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Molecular Docking &*insilico* Pharmacokinetic Parameters of Substituted Thiazolidin-4-ones as Anti-tubercular agents

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ABSTRACT

The InhA inhibitors perform a significant role in mycolic acid synthesis by blocking the fatty acid biosynthesis pathway. In this article, a molecular docking study followed by insilico pharmacokinetic parameters could be studied as an effective approach to detect newer enoylreductase inhibitors. Novel Thiazolidin-4-one derivatives were designed to perform molecular docking studies using Autodock-1.5.6 and identified the hit molecules. The hits were further evaluated for their drug likeliness using the SwissADME webserver. The binding affinity of the designed ligands towards InhA was selected based on binding affinities and interaction patterns. Almost all the compounds have good binding affinities in the range of -10.31 to -8.56 compared with that of cognate ligand -9.06. The results reveal that Thiazolidin-4-ones as InhA inhibitor and the compounds, 19, 17, 16, 15 with good binding affinities may produce significant anti-tubercular activity for further enhancement.

Keywords
InhA, insilico, Autodock, SwissADME, Discovery Studio.

Introduction

Infectious diseases are a major threat worldwide as new bacterial resistance to antibiotics inflates concerns about the recurring utilization of antibacterial agents in clinical practice. Thus, there is a need to develop new drugs for the effective treatment of bacterial infections is of major priority. One of the strategies implemented to inhibit the bacterial pathogens is to focus on the biosynthesis pathway of fatty acids (FAS-II), where the greatly conserved enoyl-acyl carrier protein reductase (InhA) plays a major role. The aptness of targeting InhA for combating tuberculosis has been validated by the first-line antitubercular drug isoniazid, a very powerful mycobacterial InhA inhibitor [1,2]. Based on several ligand- or structure-based approaches, varied classes of InhA inhibitors have been investigated, as pyrrolidinecarboxamides[3], pyridomycin, triclosan, and INH-NAD analogues [4,5]. But very few of them inhibited in vitro Mtb growth.

There is a need to design new chemical entities to inhibit the InhA enzyme. Thiazolidin-4-one is considered as a biologically active scaffold that possesses almost all types of biological activities. Thiazolidin-4-ones are successfully introduced in different categories and proved as potential moieties, such as ralitoline as a potent anticonvulsant, etozolin as an antihypertensive, pioglitazone as a hypoglycemic agent, and thiazolidomycin activity against Streptomyces species. This diversity in the biological response profile has attracted the attention of many researchers to explore this skeleton to its multiple potentials against several activities [6]. G. Cihan-U¨stu¨ndag˘ et al [7], Ur F et al [8], Küçükgüzel SG et al [9], and several more researchers have related Thiazolidin-4-ones as antitubercular agents. With certain adaptions, these compounds might generate potent chemical entities against MDR-TB & XDR-TB.

In this regard, in the current study, we had designed a diverse set of novel thiazolidin-4-ones bearing

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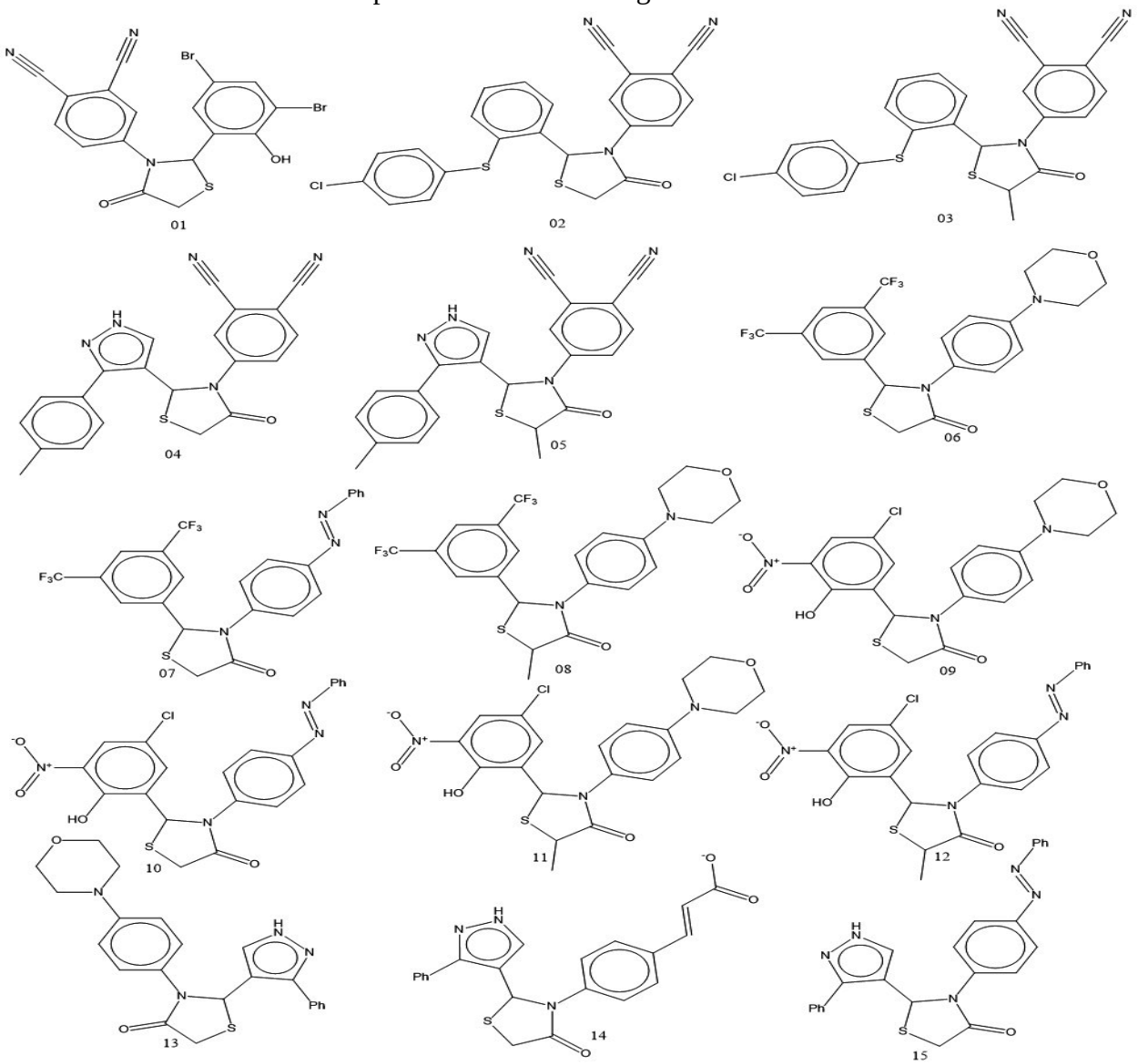
aryl and diaryl substitutions at C2 & N3 positions, unsubstituted& methyl substitution at the C5 position, and performed insilico analysis of the designed moieties [10–14].

Material and Methods

Molecular docking

Chemical structures of novel thiazolidin-4-one were designed using literature. Flexible-ligand docking simulations were executed with AutoDock version 1.5.6. X-ray crystallographic structure of InhA enzyme was taken from the protein data bank (4TZK [3];http://www.rcsb.org/) with resolution 1.62 Å. For the preparation of a target protein, crystallographic ligand ((3s)-1-cyclohexyl-n-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide), NAD, and water molecules were all removed from the original structure. All the pre-processing steps for InhA protein were executed via AutoDock Tools 1.5.6 program (ADT) [15]. ADT program was operated to fuse the non-polar hydrogens into the associated carbon atoms of the receptor and Kollman charges were allocated.



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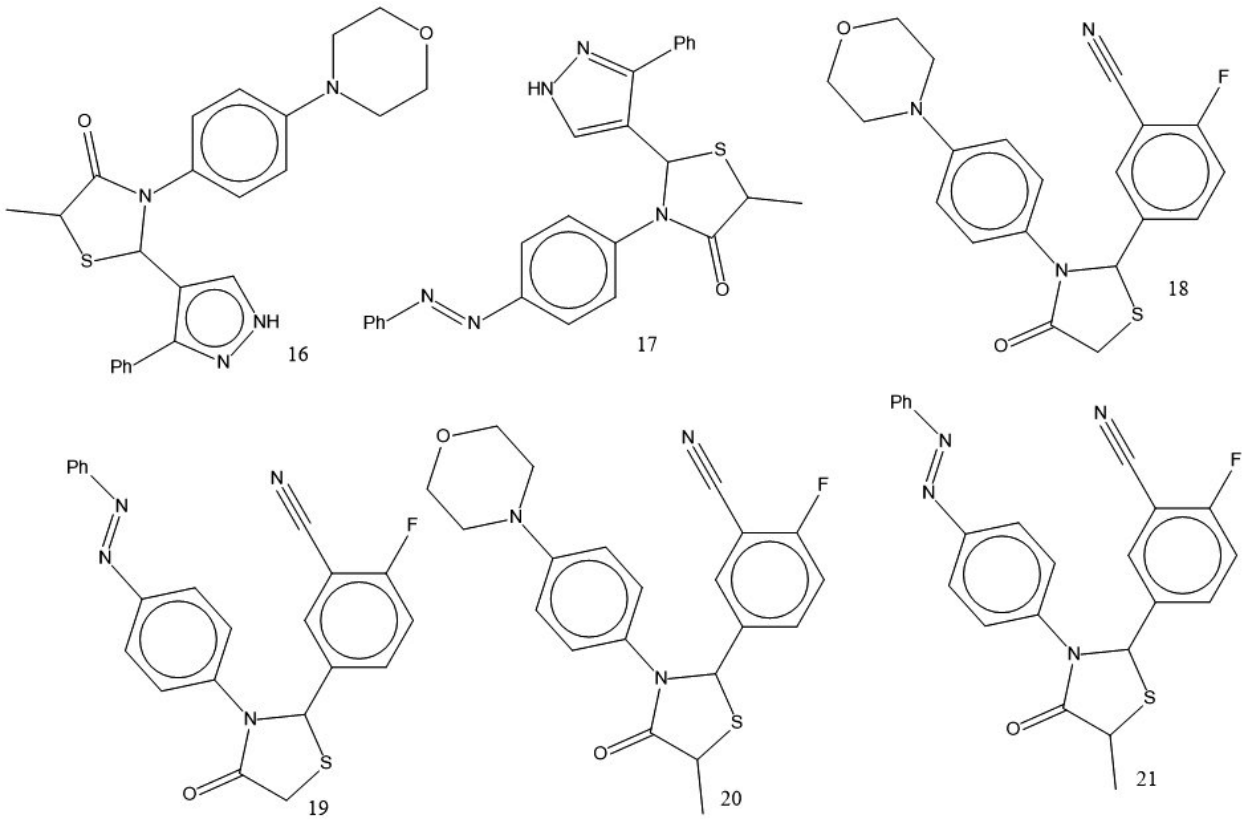

Figure-1 Structures of designed compounds (1-19)

Ligands were designed using ChemSketch [16](Figure 1), and the file formats are converted from .mol to .pdb format by using Open Babel [17]. Then non-polar hydrogens, Gasteiger charges, and torsions degrees of freedom were assigned by the ADT program. Lamarckian genetic algorithm (LGA) employed to model the interactions between thiazolidin-4-ones and InhA active site using 100 GA runs; 27000 maximum generations; a gene mutation rate of 0.02; and a crossover rate of 0.8 were applied for LGA method. Based on the validation study, the cognate (co-crystallographic) ligand was extracted and re-docked into its receptor (self-docking). Validation is performed by comparing the root mean square deviation (RMSD) of the Cartesian coordinates of the atoms of the ligand in the docked pose and crystallographic conformations.

InhA was characterized by grid maps in the actual docking procedure. The grids were calculated using the AutoGrid module. The grid included a map for every atom type within the ligand and also a map for electrostatic interactions. The size of the grid was 50 × 50 × 50 Å (distributed in the x, y, and z directions) and it was centered on the center of mass of the catalytic site of InhA with a spacing of 0.375 Å. Cluster analysis was performed on the docked results concerning RMS tolerance of 2 Å. 2D and 3D interactions of the docked ligands were analyzed using Discovery Studio Visualizer-20.1 [18].

Insilico Pharmacokinetic Parameters

The *in-silico* drug likeliness and ADME properties of the proposed molecules were determined by using the SwissADME webserver [19–21]. In this server, the structure was drawn or the SMILES format of the ligands was incorporated and executed the program to attain the desired results.

Results and Discussion

The docking study of designed thiazolidin-4-ones to the active site of protein was performed by

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Autodock for calculating the binding affinities of the designed ligands with the protein. The designed molecules were docked into the InhA (4TZK), to determine their InhA inhibitory activity. All compounds except 6, 8, 11, 18 & 20 had shown a good binding affinity to the InhA receptor compared to the cognate ligand for antitubercular activity (Table 1). From Table 1, the interactions are mainly due to the lipophilic factors and hydrogen bonding. The interactions are mainly subjugated in the region of TYR158, LYS165 & ILE194 residues which are in the active site region (Figure 2). The aryl substitutions are located in the hydrophobic pocket and the amino group is located in the hydrophilic pocket. The compound 19 exhibited hydrogen bonding with ILE21 (H-bond length 2.42 Å) residue and with LYS165 (H-bond length 2.81 Å), which are depicted in Figure 3. The best-docked poses of the compounds 19, 17, 16, 15 with significant binding affinities are shown in Figure 4.

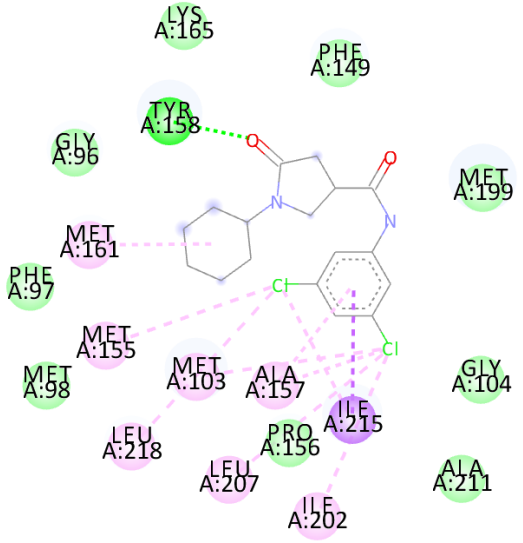

Figure- 2 2D interactions of Cognate ligand with active site of InhA (4TZK)
Green – H-bond interaction; rose – Alkyl/ Pi-Alkyl interactions; violet – Pi-Sigma interactions

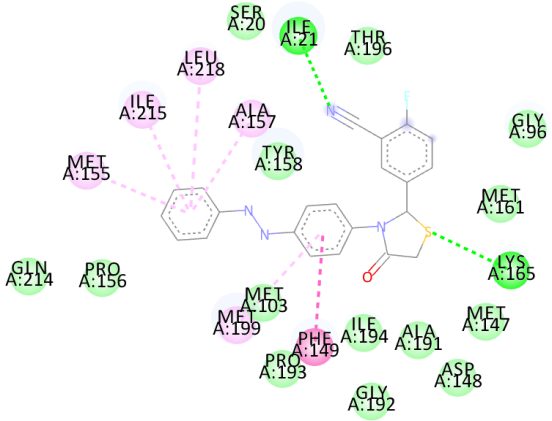

Figure- 3 2D interactions of docked compound 19 with InhA (4TZK)

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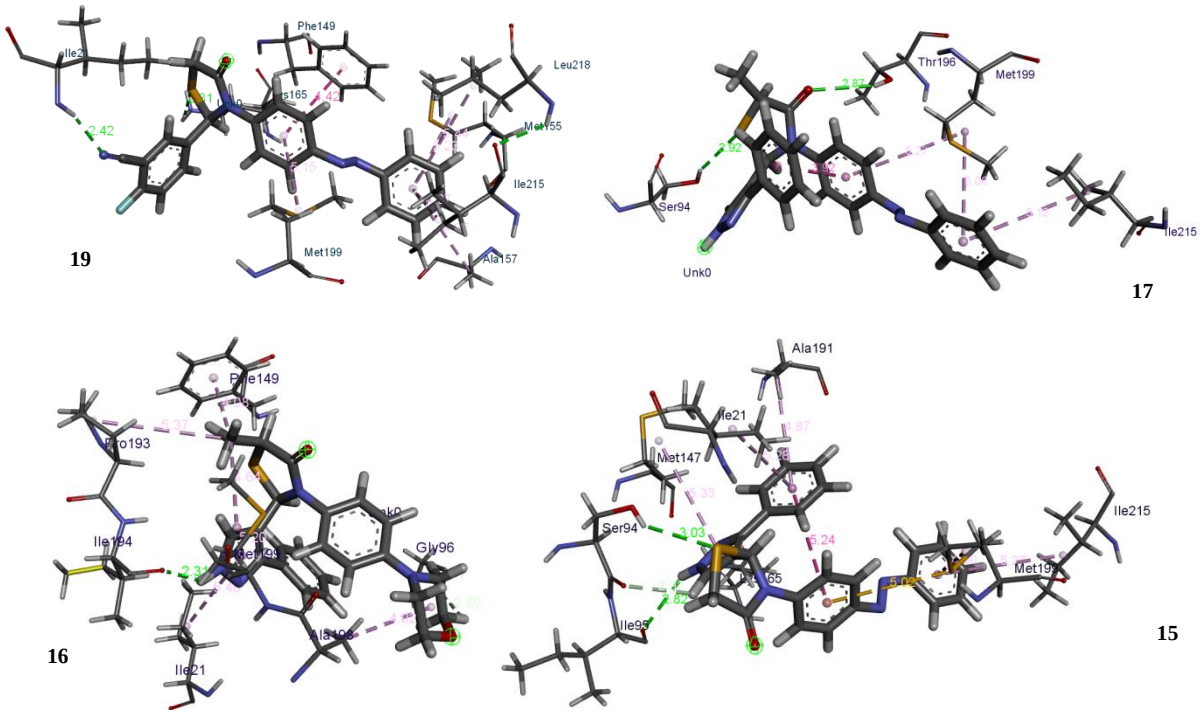

Figure- 4 Best affinity mode of docked compounds 19, 17, 16, 15 with InhA (4TZK)

Table-1 Binding energies & interacted aminoacids for compounds 1-21 with InhA (4TZK)

Compound code	Binding Energy	Number of H-bonds	Interacted aminoacid residues	Hydrogen bond length
Reference	-9.06	1	TYR158	2.22
01	-9.14	3	LYS165; MET199	2.45; 2.54,2.63
02	-9.74	1	PRO156	3.71
03	-9.85	2	TYR158; MET199	2.78; 2.68
04	-9.48	1	TYR158	3.35
05	-9.13	1	LYS165	2.64
06	-8.78	1	LYS165	2.1
07	-9.12	1	THR196	2.36
08	-8.86	1	ILE194	2.37
09	-9.60	1	LYS165	3.39
10	-9.18	1	ILE21	3.58
11	-8.56	2	LYS165; THR196	2.38; 3.55
12	-9.19	2	TYR158; ILE194	3.81; 2.58
13	-9.80	1	LYS165	2.96
14	-9.75	2	GLY96; LYS165	3.64; 2.62
15	-10.05	2	ILE21; LYS165	3.19; 2.4
16	-10.06	1	ILE194	2.31
17	-10.07	2	ILE21; LYS165	3.65; 2.61
18	-8.96	2	LYS165; PRO193	3.90; 3.38
19	-10.31	2	ILE21; LYS165	2.42; 2.81

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20	-8.82	1	LYS165	2.15
21	-9.48	2	GLY96; LYS165	3.84; 2.84

The ADMET properties for the thiazolidin-4-ones 1-21 were determined in-silico by using the SwissADME webserver of the Swiss Institute of Bioinformatics. Molecular weights of the compounds are between 383.44 and 495.44 $g.mol^{-1}$. Estimated no. of hydrogen bond donors are in the range of 0-1, hydrogen bond acceptors are in the range of 3-9. LogP, Molar refractivity, Rotatable bonds, Total polar surface area of the compounds are between 1.52 - 4.15; 105.41 - 131.56; 2 - 6; 58.08 - 136.38 $Å^2$ respectively. The compounds are in the range of Lipinski's rule of five &Veber's rule (Table-2).

Table-2 Molecular Properties for designed compounds 1-21

Compound code	MW	H-bond donors	H-bond acceptors	LogP	MR	Rotatable bonds	TPSA
01	479.15	1	4	2.8	105.41	2	113.42
02	447.96	0	3	3.02	123.56	4	118.49
03	461.99	0	3	3.46	128.37	4	118.49
04	385.44	1	4	1.98	110.53	3	121.87
05	399.47	1	4	2.31	115.34	3	121.87
06	476.44	0	8	3.46	114.29	5	58.08
07	495.44	0	9	4.15	119.18	6	70.33
08	490.46	0	8	3.75	119.09	5	58.08
09	435.88	1	5	1.52	120.14	4	124.13
10	454.89	1	6	2.74	125.03	5	136.38
11	449.91	1	5	2.09	124.94	4	124.13
12	468.91	1	6	3.46	129.83	5	136.38
13	406.5	1	3	2.46	121.86	4	86.76
14	390.44	1	4	2.01	110.86	5	114.42
15	425.51	1	4	3.17	126.75	5	99.01
16	420.53	1	3	2.9	126.67	4	86.76
17	439.53	1	4	3.51	131.56	5	99.01
18	383.44	0	4	2.98	108.95	3	81.87
19	402.44	0	5	3.56	113.85	4	94.12
20	397.47	0	4	3.21	113.76	3	81.87
21	416.47	0	5	3.8	118.65	4	94.12
Recommended values	<500 daltons	≤5	≤10	≤5	40 to 130	≤10	≤140 $Å^2$

MW- Molecular weight; MR – Molar Refractivity; TPSA – Total Polar Surface Area

All the compounds except 2, 3, 7, 8, 10, 12 & 17 are showing moderate aqueous solubility which indeed results in high Gastro-Intestinal (GI) absorption. The compounds are not crossing the Blood-Brain Barrier. The details of the ADME properties for compounds 1-21 are shown in Table - 3.
Besides, synthetic accessibility of the compounds can be predicted using Swiss ADME on a scale of 1-10 i.e., very easy to difficult to synthesize. All the designed compounds can be synthesized in the laboratory.

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Table-3 ADME & Synthetic accessability for designed compounds 1-21

Compound code	Aqueous Solubility	GI absorption	BBB permeant	log Kp (cm/s)	Synthetic Accessibility
01	Moderately soluble	High	No	-6.5	3.32
02	Poorly soluble	Low	No	-5.13	3.71
03	Poorly soluble	Low	No	-4.93	4.04
04	Moderately soluble	High	No	-6.44	3.65
05	Moderately soluble	High	No	-6.24	3.98
06	Moderately soluble	High	No	-5.74	3.74
07	Poorly soluble	Low	No	-4.42	3.91
08	Poorly soluble	Low	No	-5.54	4.09
09	Moderately soluble	High	No	-6.29	3.77
10	Poorly soluble	Low	No	-4.96	3.9
11	Moderately soluble	High	No	-6.09	4.11
12	Poorly soluble	Low	No	-4.77	4.23
13	Moderately soluble	High	No	-6.6	3.83
14	Moderately soluble	High	No	-6.36	3.64
15	Moderately soluble	High	No	-5.28	3.97
16	Moderately soluble	High	No	-6.4	4.18
17	Poorly soluble	Low	No	-5.08	4.3
18	Moderately soluble	High	No	-6.56	3.58
19	Moderately soluble	High	No	-5.23	3.74
20	Moderately soluble	High	No	-6.36	3.93
21	Moderately soluble	High	No	-5.03	4.08

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logKp – Skin Permeation

Conclusion

The docking study revealed that the thiazolidin-4-ones showed better alignment than the new experimental drugs at the active site by interacting with active site amino acid residues of InhA (4TZK). Thus, the *in-silico*method adopted in the present study helped in identifying the lead molecules to activate InhA. Results observed in the present study demonstrated that some derivatives of the designed thiazolidin-4-ones may exert interesting anti-tubercular activity. The compounds 19, 17, 16, 15 have significant InhA inhibitory activity and are likely to be useful as drugs or after further refinement in the discovery of novel anti-tubercular agents.

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Conflict of Interest

The authors declare no conflict of interest.

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