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RESEARCH ARTICLE

Screening of Anti-mycobacterial Phytochemical Compounds for Potential Inhibitors against *Mycobacterium Tuberculosis* Isocitrate Lyase

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Abstract: ***Background and Introduction*:** Tuberculosis (TB) is a leading infectious disease caused by *Mycobacterium tuberculosis*with high morbidity and mortality. Isocitrate lyase (MtbICL), a key enzyme of glyoxylate pathway has been shown to be involved in mycobacterial persistence, is attractive drug target against persistent tuberculosis.

***Methods*:** Virtual screening, molecular docking and MD simulation study has been integrated for screening of phytochemical based anti-mycobacterial compounds. Docking study of reported MtbICL inhibitors has shown an average binding affinity score -7.30 Kcal/mol. In virtual screening, compounds exhibiting lower binding energy than calculated average binding energy were selected as top hit compounds followed by calculation of drug likeness property. Relationship between experimental IC_{50} value and calculated binding gibbs free energy of reported inhibitors was also calculated through regression analysis to predict IC_{50} value of potential inhibitors.

***Results*:** Docking and MD simulation studies of top hit compounds have identified shinjudilactone (quassinoid), lecheronol A (pimarane) and caniojane (diterpene) as potential MtbICL inhibitors.

***Conclusion*:** Thus, phytochemical based anti-mycobacterial compound can further developed into effective drugs against persistence tuberculosis with lesser toxicity and side effects.

Keywords: Tuberculosis**,** Isocitrate lyase, Mycobacterial persistence, Molecular docking, MD simulation, Virtual screening.

1. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by bacillus *Mycobacterium tuberculosis* (Mtb) [1]. Tuberculosis has latently infected one third of the world population (1.7 billion) with 10 million new cases per year [2]. Relatively small population (5-15%) of total infected population develops TB disease and if not treated, higher mortality rate was reported. Tuberculosis bacteria are engulfed by alveolar macrophages with capability to replicate and cyclic reinfection confers prolonged survival and persistence [3]. Non-growing or dormant TB bacteria are not affected by most of the current available TB drugs characterize persistent tuberculosis. Most of TB drugs do not kill slowly replicating or dormant bacilli which eventually causes longer treatment duration and increased drug resistance cases [4]. Thus, a new generation of drugs that target against latent or persistent mycobacteria are needed for more effective treatment of tuberculosis [5]. In recent years, system biology approach has been utilized to study tuberculosis and host-pathogen interaction for finding suitable drug targets [6].

Glyoxylate cycle is functionally essential for the growth and survival of bacteria during the persistent phase of infection [7].Glyoxylate pathway or shunt bypasses decarboxylation steps of tricarboxylic acid (TCA) cycle to synthesize carbohydrates by utilizing fatty acids in the absence of glycolysis. In glyoxylate shunt, isocitrate lyase (encoded by *icl1 and aceA*) reversibly cleaves isocitrate into glyoxylate and succinate, second enzyme malate synthase (encoded by gene glcB) convert glyoxylate into malate. Deletion of any or both *icl* and *aceA gene* impairs the survival and decrease virulence of *M. tuberculosis* in activated macrophages [8, 9]. The crystal structure of dimer apo-form (PDB: 1F61) is available with open conformation [10]. Thus, Isocitrate lyase (MtbICL) is an attractive drug target for persistent or latent tuberculosis as absent in vertebrates including human [11].

MtbICL active site loop region includes highly conserved sequence motif (189KKCGH193) within catalytic region (Leu185-Gly196 residues) and C-terminal end (Pro411-Leu418) of the adjacent subunit. Initially, active site region adopts open conformation with cysteine (Cys191) residue placed away from other active site residues and allowing substrate isocitrate to access the binding site (Leu185-Gly196). In the binding process, Cysteine (Cys191) residue is serving as catalytic acid and interact with histidine (His193) residue (supplementary 1)[12], while one of the residue from Arg228, Glu285, Tyr89 or His180 acts as catalytic base to deprotonate the C_2 hydroxyl group of the isocitrate substrate [13]. After substrate binding, C-terminal end (Pro411-Phe427) moves to fill the space forming a lid and MtbICL protein adopts a closed conformation with substrate [14]. Polar nature of isocitrate lyase (MtbICL) binding pocket favours small and polar molecules and not easily and effectively reached by targeting compounds [15].

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Phytochemical compounds are chemical compounds obtained from plants generally through secondary metabolism. Phytochemical compounds are valuable source for novel bioactive molecules with vast chemical diversity. Phytochemical based MtbICL Inhibitors may minimize the disease treatment duration and disease reactivation. Several anti-mycobacterial phytochemical compounds were reported previously [16-18], can be new lead molecules for persistent tuberculosis. In this work, anti-mycobacterial phytochemical compounds were screened against isocitrate lyase (MtbICL) protein for identification of MtbICL inhibitor through virtual screening, docking and MD simulation study.

2. MATERIALS AND METHODS

2.1. Protein Preparation

Three dimension crystal structure of isocitrate lyase (Pdb: 1F61) was downloaded from Protein Data Bank (http://www.rcsb.org/pdb) [19]. Protein structure was prepared by assigning partial charges, removing all the water molecules and adding polar hydrogen atoms using AutoDock Tools (ADT) [20]. Energy minimization of protein was performed through standard procedure using UCSF Chimera [21].

2.2. Ligand Preparation

A dataset of phytochemical based anti-mycobacterial compounds (844) was retrieved from BioPhytol database (http://ab-openlab.csir.res.in/biophytmol/) in SDF format [22]. Three dimension chemical structures of reported MtbICL inhibitors were also downloaded from pubchem database [23, 24]. Corresponding chemical structures were converted into 3D structure and saved to pdbqt format by Open Babel [25]. Pubchem sketcher and CACTUS online tools were used for sketching and generating compound structures [26].

2.3. Virtual Screening

Virtual screening of compounds against MtbICL protein was performed using molecular docking program AutoDock Vina in PyRx 0.8 [27, 28]. Swiss PDB viewer was used to visualize protein-ligand complex [29].

2.4. Drug Likeness Property

Top hit phytochemical compounds with significantly lower binding energy were evaluated for drug likeness property using DruLito software [30]. DruLito consists of several parameters including Lipinski's rule of five to screen the drug like chemical compounds [31].

2.5. Molecular Docking

AutoDockv4.2 software was used for docking study of ligand-protein interaction [32]. Protein structure was kept rigid and grid box (33 Å x 73 Å x 10 Å) was used for docking calculations. Docking was performed using genetic algorithm, population size of 150, maximum number of energy evaluations set to 2,500,000, gene mutation rate at 0.02 and rate of crossover of 0.8. Hydrogen bond and vander wall interaction diagrams of ligand-protein docking complex were drawn using Ligplot [33].

2.6. Molecular Dynamics (MD) Simulation

Molecular dynamics simulation provides an understanding of biomolecular structure and function at different timescales [34]. MD simulation of the ligand-protein systems were performed for 100 ns time period for each system and trajectories were saved at every 10 ps using GROMACS 4.6.5 [35]. Energy minimization was performed for each system using steepest descent method and protein-ligand system equilibrated for constant volume, pressure (1 atm) and temperature (300 K) using NVT and NPT ensemble. Binding free energy of protein-ligand complexes was calculated by MM/PBSA method [36]. The whole process of MD simulation was repeated twice to ensure statistical significance and reproducibility of the results.

3. RESULTS AND DISCUSSIONS

3.1. Docking Study of Reported MtbICL Inhibitors

Some MtbICL inhibitors including 3-nitropropionate (3-NP), 3-bromopyruvate (3-BP) and itaconic acid has been reported in the previous works [37-39], but these compounds are not suitable for drug development due to higher toxicity and non-selective binding with other key enzymes (Suppl. **2**) [40]. MtbICL crystal structures available with glyoxylate and 3-nitropropionate (3-NP) substrates (Pdb: 1F81) and 3-bromopyruvate (3-BP) substrate (Pdb: 1F8M) gives an insight into binding mechanism and residues involve in MtbICL inhibition (Fig. **1a**-**d**). Molecular docking of reported inhibitors against MtbICL protein was performed to obtain average binding affinity *i.e.* -7.30 Kcal/mol (Table **1**). Average binding energy and interacting protein residues information can be helpful for virtual screening of phytochemical compounds against MtbICL protein.

3.2. Virtual Screening and Drug Likeness Property

Virtual screening is important tool for screening and prioritizing compounds with lower binding energy against target protein [41]. Anti-mycobacterial phytomolecules (844) were screened against apo-MtbICL protein (Pdb: 1F61) with open conformation. Search space for docking calculation was focused on grid size (33.27 Å x 71.26 Å x 10.37 Å) enclosing active site residues. Binding energy of ligand-protein complex was calculated with different nine poses and subsequently selected best pose with lowest binding affinity. Phytoligands exhibiting lower binding affinity than average binding score of reported inhibitors (-7.30 Kcal/mol) were selected as top hit compounds. Top hit compounds following drug likeness property *i.e.* lipinski's rule of five were selected as potential inhibitors (Table **2**). These top hit drug like compounds mostly include phytochemical class flavonoid, quinone and terpene with phenol and alcohol functional groups (Suppl. **3**).

3.3. Docking Analysis

Molecular docking investigates binding conformation and orientations of phytoligands within MtbICL binding site. Focused docking enclosing MtbICL active site residues has

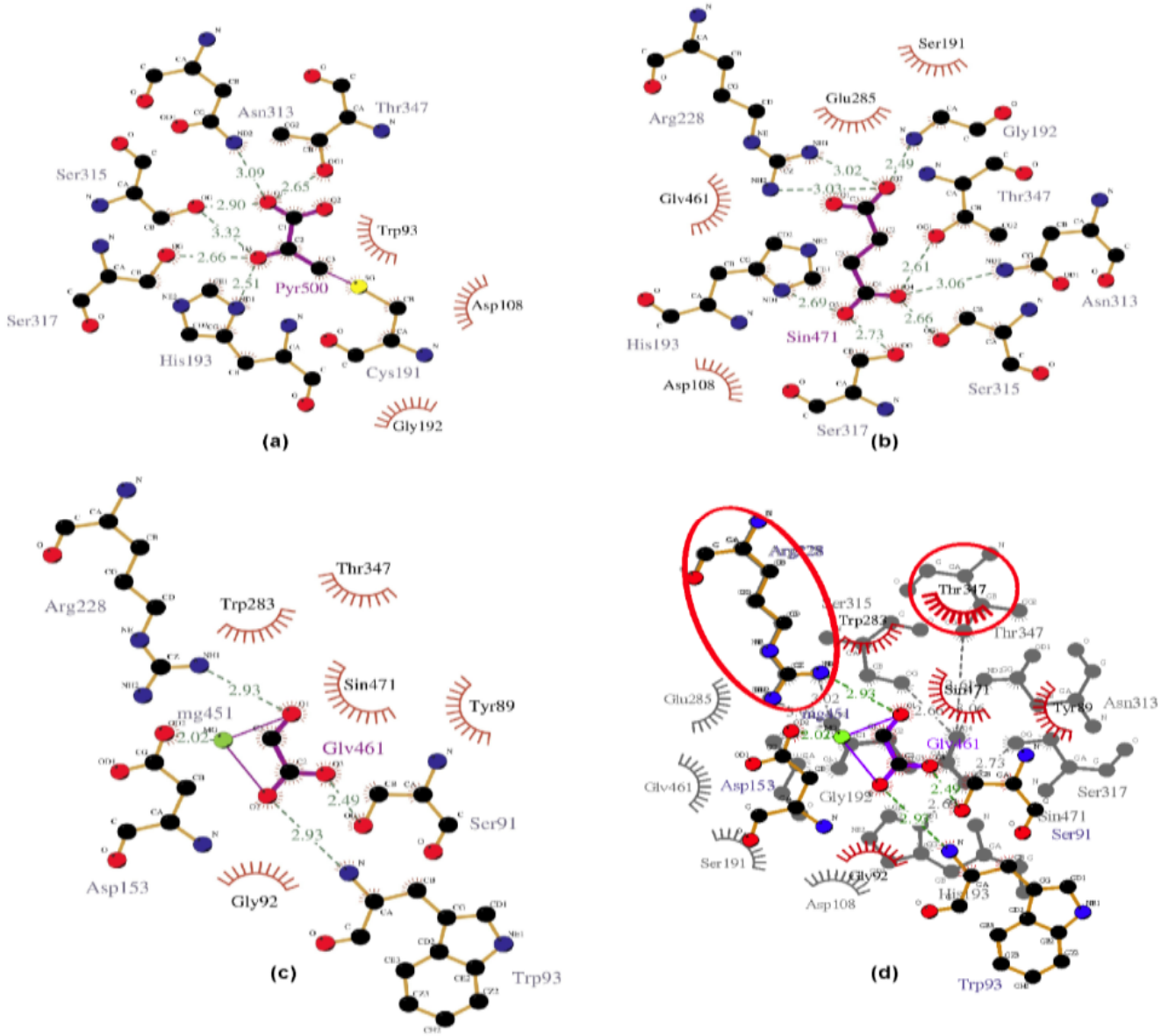

Fig. (1). Molecular interaction of MtbICL protein with previously reported inhibitors (**a**) 3-NP-1F8M (**b**) Succinate-IF8I (**c**) Gly-1F8I (**d**) Sin+Gly-1F8I.

been performed with selected top hit (10) compounds to analyse their binding pattern (Table **3**). In docking studies, lecheronolA (diterpene), shinjudilactone (quassinoid) and caniojane (diterpene) showed lower ligand-protein binding energy -7.77, -7.51 and -7.46 kcal/mol, respectively in comparison with 3-BP and 3-NP (-3.23 kcal/mol and -3.04 kcal/mol, respectively). Other top hit compounds ailanthone (quassinoid), isodiospyrin (naphthoquinone), engelhardione and 5-epi-isocentratherin (sesquiterpene) have also shown lower binding energy -7.41, -7.00 and -6.84 kcal/mol, respectively (Table **3**).

Active site loop (Lys189 and Lys190) and C-terminal lid (Glu423 and Glu424) residues are essential for catalytic activity of MtbICL protein [14]. MtbICL active site residues Lys189, Cys191, Lys197 along with Trp93, Asp108, Arg228, Asn313, Ser315 and Thr347 were involved in hydrogen-bond formation with 3-BP and 3-NP as reported previously (Fig. **1**). Molecular docking result also found these residues interacting with selected phytoligand compounds. In the docking process, phytoligand-MtbICL complexes also showed stable hydrogen bonding with active site residues Lys189, Cys191, Arg228, Asn313, Ser315 and Thr347 (Fig. **2a-f**).

3.4. Prediction of IC_{50} Value

The subset of ten (n=10) reported inhibitors with experimental IC_{50} value was used as training set to derive a relationship with binding gibbs free energy ($\Delta G_{binding}$) calculated by AutoDock (supplementary 4). Correlation between $\Delta G_{binding}$ and experimental IC_{50} value of reported inhibitors is shown in Fig. (**3a**). This linear dependency can be used for prediction of IC_{50} value of new lead compounds. Regression analysis (R^2 = 0.04341) was performed to predict corre-

Table 1. Binding energy calculation of isocitrate lyase (MtbICL) inhibitors.

S. No.	Name of Inhibitor (Pubchem ID)	Calculated Binding Energy (kcal/mol)		Experimental Inhibition (Ki/IC_{50})	Calculated Interacting Residues (H-bonds)	Reference
		(AutodockVina)	(Autodock)			
1.	3-Nitropropionate (1678)	-4.8	-3.23	Ki= 3 μM	Tyr89, Ser91, His180, Ser191, Gly192, His193, Asn313, Ser315, Ser317, Thr347 and Leu348.	[37]
2.	3-Bromopyruvate (70684)	-4.1	-3.04	Ki= 120 μM	His193, Asn313, Ser315, Ser317, Thr347 and cys191	[38]
3.	Itaconate (811)	-5.1	-3.08	Ki=120 μM	Lys189, Gly192	[39]
4.	Pyruvate-isoniazid analog with their copper complex	-5.4	-4.02	Inhibition rate 6-92%	Lys189, Lys190, Gly192, Gly287	[41]
5.	3-Nitropropionamides derivatives (54180692)	-6.7	-6.93	0.1 μM	Ser315, Ser317, Ser191, His193, Asn313, Leu194	[42]
6.	Quercetin(5280343)	-8.1	-6.76	3.57 μM	Gln39, Val42, His46,Lys169, Val218 and Ala219.	[14]
7.	Pthalazinyl derivatives	-8.1	-7.51	45-61% inhibition at 10 μM	Arg228, His393	[43]
8.	Phthalazin-4-ylacetamides	-8.7	-9.24	40.62-66% inhibition at 10 μM	Cys191, Leu194 His 193, Ser 315 Ser 317, Thr347	[44]
9.	5-Nitro-2-furoic acid hydrazones with furan-2-carbaldehyde	-7.0	-6.01	86.8% inhibition at 10mM	Lys189, Lys192, Arg260	[45]
10.	5-Nitro-2,6-dioxohexahydro-4-Pyrimidine carboxamides	-7.9	-5.95	45.7% inhibition at 10mM	Lys189, Gly192, Lys392, Arg395	[46]
11.	Isatinylthiosemicarbazones derivatives	-8.4	-6.48	63.44% inhibition at 10mM	Lys189, Asn263	[47]
12.	Extract of traditional Chinese medicine (I2906)	-6.6	-5.05	134 μg/mL	Ser317, Asn319	[48]
13.	Mannich base, Ydcm67	-7.5	-6.24	57.4% inhibition at 0.05mg/mL	Lys189, Asn263	[49]
14.	Thiobenzanilide (2-methoxy-2'-hydroxythiobenzanilide) derivatives	-6.2	-6.34	21-23% inhibition at 10 μmol/L	Leu194, Gly195	[50]
15.	Salicylanilide(2-hydroxy-N-phenylbenzamides) derivatives	-7.9	-6.46	22-59% inhibition at 10-100 μmol/L	Asp108,Gln184, Arg228, Glu285, Thr347	[51]

sponding IC_{50} value for calculated $\Delta G_{binding}$ of phytoligand-MtbICL complexes (Table **1**, Fig. **3b**).

3.5. Molecular Dynamics (MD) Simulation

Five simulation systems *i.e*. apo-MtbICL (ligand-free structure), 3-nitropropionic acid (3-NP)-MtbICL alongwith three phytoligand-MtbICL docked confirmations *i.e*. Shinjudilactone-MtbICL, Lecheronol-MtbICL and caniojane-MtbICL confirmations were prepared for MD simulation study to evaluate stability of protein-ligand complexes.

3.5.1. Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF)

RMSD value measures structural deviation of protein backbone and stability of protein-ligand complex. Average RMSD value for 3-NP-MtbICL, Shinjudilactone-MtbICL, Lecheronol-MtbICL and caniojane-MtbICL (0.26 nm, 0.24

Table 2. Calculation of drug likeness property of top hit phytochemical based anti-mycobacterial compounds.

S. No.	Name of Compound (BioPhytol ID)	Molecular weight (MW)	logP	HBA	HBD	TPSA
1.	Ailanthone (1085)	376.15	-0.429	7	3	113.29
2.	Shinjudilactone (1086)	376.15	0.413	7	2	110.13
3.	3-Hydroxyxanthyletin (1743)	244.07	2.243	4	1	55.76
4.	Isodiospyrin (3111)	374.08	0.516	6	2	108.74
5.	Erygibisoflavone (3390)	354.11	1.551	6	3	96.22
6.	Engelhardione (3669)	312.14	2.164	4	2	66.76
7.	Lecheronol A (3986)	306.22	1.534	3	2	57.53
8.	5-epi-isocentratherin (4010)	374.14	1.152	7	1	99.13
9.	Caniojane (4028)	356.2	2.842	4	0	44.76
10.	Engelharquinone (4085)	348.06	-1.337	6	3	111.9

Table 3. Docking analysis of selected phytochemical based anti-mycobacterial compounds.

S. No.	Name of Compound (Bio-Phytol ID)	Calculated Binding Energy (kcal/mol)		Calculated Inhibitory Value (Ki) (μM)	Predicted IC_{50} Value (μM)	Interacting Residues (H-Bonds)
		(Auto-DockVina)	(AutoDock)			
1.	Ailanthone (1085)	-10.40	-7.41	3.10	8.51	Arg255, Cys191, Ser413
2.	Shinjudilactone (1086)	-9.60	-7.51	3.43	6.91	Asp108, Arg228
3.	3-Hydroxyxanthyletin (1743)	-9.10	-6.98	7.60	9.12	Ser315, Arg228
4.	Isodiospyrin (3111)	-9.10	-7.00	7.43	7.94	Cys191
5.	Erygibisoflavone (3390)	-9.50	-7.33	3.73	5.62	Ser315, Arg228, Glu155, Asn313, Glu285
6.	Engelhardione (3669)	-9.70	-6.93	8.26	12.00	Cys191, Arg255
7.	Lecheronol A (3986)	-9.20	-7.77	2.03	4.89	Trp93, Thr347, His393
8.	5-epi-isocentratherin (4010)	-9.10	-6.84	9.72	9.77	Glu285, Arg228, Lys197, Gln184
9.	Caniojane (4028)	-9.40	-7.46	4.20	6.92	Lys189, Lys197
10.	Engelharquinone (4085)	-10.40	-6.97	7.82	9.33	Trp93, Asp153, Glu285, Arg228, Gln394

nm, 0.26 nm and 0.26 nm, respectively) were found lower than apo-MtbICL (0.31 nm) (Fig. **4a**). RMSF gives insight into the flexible regions of the protein-ligand complex and fluctuation about its average position. RMSF plot showed higher flexibility in active site region (Lys190 to Gly195) and C-terminal end (Pro411- Leu418) of apo-MtbICL suggesting movement of this region during catalytic reaction (Fig. **4b**). Further, RMSF plot of ligand-MtbICL complexes showed less flexibility than apo-MtbICL complex over flexible region suggesting stability of protein after ligand binding.

3.5.2. Radius of Gyration (Rg) and Solvent Accessible Surface Area (SASA)

Rotation of gyration measure compactness of the ligand-protein structure and SASA determines buried area of protein ligand complex. 3-NP and phytoligand-MtbICL complexes have shown lesser Rg value (2.95, 2.95, 2.96 and

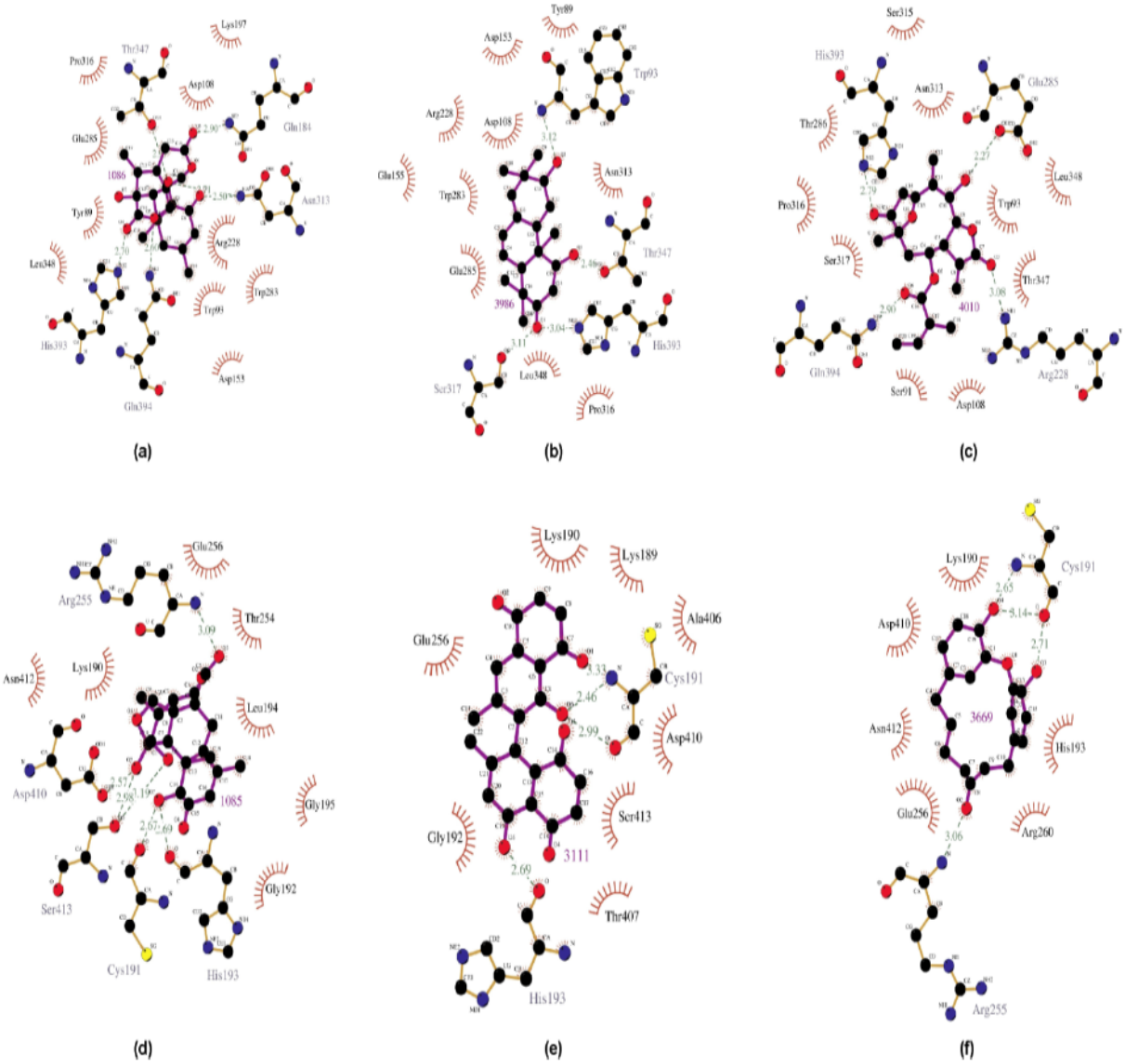

Fig. (2). Ligand- MtbICL protein interaction in docked complexes (**a**) Shinjudilactone - MtbICL (**b**) Lecheronol- MtbICL (**c**) 5-epi-isocentratherin - MtbICL (**d**) Ailanthone - MtbICL (**e**) Isodiospyrin - MtbICL (**f**) Engelhardione - MtbICL.

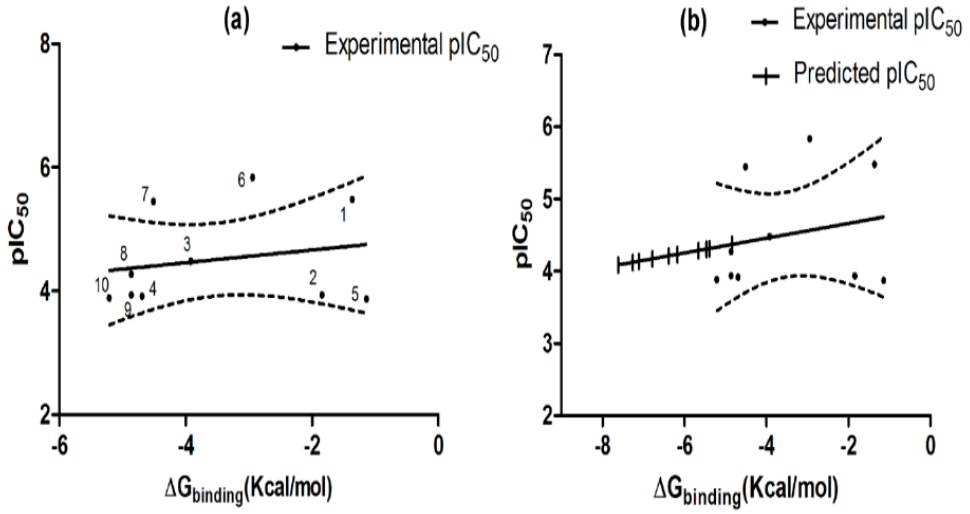

Fig. (3). (**a**) Relationship between calculated binding energy and experimental extent of inhibition (pIC_{50}) (**b**) Prediction of pIC_{50} value for selected anti-mycobacterial compounds.

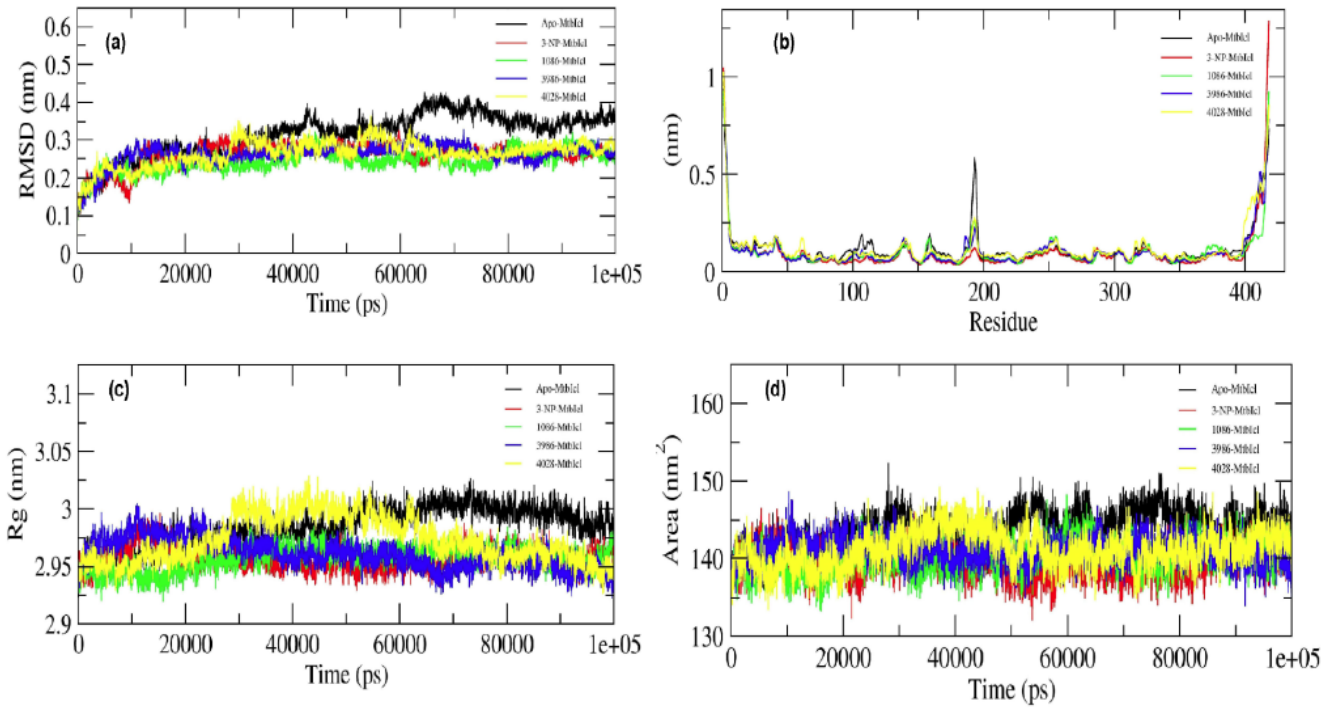

Fig. (4). (**a**) MD simulation of ligand-MtbICL complexes (**a**) RMSD, (**b**) RMSF, (**c**) Radius of gyration (Rg), (**d**) Solvent accessible surface area (SASA).

Table 4. Calculated binding energy (KJ/mol) of ligand-MtbICL protein-complexes.

Ligands	van der Waal Energy	Electrostatic Energy	Polar Solvation Energy	SASA Energy	Binding Energy
3-NP	-53.42 ±10.80	-89.33 ±16.22	137.10 ±21.66	-7.61 ±0.53	-13.27 ±13.68
3-BP	-4.37±9.41	-1.74±6.97	-0.16 ±97.91	-0.58±2.47	-6.84±98.26
Shinjudilactone	-144.19±11.84	-80.69±9.70	189.16±13.98	-13.79±0.54	-49.52±12.91
Lecheronol A	-109.84±11.53	-58.05±10.57	146.88±22.52	-12.28±0.84	-33.28±19.87
Caniojane	-75.86±16.52	-20.40±16.43	56.26±29.78	-7.47±1.66	-47.47±13.39

2.97 nm, respectively) than apo-MtbICL (2.98 nm) suggesting stability to their complex structures (Fig. **4c**). SASA value of 3-NP-MtbICL, Shinjudilactone -MtbICL, Lecheronol -MtbICL and caniojane -MtbICL complex (139.63, 140.15, 141.01 and 141.20 nm^2,respectively) were also found lower in comparison to apo-MtbICL (143.5 nm^2) that suggested system acquired compactness after binding with ligands (Fig. **4d**).

3.5.3. Calculations of Binding Free Energy

Binding free energy of standard inhibitors *i.e.* 3-nitropropionic acid (3-NP) and 3-bromopyruvic acid (3-BP) along with all phytoligand-MtbICL complexes were calculated. Other binding energy forms such as vander waal energy, electrostatic energy, polar salvation and SASA energy has also been calculated for the ligand-MtbICL complexes (Table **4**). The results showed stable binding of all three ligand-MtbICL complexes in comparison with 3-NP-MtbICL and 3-BP-MtbICL complex and binding free energy of all phytoligand -MtbICL complex found lower than standard inhibitors.

In docking study, Shinjudilactone (quassinoid), Lecheronol A (diterpene) and Caniojane (diterpene) have shown lower binding free energy in comparison to standard inhibitors and binding orientations with catalytic residues. MD simulation showed stable hydrogen bonding and lower free energy of all phytoligand-MtbICL complexes in comparison with the standard inhibitors. Thus, MD simulation results showed stable binding and proper bonding interaction with active site residues among all selected phytoligand -MtbICL complex. Thus, reported compounds constitute interesting starting points for future anti-TB drug candidates as few previous reports confirm their anti-tubercular property [52-54].

CONCLUSION

Present study found some potential inhibitor compounds (Table **2**) against MtbICL that represent flavonoid, quinone and terpene phytochemical class. In virtual screening, Phy-

toligands-MtbICL docked confirmations with lower binding energy were identified as potential MtbICL inhibitors. Among these compounds, shinjudilactone (quassinoid), lecheronol A (pimarane) and caniojane (diterpene) were potential MtbICL inhibitors based on molecular docking and MD simulation studies. Thus, phytochemical based anti-mycobacterial compound can further developed into effective drugs against persistence tuberculosis with lesser toxicity and side effects.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available in the supplementary file of this research article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material (1-4) of this research article can be found in supplementary file.

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