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A N I N T E R N A T I O N A L J O U R N A L

Insilico Analysis of Phytoconstituents from *Allium sativum* as Potential Inhibitors of Inha in *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis is leading cause of death among the global bacterial infections. The main causative for tuberculosis is Mycobacterium tuberculosis, *which will survive in its host human being for decades in latent or chronic levels. In addition, the late multidrug resistance at a disturbing rate accompanies the appearance of tuberculosis. The quick spread of resistance to initial stage treatment medications has redirected the focus of the medical community in the creation of an array of new drug against* Mycobacterium tuberculosis. *The InhA protein is a component of Fatty acid synthetase (FAS) II and exhibits an NADH reliant enoyl-ACP reductase activity. InhA is a vital enzyme of* M.tuberculosis *in control of cell wall synthesis, which can turn out to be a great focus for the synthesis of anti-tubercular treatment. Inspired from the offering biological actions of phytoconstituents from* Allium sativum, *the current research concentrates on looking at novel lead compounds from the plant. Molecular docking studies were carried out employing specific phytoconstituents from* A.sativum *with the protein InhA target. Ajoene shows much more encouragingresults with a Mol Dock rating of 80.6047Kcal/mol, as opposed to the typical initial line drug isoniazid (Moldock score: -58.7028 Kcal/mol). Molecular docking prediction indicate that Ajoene could be formulated into a possible treatment drug for* Mycobacterium tuberculosis.

Key words: Tuberculosis, InhA, Enoyl ACP reductase, *Allium sativum*, Moldock

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INTRODUCTION

Tuberculosis is one of the major global health care issues, caused by the infection of *Mycobacterium tuberculosis. M.tuberculosis* has about 4111 genes in the circular genome of size 4.4 Mb and 65% of GC content (Schürch AC, et al., 2010). Almost One third of the human population was being affected by *M.tuberculosis* of which a major population is from developing countries (Lönnroth K, et al., 2010).

Due to inadequate administration and prolonged use of the first-line treatment drugs like isoniazid rifampicin, ethambutol and streptomycin (VanderWerf MJ, et al., 2012)the emergence of Multidrug resistance in *M.tuberculosis* strains have been observed around the world with high mortality rates among HIV-positive patients (Jacobs RF, 1994). Improved MDR scenario in *M.tuberculosis* is also emerging in HIV negative populations (vanRie A, et al., 1999). According to WHO 3.7% of the new cases of the previously treated patients have been infected with the multidrug-resistant (MDR) *M.tuberculosis* The reason behind this drug resistance is the primary transmission which is a growing concern in recent days (Zhao Y, et al., 2012). The above mentioned serious concern~~s~~ has leads to the development of new anti-tubercular drugs.

The internal resistance of *M.tuberculosis* is due to several factors such as active drug efflux mechanism, highly permeable mycolic acid containing cell wall and the production of drug modifying and drug inactivating enzymes (Ramaswamy S, et al., 1998). *M.tuberculosis* contains unique signature fatty acids and mycolic acids that are unusually long chain α-alkyl and β-hydroxy fatty acids of 60-90 carbons (Takayama K, et al., 2005). Mycolic acids are the central constituents of the mycobacterial cell wall and the biosynthesis of mycolic acid involves several successive enzymatic reactions involving two enzyme systems FAS-I (Fatty acid Synthase – I) and FAS-II (Fatty acid Synthase – II). In the present study InhA protein a part of Fatty acid Synthase system was selected as a novel drug target.

Plant derived phytochemicals have been traditionally used as natural remedy in treatment of diverse ailments. Usage of medicinal plants demonstrate promising and potential effect in treatment against many human diseases (Ronaldo Anuf, et al., 2014). In this content there is an interest in developing novel lead molecules from plant sources (Ramaraj, et al., 2014).

Garlic (*Allium sativum*) is a potent medicinal plant widely used as a food item and folk medical treatment for hundreds of years, globally (Rivlin RS, 2001). Garlic is reported to have powerful biological characteristics such antioxidant, antimicrobial, hypoglycemic, anti-cancer, anti-inflammatory, immunomodulatory and anticardiovascular effects (Reuter HD, et al., 1996). Various garlic herb extracts have shown highly effective activity against Gram-positive and Gram-negative bacteria such as different strains of *Escherichia*, *Bacillus*, *Clostridium*, *Klebsiella*, *Salmonella*, *Proteus*, *Staphylococcus*, *Streptococcus*, *Helicobacter pylori* (Cellini L, et al., 1996) possibly even acid-fast *bacilli* (AFB) like MTB (Uchida Y, et al., 1975).

In this study, we aim at exploring novel phyto-constituents from *A.sativum* as potential lead molecules against the target protein enoyl- ACP reductase (InhA) using Molegro Virtual Docker.

MATERIALS AND METHODS

Ligand Preparation

The structure of different phytochemicals from *A.sativum* were retrieved from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and Chemspider database (http://www.chemspider.com/). The compounds were converted into compatible format (.sdf) using Open Babel tool. The root mean square gradient value was selected less than 0.001 kcal/mol. The energy minimized structure was used for docking studies.

Protein Preparation

The three dimensional crystal structure of the protein enoyl- ACP reductase was retained from Protein DataBank (http://www.rcsb.org/pdb/). The bonds, bond orders, explicit hydrogen, charges (calculated by MVD), flexible torsion and Tripos atom types were assigned if they were missing by using 'Protein Preparation' module of Molegro Virtual Docker for the protein enoyl- ACP reductase.

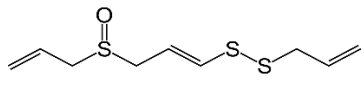
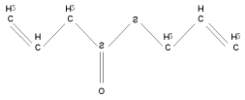
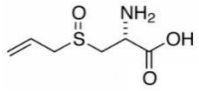
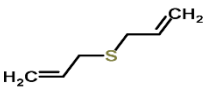
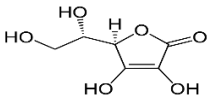
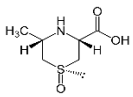
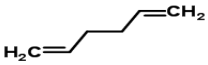
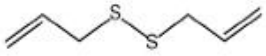
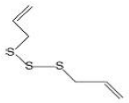
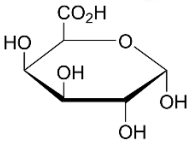
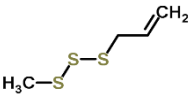
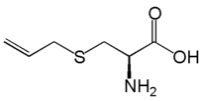
Molecular Docking

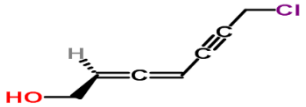
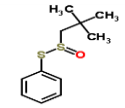
Molgro virtual docking computer program relies upon the innovative hybrid search technique referred to as guided differential evaluation. Docking was carried out applying a grid resolution of 0.30 Å and for all of the 5 independent runs; the highest possible amount of 1,500 iterations were

carried out on a single population of 50 individuals. The active binding location was regarded as a rigid molecule, however the ligands were regarded to be flexible, i.e. any non-ring torsions were permitted. Default configurations were employed for every calculation.

Phyto-constituents from *A.sativum* (Table 1.) were docked with enoyl- ACP reductase using Moldock module in the Docking wizard of MolDock Virtual docking software. Moldock uses the Scoring function derived from Piecewise Linear Potential (PLP) scoring function.

Table 1. Selected phyto-compounds of *A.sativum*

S.No	Compound Name	Compound ID (CID)	Structure
1.	Ajoene	5386591	
2.	Allicin	65036	
3.	Alliin	87310	
4.	Allyl sulfide	11617	
5.	Ascorbic acid	54670067	
6.	Cycloalliin	193294	
7.	Diallyl	11110 (Chemspider ID)	
8.	Diallyl Disulfide	16590	
9.	Diallyl Trisulfide	16315	
10.	Galacturonic acid	441476	
11.	Methylallyl trisulfide	61926	
12.	S-allylcysteine	98280	

13.	Scorodonin	189818	
14.	Thiosulfinate	25244242	

RESULTS AND DISCUSSIONS

Natural products and some of their derivatives have shown promising activity against *M.tuberclosis* highlighting their potential as a powerful source of new class of drugs. A number of genetic mutation were seen to have taken place generally between codons 138 and 328. The Ser315Thr mutation is most frequent and present in more than 40% of every INH resistant strains. These strains needs to be targeted with potent drug molecues to attain the best possible medicinal effects. Therefore, In the present study library of natural compounds from *A.sativum* has been screened against enoyl- ACP reductase and concluded our results using average binding affinity of these compounds against them. The selected phytocompounds from *A.sativum* has shown potent binding affinity against enoyl- ACP reductase.

Prediction of Binding site

Detection of cavities is the more important step in the protein-ligand interaction. The cavities present in the protein enoyl- ACP reductase is detected based on the cavity detection algorithm in MVD tool (Thomsen R, et al., 2006). The volume of the cavities is listed in Table 2. In most cases, the cavities with the largest size and volume is associated with the binding site. Cavity 2 and Cavity 3 have the larger volume of 928.256 $Å^3$ "(Fig. 1 A)" and 501.248 $Å^3$ "(Fig. 1 B)" respectively. The cavity with larger size has been selected as the binding site for the protein enoyl-ACP reductase during docking with Molgro Virtual Docker "(Fig. 2)".

Table 2. Volume of cavities

S.No	Cavities	Volume of cavities ($Å^3$)
1.	Cavity 1	416.256
2.	Cavity 2	928.256
3.	Cavity 3	501.248
4.	Cavity 4	428.544
5.	Cavity 5	418.204

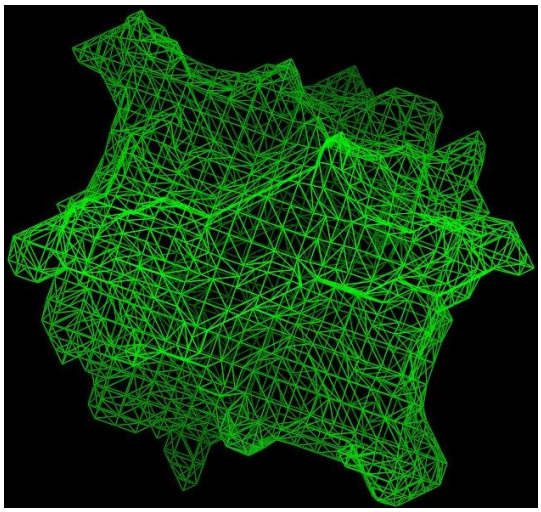


Fig. 1 A Binding site for enoyl- ACP reductase cavity-1

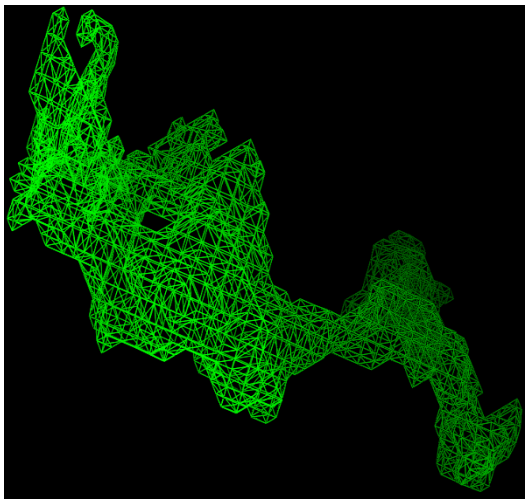


Fig. 1 B Binding site for enoyl- ACP reductase cavity – 2

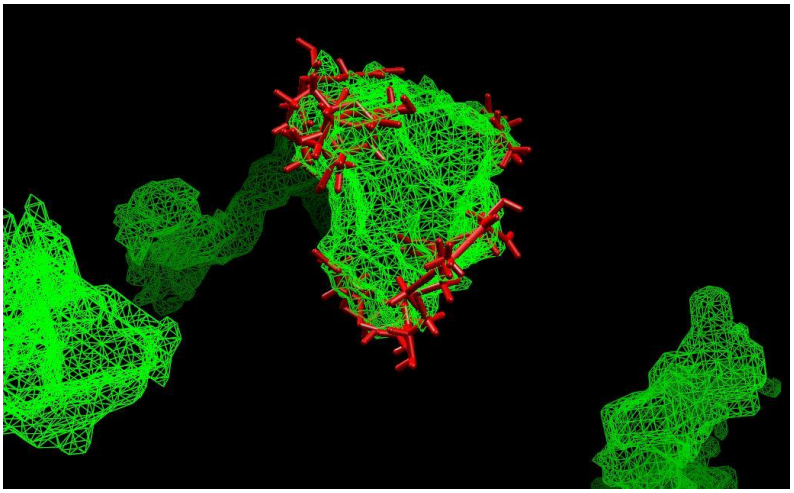


Fig. 2 Selected ligands bind with cavity – 1 predicted by MVD tool

Molecular Docking

In recent scenario, computational methods such as molecular docking tools are used to design novel lead molecules by exploring the interaction between the protein and the ligand molecules (Srivastava, et al., 2010). The docking analysis of the selected phyto-compounds from *A.sativum* with the protein enoyl- ACP reductase was performed using MVD. The inhibition susceptibility was determined using moldock score which was generated by MVD.

The receptor bound ligand was docked deeply in binding pocket region by strong interaction. The active compound Ajoene binds with the receptor with a moldock score of -80.6047 (Table 3). It binds to the Ile – 257 amino acid in the cavity 2 (Fig. 3 A) and has only one Hydrogen bond interaction. Allin expressed a hydrogen bond score of -9.707 Kcal/mol and with four amino acid interactions at different domains of the target protein "(Fig. 3 B)". Ascorbic acid had a moldock score of -71.4068 Kcal/mol and the hydrogen bond interactions were high as compared to that of Ajoene. Compounds such as methylallyl trisulfide and diallyl shows very less Moldock score and hence have a very less affinity for the target.

The H-Bond score for Ascorbic acid was -14.5056 Kcal/mol. There are 9 amino acid residues from different domains of enoyl- ACP reductase involved in interaction with Ascorbic acid "(Fig. 3 C)". Scorodonin had four hydrogen bond interaction with 2 amino acid residues "(Fig. 3 D)". Table 4. indicated that the amino acids, Ile 257, Ser 152, and Arg 173 are found common in most compounds.

Table 3. Docking energies of *A.sativum* for enoyl- ACP reductase

S.No	Compound Name	Moldock Score (Kcal/mol)	Rerank score (Kcal/mol)	HBond (Kcal/mol)	Docking score (Kcal/mol)
1.	Ajoene	-80.6047	-67.878	-0.114737	-80.048
2.	Scorodonin	-74.033	-58.872	-4.62487	-76.96
3.	Alliin	-72.1619	-61.8725	-9.70757	-77.939
4.	Ascorbic acid	-71.4068	-63.597	-14.5056	-85.060
5.	Thiosulfinate	-69.7631	-56.3802	-4.83278	-69.009
6.	Cycloalliin	-67.5228	-56.5924	-5.39142	-74.059
7.	Allicin	-66.6876	-52.3595	-1.72242	-65.644
8.	S-allylcysteine	-63.5625	-37.6933	-9.19796	-68.176
9.	Diallyl trisulfide	-62.0729	-46.8931	0	-61.558
10.	Diallyl disulfide	-60.7435	-51.4489	0	-59.583
11.	Galacturonic acid	-54.8406	-56.9334	-16.1429	-69.654
12.	Allyl sulfide	-51.9668	-43.8319	0	-51.724
13.	Methylallyl trisulfide	-49.9732	-40.1364	0	-49.356
14.	Diallyl	-49.4581	-40.5511	0	-48.913

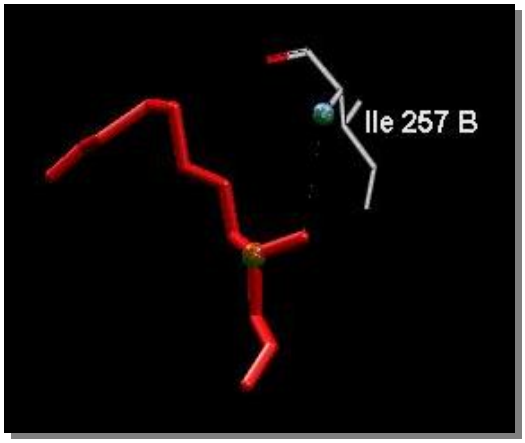


Fig. 3 A Interaction of Ajoene H_2 bond

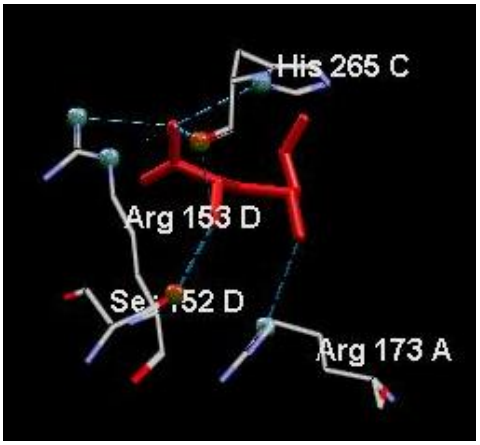


Fig. 3 B Interaction of Allin H_2 bond

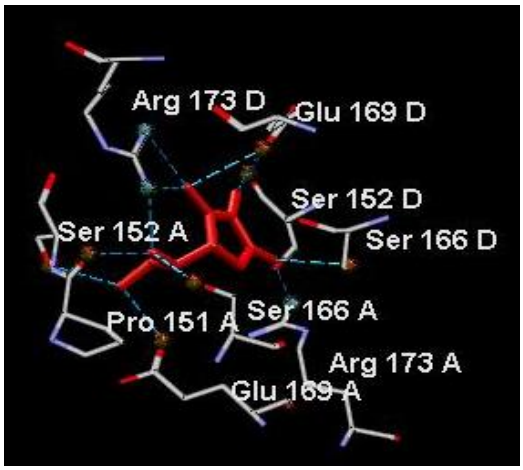


Fig. 3 C Interaction of Ascorbic acid H_2 bond

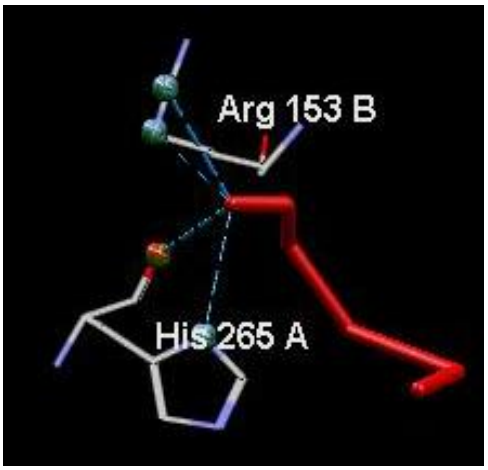


Fig. 3 D Interaction of Scordinin H_2 bond

Table 4. Amino acid residue of enoyl- ACP reductase involved in binding with phytoconstituents from *A.sativum*

S.No	Compound Name	H_2 –interaction with Amino acid	No. of H_2 bond
1.	Ajoene	Ile 257	1
2.	Scorodonin	Arg 153, His 256	4
3.	Alliin	Ser 152, Arg 153, Arg 173, His 265	7
4.	Ascorbic acid	Pro 151, Ser 152, Ser 166, Glu 169, Arg 173	12
5.	Thiosulfinate	Arg 173, Gly 255	2
6.	Cycloalliin	Ser 152, Arg 173, Gly 255, Asp 256, Ile 257	5
7.	Allicin	Arg 173	1
8.	S-allylcysteine	Asp 256, Ile 257, Tyr 259	7
9.	Diallyl trisulfide	--	--
10.	Diallyl disulfide	--	--
11.	Galacturonic acid	Ser 166, Glu 169, Ser 170, Arg 173,	12
12.	Allyl sulfide	--	--
13.	Methylallyl trisulfide	--	--
14.	Diallyl	--	--

The rate limiting step and final step Mycobacterium Enoyl-ACP reductase catalyzes the final step and rate limiting step in the fatty acid synthesis (mycolic acid) by utilizing NADH to reduce trans double bond of longer fatty acyl substrates (Moir DT, 2005, Rozwarski DA, et al., 1998) and are validated as the excellent target for drug development against *M.tuberculosis* (Yamada H, et

al., 1995). The replacement of an amino acid in the NADH binding site of *InhA* apparently results in INH resistance, preventing the inhibition of mycolic acid biosynthesis (Zhang YM, et al., 2006). Various genetic mutations have been observed to occur usually between codons 138 and 328 (Ramaswamy S, et al., 1998). The Ser-315-Thr mutation is most frequent and found in about 40% of all INH-resistant strains (Marttila HJ, et al., 1998, Zhang YM, et al., 2006). The mutation leads to the enzyme lacking the ability to activate INH, but preserves 50% of catalase peroxidase activity. The modified catalase-peroxidase offers high-level resistance to INH (Telenti A, et al., 1993). So the biological activity of INH is reduced due to the mutation in *InhA*. Therefore, there is an urgent need for novel intervention strategies to target TB.

Ajoene shows the potent inhibitory activity against the InhA protein. Ajoene (IUPAC name: (Z)-1-(prop-2-enyldisulfanyl)-3prop-2-enylsulfinylprop-1-ene) is the organosulfur compound, which contains sulfoxide and disulfide groups. Ajoene is reported for the anti-cancer activity (Roger, et al., 2008), anti-bacterial activity (Torres, et al., 2012), anti-fungal activity (Ledezma, et al., 2006) and wide possess wide range of biological applications. Development of Ajoene as a potential inhibitor would assist in effective medication for the disease with minimal or less toxic effects.

CONCLUSION

The present molecular docking studies provide insights into inhibition of InhA protein by phytoconstituents from *A.sativum*. Docking study suggest that Ajoene has a high binding affinity for InhA protein, which is much higher than the known inhibitor isoniazid. This study has led to the development of novel lead molecules for the treatment of Tuberculosis. Further these lead compounds can be used for designing more effective inhibitors of InhA.

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REFERENCE

Cellini L, DiCampli E, Masulli M, DiBartolomeo S and Allocati N, Inhibition of Helicobacter pylori by garlic extract (Allium sativum), FEMS Immunol. Med.Micrbiol., 13, 1996, 273-277.

Jacobs RF, Multidrug-resistant tuberculosis, Clinical Infectious Diseases, 19 1994, 1-10.

Ledezma E and Apitz-Castro R, Ajoene the main active compound of garlic (Allium sativum): a new antifungal agent, Revista Iberoamericana de Micología, 23, 2006, 75-80.

Lönnroth K, Castro KG and Chakaya JM, Tuberculosis control and elimination 2010–50: cure, care, and social development, Lancet, 375 2010, 1814-1829.

Marttila HJ, Soini H and Eerola E, A Ser315Thr substitution in KatG is predominant in genetically heterogeneous multidrug-resistantMycobacterium tuberculosis isolates originating from the St. Petersburg area in Russia, Antimicrob Agents Chemother, 42, 1998, 2443-2445.

Moir DT, Identification of inhibitors of bacterial enoylacyl carrier protein reductase, Infectious Disorders - Drug Targets, 5, 2005, 297-305.

Ramaraj K and Ronaldo Anuf A, Inhibition of FGFR2 signal transduction in Acne Vulgaris using Bioactive Flavonoids: An Insilico approach, Asian Journal of Medical and Pharmaceutical Sciences, 2, 2014, 136-142.

Ramaswamy S and Musser JM, Molecular genetic basis of antimicrobial agent resistance inMycobacterium tuberculosis: 1998 update, Tubercle and Lung Disease, 79, 1998, 3-29.

Reuter HD, Koch HP and Lawson LD, Therapeutic effects and applications of garlic and its preparations. In: Garlic. The Science and Therapeutic Application of Allium sativum L. and Related Species, Baltimore: Williams & Wilkins, 1996, 135-212.

Rivlin RS, Historical perspective on the use of garlic, J. Nutr., 131, 2001, 951-954.

Roger H, Catherine HK, Parker IM, Mino RC, Philip R, Susan T, Francois T and Thozama Q, Substituted ajoenes as novel anti-cancer agents, Bioorganic & Medicinal Chemistry Letters, 18, 2008, 5277-5279.

Ronaldo Anuf A, Breethi N, Shahila S and Ramaraj K, Insilico Analysis of Plumbagin and its synthetic analogues as potential Bcl-2 Inhibitors, Asian Journal of Medical and Pharmaceutical Sciences, 2, 2014, 108-114.

Rozwarski DA, Grant GA, Barton DHR, JacobsJr WR and Sacchettini JC, Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis, Science, 279, 1998, 98-102.

Schürch AC, Kremer K, Kiers A, Daviena O, Boeree MJ, Siezen RJ, Smith NG and vanSoolingen D, The tempo and mode of molecular evolution of Mycobacterium tuberculosis at patient-to-patient scale, Infect Genet Evol, 10 2010, 108:114.

Srivastava V, Gupta SP, Siddiqi MI and Mishra BN, Molecular docking studies on quinazoline antifolate

derivatives as human thymidylate synthase inhibitors, Bioinformation, 4, 2010, 357-365.

Takayama K, Wang C and Besra GS, Pathway to synthesis and processing of mycolic acids inMycobacterium tuberculosis, Clin. Microbiol. Rev., 18 2005, 81-101.

Telenti A, Imboden P and Marchesi F, Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis, Lancet, 341 1993, 647-650.

Thomsen R and Christensen MH, MolDock: A new technique for high-accuracy molecular docking., J Med Chem, 49, 2006, 3315-3321.

Torres J and Romero H, In vitro antifungal activity of ajoene on five clinical isolates of Histoplasma capsulatum var. capsulatum, Revista Iberoamericana de Micología, 29, 2012, 24-28.

Uchida Y, Takahashi T and Sato N, The characteristics of the antibacterial activity of garlic, Jpn. J. Antibiotics, 28, 1975, 638-642.

VanderWerf MJ, Langendam MW, Huitric E and Manissero D, Multidrug resistance after inappropriate tuberculosis treatment: a meta-analysis, Eur Respir J, 39 2012, 1511-1519.

vanRie A, Warren RM and Beyers N, Transmission of a multidrug-resistantMycobacterium tuberculosis strain resembling ‘strain W’ among non-institutionalised, human immunodeficiency virus-seronegative patients, J. Infect. Dis., 180 1999, 1608:1615.

Yamada H, Shiomi K, Xu Q, Nagai T, Shibata M and Oya L, New glycosidases inhibitors, panosialins D and wD produced by Streptomyces sp. OH-5186, J. Antibiot., 48, 1995, 205-210.

Zhang YM, White SW and Rock CO, Inhibiting bacterial fatty acid synthesis, J. Biol. Chem., 281, 2006, 17541-17544.

Zhao Y, Xu S and Wang L, National survey of drug-resistant tuberculosis in China, The New England Journal of Medicine, 366, 2012, 2161-2170.

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