabulated in **Figure 2 a-d**.

4. **Virtual Screening of The Ligand Library of 1,2,4-Triazole For Minimum Energy Calculation**

   Docking was done to achieve an overall "best-fit" conformation between the ligand and the protein. Docking score of different derivatives with enzyme cyp P450 were as shown below in **Table 1**.

The re-rank-score provides an estimate of the strength of the interaction. 3-[3'-(4''-m-chlorobenzyl)-4',5'-dihydro-1'H-1',2',4'-triazol-5'-on-4'-yl]-iminoisatin shows the minimum re-rank score (-75.0732) and 3-[3'-(4''-o-ethylbenzyl)-4',5'-dihydro-1'H-1',2',4'-triazol-5'-on-4'-yl]-imino isatin has maximum re-rank score (-68.836).

The active site i.e. amino acids interaction with the drug molecules has been represented with the help of pictures captured by MVD in **Figure 3 a-d**.

The pharmacological properties of triazole having chlorine substituted phenyl ring can be justified by the observation **[10]**.It can also be explained by the study of **[11]**, according to which alkyl substituent ring resulted in no improvement or loss in antibacterial activity **(Figure 4)**.

5. Preclinical Pharmacology/Toxicology of Drug Like Molecules (ADME/TOX Properties)

After selection of lead molecule preclinical pharmacology and toxicology study was carried out to find out the druglikeness of molecule **(Table 2)**. Factors such as absorption, distribution, biotransformation (metabolism) and elimination govern the ability of a drug to reach the active site soon after its administration **[12, 13]**.

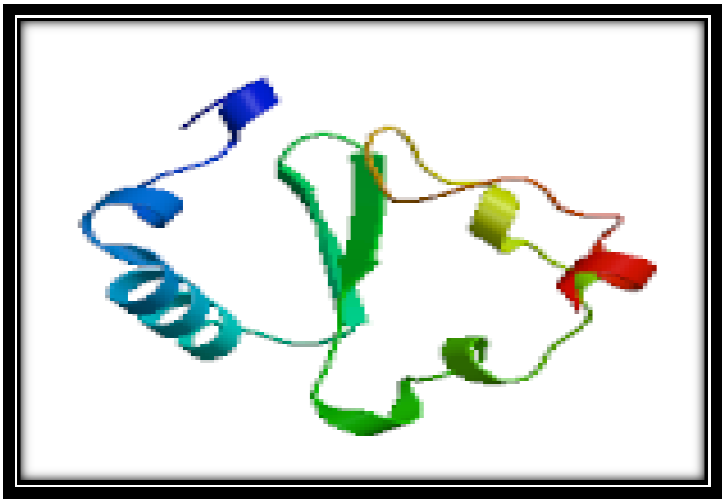


Figure 1(a): Secondary Structure of Enzyme 14-α Sterol Demethylase (Cyp P450) in *Aspergillus flavus*

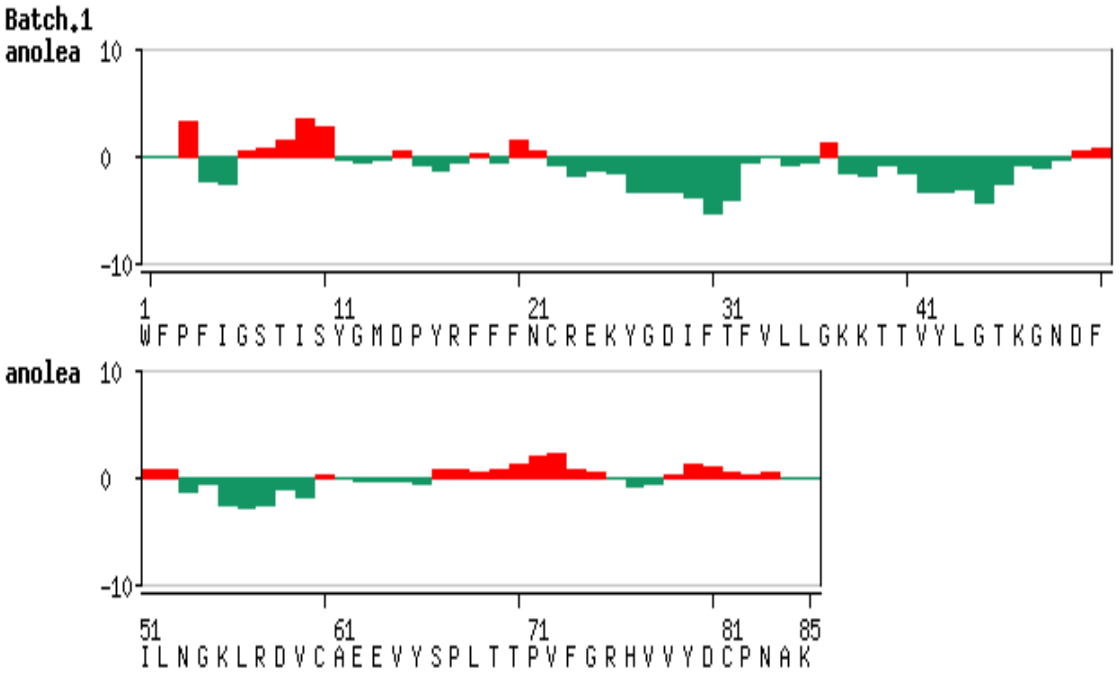
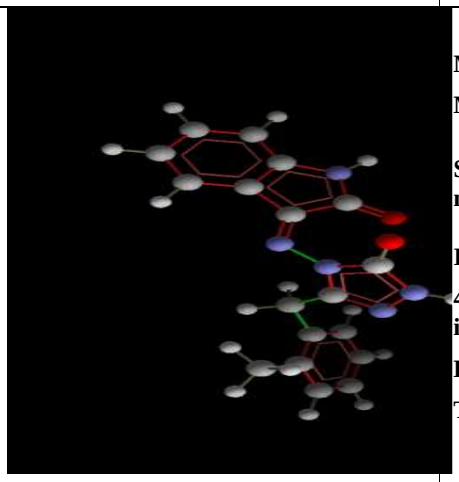


Figure 1(b): Amino Acid Sequence (residue) in 14-α Sterol Demethylase (Cyp P450) in *Aspergillus flavus*

Nitrogen *Carbon* *Sulphur* *Oxygen* *Chlorine* *Fluorine* *Bromine*		
S.No	**3D Structure**	**Properties**
2.(a)		**Molecular Weight : 333.344** **Molecular Formula: $C_{18}H_{15}N_5O_2$** **Smile:Cc1ccccc1Cc2n[nH]c(=O)n2N=c3c(=O)[nH]c4ccccc34** **IUPACName:3-[3'-(4''-o-ethylbenzyl)-4',5'-dihydro-1'H-1',2',4'-triazol-5'-on-4'-yl]-iminoisatin** **Heavy atoms: 25** **Torsion 3**

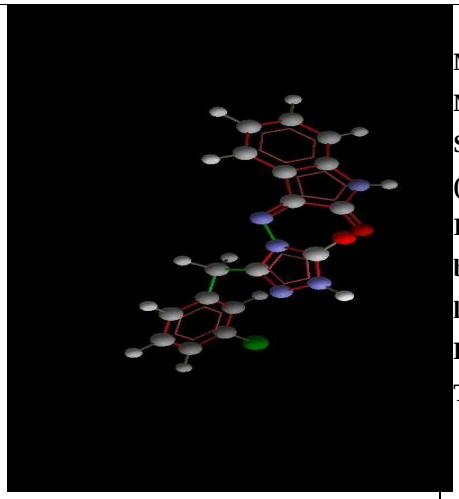
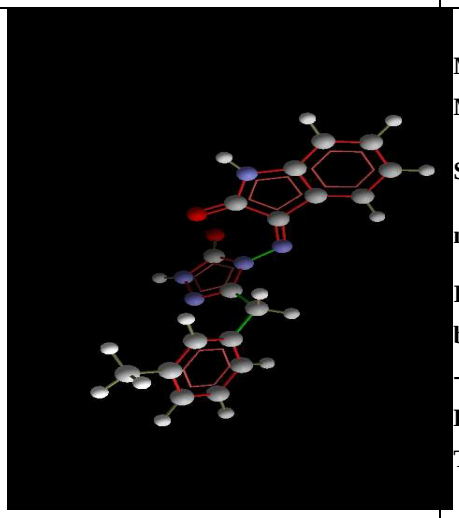
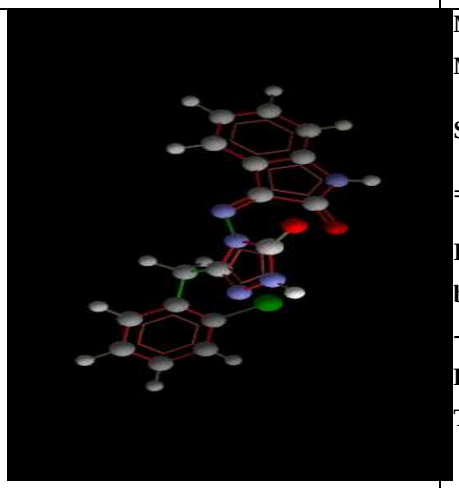
2.(b)		**Molecular Weight : 333.344** **Molecular Formula: $C_{17}H_{12}N_5O_2Cl$** **Smile: Clc4cccc(Cc1n[nH]c(=O)n1N=c2c (=O)[nH]c3ccccc23)c4** **IUPAC Name: 3-[3'-(4''-m-chloro benzyl)-4',5'-dihydro-1'H-1',2',4'-triazo l-5'-on-4'-yl]-iminoisatin** **Heavy atoms: 25** **Torsion 3**
2.(c)		**Molecular Weight : 353.762** **Molecular Formula: $C_{18}H_{15}N_5O_2$** **Smile: Cc4cccc(Cc1n[nH]c(=O) n1N=c2c(=O)[nH]c3ccccc23)c4** **IUPAC Name: 3-[3'-(4''-m-methyl benzyl)-4',5'-dihydro-1'H-1',2',4'-triazol -5'-on-4'-yl]-iminoisatin** **Heavy atoms: 25** **Torsion 3**
2.(d)		**Molecular Weight : 353.762** **Molecular Formula: $C_{17}H_{12}N_5O_2Cl$** **Smile: Clc1ccccc1Cc2n[nH]c(=O)n2N =c3c(=O)[nH]c4ccccc34** **IUPAC Name 3-[3'-(4''-o-chloro benzyl)-4',5'-dihydro-1'H-1',2',4'-triazol -5'-on-4'-yl]-iminoisatin** **Heavy atoms: 25** **Torsion 3**

Figure 2(a-d): Derivatives of Isatin –3 imine and 4-amino-4,5 dihydro-1H-1,2,4 triazole-5 ones

Table 1: Docking Score of "Isatin –3 imine and 4-amino-4,5 dihydro- 1H-1,2,4 triazole-5 ones" With Cytochrome *A. flavus*

Molecular Formula	Mol Dock Score	Re-rank Score	H-Bond
$C_{18}H_{15}N_5O_2$	-101.245	-68.836	0
$C_{17}H_{12}N_5O_2Cl$	-108.163	-75.0732	-2.5
$C_{18}H_{15}N_5O_2$	-104.56	-71.8035	-2.48124
$C_{17}H_{12}N_5O_2Cl$	-99.0221	-71.6824	-2.5

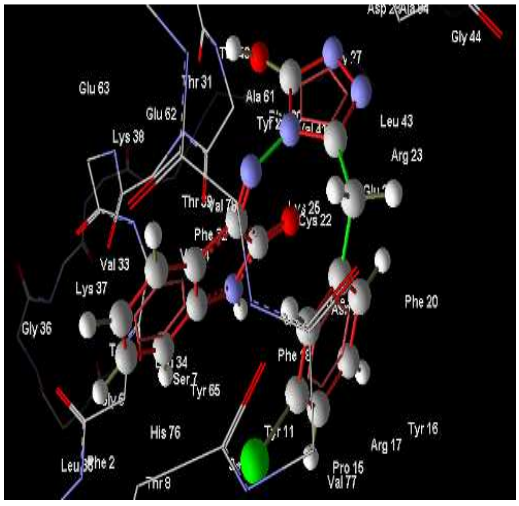


Figure 3(a): Residue Around the Cavity With Which Receptor Binds

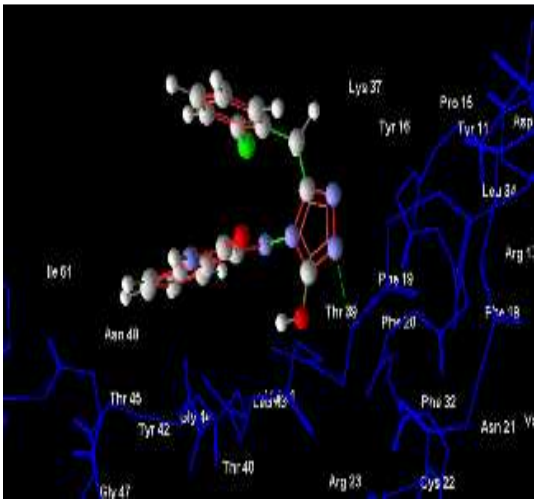


Figure 3(b): Hydrogen Bond Interaction Between Lead Compound and Thr 123 Residue

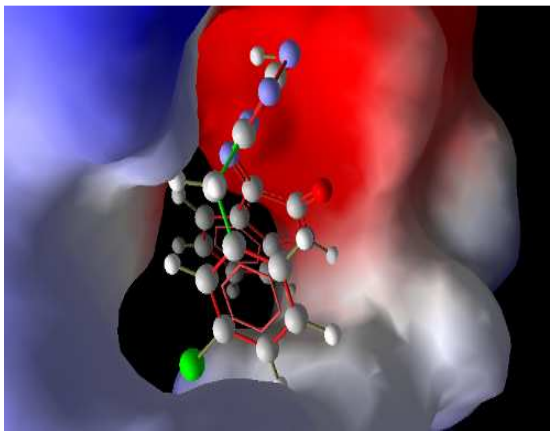


Fig 3.(c) Binding Position of Ligand at the Surface of Receptor

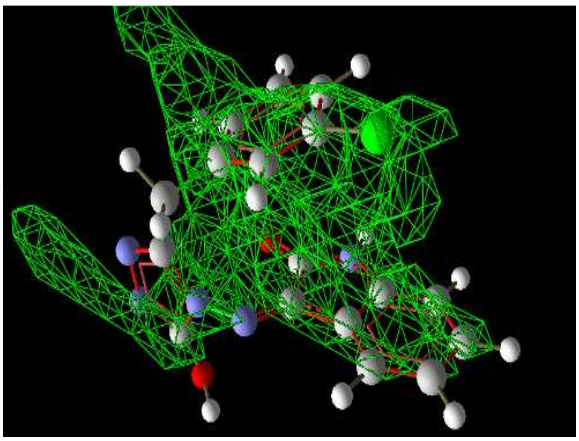


Fig 3.(d) Binding of Ligand at the Cavity of Enzyme

Fig 3 (a-d): Interaction Between Top Scorer Derivative " 3-[3'-(4''-m-chloro benzyl)-4',5'-dihydro-1'H-1',2',4'-triazol-5'-on-4'-yl]-iminoisatin" and *A. flavus*.

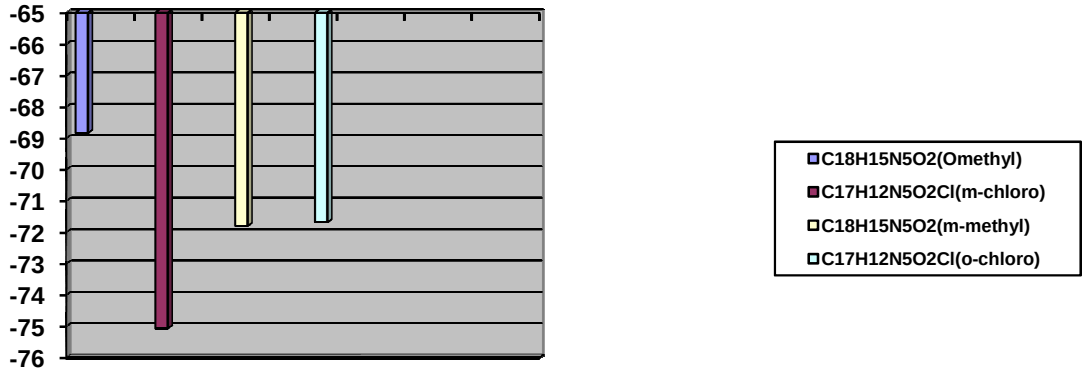


Figure 4: Comparative Inhibitory Effect of Isatin –3 Imine and 4-Amino-4,5 Dihydro-1h-1,2,4 Triazole-5-Ones Derivatives Against *A. flavus*

CONCLUSION

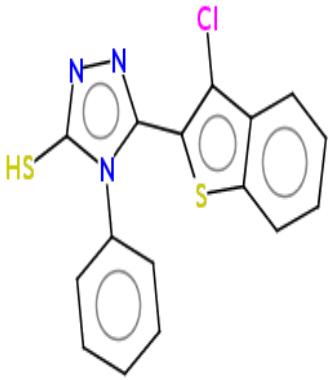
The study was concerned with virtual screening of Isatin substituted 1,2,4 triazole derivatives and their potential application on *A.flavus.* The work was performed to get the effective drug from the derivatives of Isatin –3 imine and 4-amino-4,5 dihydro-1H-1,2,4 triazole-5 ones through the chemoinformartics approach.

The structure of drug like molecule was drawn and submitted by a server ie; CADD groups to derive the pdb library of Isatin –3 imine and 4-amino-4,5 dihydro-1H-1,2,4 triazole-5 ones in mol 2 format. The sequence of amino acid of *Aspergillus flavus* was retrieved from NCBI in Fasta format. This sequence was then used to get the 3D structutre of cypP450 with the help of SWISS model. Drug target identification and docking of the ligand molecule with the protein was done with the help of MVD software. The derivative with least negative re-rank score is supposed to be the most effective drug. The bioavailability, absorption, and toxicity of the drug like molecules were studied by pharma-algorithm. Absorption rate should be high, so that these molecules must be available for biological system.

The results obtained demonstrate that meta chloro derivative is effective antifungal agent against *A.flavus.* It shows that heterocycle containing electron withdrawing group has potential pharmacological properties.

Therefore, homology based rational drug designing can be a successful approach for designing of potent antifungal drug.

Table 2: ADME/Tox properties of " 3-[3'-(4''-m-chloro benzyl)-4',5'-dihydro-1'H-1',2',4'-triazol-5'-on-4'-yl]-iminoisatin



Oral bioavailability more than 70%

Maximum passive absorption: 100%

Contribution from:

Trancellular route = 100%

Paracellular route = 0%

Permeability:Human Jejunum scale (pH=6.5):

Pe, Jejunum = 2.98×10^{-4} cm/s

Caco-2 scale (pH=7.4, 500 rpm):

Pe, Caco-2 = 141.37×10^{-6} cm/s

Absorption rate:

Ka = 0.090 min^{-1}

Probability of Effect on

	Lower limit	Upper limit
Blood	0.53	
Cardio-vascular system	0.82	
Gastro-intestinal system	0.31	
Kidney	0.35	
Liver	0.04	
Lung	0.46	

	LD50	pLD50	Lower limit	Upper limit
Intra Peritoneal	420 mg/kg	-0.13	-0.87	0.49
Oral	720 mg/kg	-0.36	-1.84	-0.07
Intra venous	190 mg/kg	0.21	-0.69	1.41
Sub cutaneous	620 mg/kg	-0.30	-2.04	0.82

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