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Synthesis of novel 2, 5-disubstituted tetrazole derivatives as potent biological agents

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ADME-toxicology studies

A B S T R A C T

In the present work, we have discussed the synthesis of a series of 1-((2'-(2-(2-(substitutedphenyl))-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid derivatives by the reaction of 1-((2'-(2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid with phenacyl bromides using potassium carbonate. The structures of synthesized derivatives have been characterized using different spectroscopic techniques like FT-IR, ^{1}H-NMR, ^{13}C-NMR, and Mass. Synthesized compounds were screened for their antibacterial and anti-TB activities all the compounds have found better activity against selected pathogenic strains. In addition, in silco molecular docking studies were carried out on targeted enzymes DNA gyrase and *3-oxoacyl-ACP reductase* with ADME-toxicology studies.

1. Introduction

An increase in the population, as well as an increase in diseases caused by pathogenic organisms, leads to death. One-third of the population who died was often infected by Tuberculosis (TB) disease which is caused by Mycobacterium tuberculosis (MTB). According to the World Health Organization's (WHO) Global Tuberculosis (2019), about 10 million people were affected and approximately 1.4 million deaths were caused by TB.[1-3] Still, the researchers were facing challenges in the treatment of TB due to the emergence of (MDR TB) Multidrug-resistant TB, (XDR TB) extensively drug-resistant TB, and an increase in the number of TB-HIV co-infections.[4]

Lipophilicity is an important parameter related to the membrane permeation of compounds into MTB cells, and simply an increment of the lipophilic character of drugs may increase their anti-TB activities accordingly.[5] Tetrazole can increase lipophilicity that facilitates compounds to penetrate the plasma membrane, so Tetrazole derivatives are the potential as anti-TB agents.[6] Tetrazole is a most advantageous scaffold which represents an important class of heterocyclic compounds, well-endowed with interesting pharmacological activities and broad applications in different scientific research fields. Primarily, due to the role played by Tetrazole in medicinal chemistry as this moiety offers a more complementary pharmacologic profile and a metabolically stable substitute for carboxylic acid functionalities. The heterocyclic Tetrazole moiety has admirable biological, pharmaceutical and clinical applications.[7-9]

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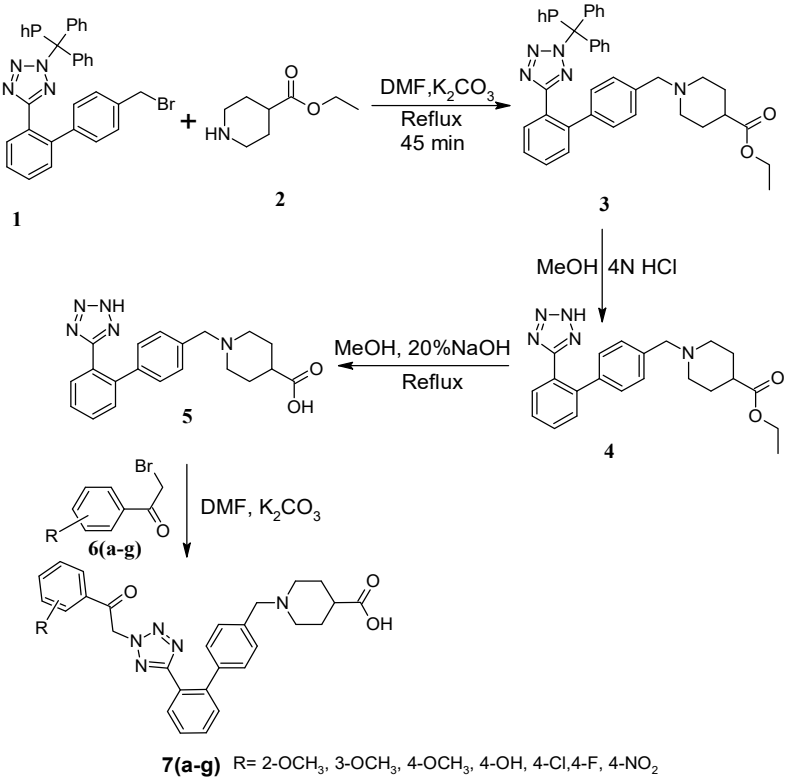
In 1994, the first member of Sartan's family, Losartan, was synthesized and applied to clinical treatments. After that, scientists have been decorating its molecular structure with chemical reactions on the mother nucleus to improve its curative effects of lowering blood pressure, prolonging its process, and Losartan's outstanding performance accelerated the emergence of other members of the Sartan's family meanwhile promoted the development of medicinal chemistry. These novel medicines can be used for the treatment of hypertension, heart failure, and kidney diseases.[10] Until now, the angiotensin II acceptor depressant family has many members, such as Losartan, Irbesartan, Candesartan, Tazobactam, Valsartan, and Olmesartan. In these medicinal molecules, biphenyl tetrazoles are a type of heterocyclic compounds having therapeutic applications in the field of drugs such as antibacterial[11], antifungal[12], anticancer[13], analgesic[14], anti-inflammatory[15], and anti-diabetic.[16] The biphenyl tetrazoles are molecules having diverse activity and a potential role in biosciences.[17]

Initially, biphenyl tetrazole compounds were used in chemical and agrochemical industries as an intermediate, and they were prepared by researchers and evaluated for their therapeutic significance. Usually, these moieties were known for hypertension drugs[18] and other pharmacologically potential antibacterial and anti-TB agents. Till now several biphenyl tetrazole derivatives have been reported and were screened for many biological activities and several attempts were made to improve and explore the pharmacological properties by varying the substitution. Combining biphenyl tetrazole with substituted phenacyl bromides by using a base catalyst is expected to give novel heterocyclic compounds with better biological activities.[19]

2. Results and Discussion

2.1. Chemistry

In this study, we carried out synthesis of novel 2, 5-disubstituted tetrazole derivatives (**7a-g**) through multi step reaction. Initially, *n-(triphenylmethyl)-5-(4'-bromomethylbiphenyl-2-yl-) terazole* (**1**) reacted with ethyl-piperidine-4-carboxylate (**2**) in DMF using potassium carbonate as a catalyst the reaction proceeds elimination of hydrogen bromide and to get yield 2-(N-triphenylmethyl tetrazolyl)-methyl biphenyl ethyl piperidine-4-carboxylate (**3**). Further, triphenyl was removed using mixture of 4N HCl in methanol (20 mL) solvent to get methyl biphenyl tetrazolyl-ethyl piperidine-4-carboxylate (**4**) and on hydrolyzing the compound leads to get stable compound (**5**), and then reacted with substituted phenacyl bromides (**6a-g**) using potassium carbonate in solvent media to afford final 1-((2'-(2-(2-(substituted phenyl))-2-oxoethyl)-2*H*-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid derivatives (**7a-g**) solid product. The reaction pathway involved in the synthesis of target compounds **(7a-g)** has been given in S**cheme-1**.



Scheme1. Reaction pathway for the synthesis of compounds **7(a-g)**

The structures of novel 2, 5-disubstituted tetrazole derivatives (**7a-g**) were confirmed by their IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra.

2.2. Pharmacological activities

2.2.1. Antibacterial activity

The *in vitro* antibacterial activity of the synthesized compounds (**7a-g**) was evaluated against four bacterial strains *S. aureus*, *B. substilis*, *E. coli,* and *Shigella* at different concentrations of 3, 6, 9, and 12µg/mL. Obtained results were expressed as a zone of inhibition and minimum inhibitory concentrations (MIC) and the results were compared with the standard drug Streptomycin and obtained results were summarized in **Table1** and **2.**

The investigation of antibacterial activity revealed that the tested compounds showed an outstanding zone of inhibition against all four strains. In that, the compound **7g** exhibited the highest zone of inhibition of 14±0, 14±0, 16±0 and 12±0 mm against strains *S. aureus*, *B. substilis E. coli,* and *Shigella* respectively at the concentration of 12 µg/mL as compared to other compounds in the series. Minimum inhibitory concentration (MIC) results indicate that the compounds show almost similar MIC values in the range of 2.3 ± 0.26 to 2.5 ± 0.26, 2.1 ± 0.29 to 2.4 ± 0.25, 2.1 ± 0.23 to 2.3 ± 0.25 and 2.2 ± 0.23 to 2.5 ± 0.29 µg/mL of *B. substiles*, *S. aureus*, *E. coli,* and *Shigella* respectively. In that, the compounds **7a**, and **7e** and **7g** shows MIC a value of 2.3 µg/mL against *B.substilis* the compounds **7b**, **7e,** and **7g** show a MIC value of 2.1 µg/mL against *S. aureus*, **7a**, **7c**, **7e**, **7f** and **7g** exhibited MIC value 2.1 µg/mL against *E. coli* and **7a**, **7b** and **7e** shows MIC value 2.2 µg/mL against *Shigella* strain. These results suggested that the compound **7g** exhibited the highest antibacterial activity due to the presence of the electron-withdrawing nitro group in their ring structure.[20-24]

Table1. Antibacterial activity results of synthesized compounds **7(a-g)**

		Zone of inhibition in mm (mean ± SD)			
Compd	Test volume (µL)	*S. aureus*	*B. subtilis*	*E. coli*	*Shigella*
7a	3	6±1	6±1	6±1	5±1
	6	6±1	6±1	7±1	5±1
	9	7±0	7±0	9±0	6±0
	12	9±0	10±0	12±0	9±0
7b	3	6±1	6±1	8±1	5±1
	6	7±1	7±1	8±1	8±1
	9	8±0	8±0	8±0	9±0
	12	10±0	12±0	12±0	10±0
7c	3	6±1	6±1	6±1	5±1
	6	7±1	7±1	7±1	6±1
	9	7±0	8±0	9±0	9±0
	12	10±0	12±0	12±0	10±0
7d	3	6±1	6±1	6±1	5±1
	6	7±1	8±1	8±1	6±1
	9	9±0	9±0	8±0	9±0
	12	13±0	12±0	12±0	10±0
7e	3	6±1	6±1	6±1	6±1
	6	7±1	8±1	8±1	7±1
	9	9±0	9±0	10±0	8±0
	12	11±0	12±0	12±0	11±0
7f	3	6±1	6±1	6±1	5±1
	6	7±1	7±1	8±1	7±1
	9	8±0	9±0	10±0	9±0
	12	10±0	13±0	13±0	10±0
7g	3	6±1	6±1	6±1	7±1
	6	7±1	7±1	9±1	8±1
	9	9±0	10±0	11±0	9±0
	12	14±0	14±0	16±0	12±0
Streptomycin	3	20±1	23±1	18±1	21±1
	6	22±2	25±2	20±2	22±2
	9	22±2	24±2	22±2	22±2
	12	27±2	27±2	25±2	24±2

Table 2. MIC results of synthesized compounds **7(a-g)**

	MIC µg/mL (mean ± SD)			
Compd	*B. subtilis*	*S. Aures*	*E. coli*	*Shigella*
7a	2.3 ± 0.26	2.4 ± 0.25	2.1 ± 0.25	2.2 ± 0.26
7b	2.4 ± 0.31	2.1 ± 0.29	2.3 ± 0.25	2.2 ± 0.25
7c	2.4 ± 0.24	2.3 ± 0.26	2.1 ± 0.23	2.3 ± 0.24
7d	2.5 ± 0.26	2.4 ± 0.24	2.2 ± 0.30	2.5 ± 0.29
7e	2.3 ± 0.21	2.1 ± 0.22	2.1 ± 0.26	2.2 ± 0.23
7f	2.5 ± 0.21	2.4 ± 0.23	2.1 ± 0.25	2.3 ± 0.26
7g	2.3 ± 0.26	2.1 ± 0.29	2.1 ± 0.23	2.5 ± 0.29
Streptomycin	2.5 ± 0.30	2.5 ± 0.30	2.2 ± 0.26	2.3 ± 0.28

2.2.2. Anti-tubercular activity

The anti-tubercular activity of the synthesized compounds (**7a-g**) was evaluated against H37RV strain at concentrations of 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50, and 100 µg/mL and the results have been shown in **Table 3** and **Fig 1**. Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, and Streptomycin were used as reference standards for comparison. The activity results suggested that the compounds **7b, 7f,** and **7g** exhibited very good sensitivity with a MIC value of 1.6 µg/mL, and they have sensitivity similar to reference standards Isoniazid & Ethambutol (MIC= 1.6µg/mL). Compound **7d** showed sensitivity at 6.25 µg/mL, and the remaining compounds also exhibited moderate sensitivity with a MIC value range of 12.5-50 µg/mL as compared to standards.

Table 3. The anti-tubercular activity results of the synthesized compounds **7(a-g)**

Compd	100 µg/mL.	50 µg/mL.	25 µg/mL.	12.5 µg/mL.	6.25 µg/mL.	3.12 µg/mL.	1.6 µg/mL.	0.8 µg/mL.
7a	S	S	R	R	R	R	R	R
7b	S	S	S	S	S	S	S	R
7c	S	S	S	S	R	R	R	R
7d	S	S	S	S	S	R	R	R
7e	S	R	R	R	R	R	R	R
7f	S	S	S	S	S	S	S	R
7g	S	S	S	S	S	S	S	R

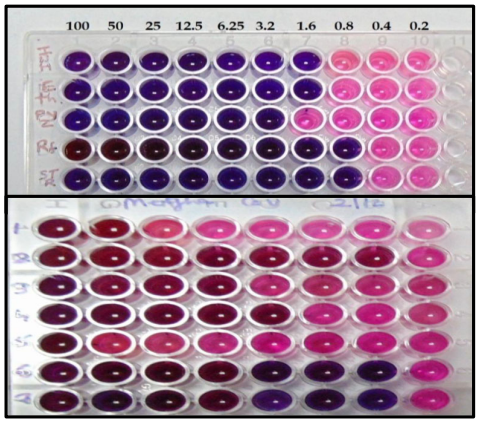


Fig. 1. Anti-tubercular activity results of the synthesized compounds (**7a-g)**

2.3. in silico molecular docking studies

The binding affinity and interaction of the synthesized compounds with active sites of the enzymes were analyzed using *in silico* molecular docking studies. The docking of receptors DNA gyrase for antibacterial activity and 3-oxoacyl-ACP reductase was chosen for the anti-tubercular activity. The 2D and 3D representations of compounds was shown in **Fig. 2** and **Fig. 3** with streptomycin as the standard drug. The binding energies & bonds with amino acids such as ARG47, GLY90, LEU91, GLY22, ASN24, SER92 and HIS95, ALA96, VAL120, SER121, ILE90, VAL93, ASN24, ARG47, GLY90, SER92 for antibacterial and anti-tubercular activities respectively.[25] The docking study of antibacterial activity results showed that the docking scores of compounds (**7a-g**) with DNA gyrase were found to be in the range of -8.9 to-10.4 kcal/mol. The compound **7d** shows the least binding energy of -10.4 kcal/mol and five hydrogen bonds. The most active compound was **7d** from the series (**7a-g**) interaction with the protein DNA gyrase (PDB:1KZN) exhibited conventional hydrogen bond interactions with HIS95, ALA96, SER121, SER121, and ILE90. Carbon Hydrogen Bond GLY119, ASN46, Pi-Anion GLU50, Pi-Sigma THR165, Alkyl ILE90, Pi-Alkyl. PRO79, ILE78, ILE90, ILE78, ALA47, VAL167. The streptomycin was used as the reference standard for comparison (-7.6 Kcal/mol). Streptomycin interactions with the protein Conventional Hydrogen Bond ASP73, THR165, VAL71, VAL43, Results were tabulated in **Table 4**.

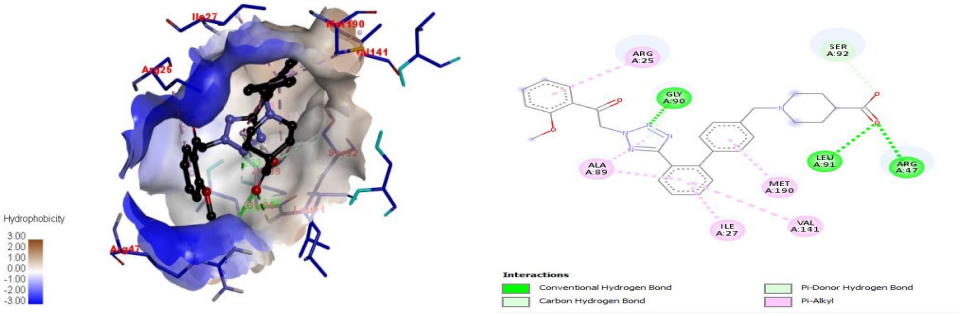
The docking results of anti-tubercular activity revealed that the compounds (**7a-g**) had significant binding modes with docking scores in the range of -10.1 to -10.8 kcal/mol and 3-4 hydrogen bonds in their receptor active pocket. Streptomycin was used as a reference standard for comparison (-8.8 kcal/mol). All the synthesized compounds showed encouraging binding energies and established one or more hydrogen bonds with amino acids in the active pockets. In that, the compound **7g** showed the least binding energy of -10.8 kcal/mol and four hydrogen bonds. The most active compound was **7g** from the series (**7a-g**) interaction with the protein 3-oxoacyl-ACP reductase (PDB: 1UZL). Exhibited conventional hydrogen bond interactions with GLY22, ARG47, ARG47, and LEU91.Pi-Donor Hydrogen Bond interaction SER92, Alkyl interaction ARG47, Pi-Alkyl interaction, ILE27, ALA89, MET 190. The streptomycin was used as a reference standard for comparison. (-8.8 Kcal/mol).[26,27] Conventional Hydrogen Bond ASN24, ARG47, GLY90, SER92 (**Table 5** and **Fig. 3**).

Table4. Molecular interactions for antibacterial activity of synthesized compounds (**7a-g**) and standard with DNA gyrase protein

Compd.	Binding affinity (kcal/mol)	Hydrogen bond interaction	Hydrogen bond length in Å	Hydrophobic and other interactions
7a	-9.1	HIS95 ALA96 VAL120 SER121 ILE90	2.86 2.46 2.65 2.64 2.14	GLU50, ASN46, ASN46, ALA47, ILE90, PRO79, ILE78, ILE78, ILE90, ILE78, PRO79, ILE90
7b	-9.1	HIS95 ALA96 VAL120 SER121	2.99 2.36 2.65 2.74	GLU50, ASN46, ASN46, ALA47, ALA86, ILE90, PRO79, ILE78, ILE90, ILE78, PRO79, PRO79, ILE90
7c	-8.9	VAL120 SER121	2.66 1.99	GLU50, ASN46, ASN46, ALA47, ALA86, ILE90, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79, PRO79
7d	-10.4	HIS95 ALA96 SER121 SER121 ILE90	2.43 2.48 2.65 2.90 2.29	GLY119, ASN46, GLU50, THR165, ILE90, PRO79, ILE78, ILE90, ILE78, ALA47, VAL167
7e	-9.7	VAL120 SER121	2.67 1.98	GLU50, ASN46, ASN46, ALA47, ALA86 , ILE90, PRO79, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79
7f	-9.7	HIS95 VAL120 SER121 SER121 VAL93	2.96 2.67 1.98 2.69 2.60	GLU50, ASN46, ASN46, ALA47, ALA86, ILE90, PRO79, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79, ILE90
7g	-9.4	VAL120 SER121 SER121	2.64 2.02 2.65	GLU50, ASN46, ASN46, ALA47, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79, ILE90
Streptomycin	-7.6	ASP73 THRI65 VAL71 VAL43 VAL43	2.23 2.45 2.72 2.50 2.58	-

Table 5. Molecular interactions for anti-TB activity of synthesized compounds (**7a-g**) and standard with **3-oxoacyl-ACP reductase** protein

Compd.	Binding affinity (kcal/mol)	Hydrogen bond interaction	Hydrogen bond length in Å	Hydrophobic and other interactions
7a	-10.2	HIS95 ALA96 VAL120 SER121 ILE90	2.86 2.46 2.65 2.64 2.14	GLU50, ASN46, ASN46, ALA47, ILE90, PRO79, ILE78, ILE78, ILE90, ILE78, PRO79, ILE90
7b	-10.6	HIS95 ALA96 VAL120 SER121	2.99 2.36 2.65 2.74	GLU50, ASN46, ASN46, ALA47, ALA86, ILE90, PRO79, ILE78, ILE90, ILE78, PRO79, PRO79, ILE90
7c	-10.4	VAL120 SER121	2.66 1.99	GLU50, ASN46, ASN46, ALA47, ALA86, ILE90, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79, PRO79
7d	-10.5	HIS95 ALA96 SER121 SER121 ILE90	2.43 2.48 2.65 2.90 2.29	GLY119, ASN46, GLU50, THR165, ILE90, PRO79, ILE78, ILE90, ILE78, ALA47, VAL167
7e	-10.1	VAL120 SER121	2.67 1.98	GLU50, ASN46, ASN46, ALA47, ALA86 , ILE90, PRO79, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79
7f	-10.4	HIS95 VAL120 SER121 SER121 VAL93	2.96 2.67 1.98 2.69 2.60	GLU50, ASN46, ASN46, ALA47, ALA86, ILE90, PRO79, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79, ILE90
7g	-10.8	VAL120 SER121 SER121	2.64 2.02 2.65	GLU50, ASN46, ASN46, ALA47, ILE90, ILE78, ILE78, ILE90, ILE78, PRO79, ILE90
Streptomycin	-8.8	ASN24 ARG47 GLY90 SER92	2.21 2.31 2.37 2.45	GLY22, LEU91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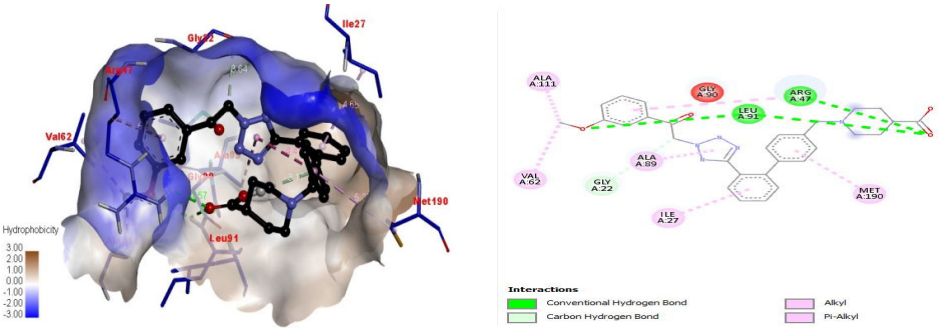
Pi-Alkyl

7a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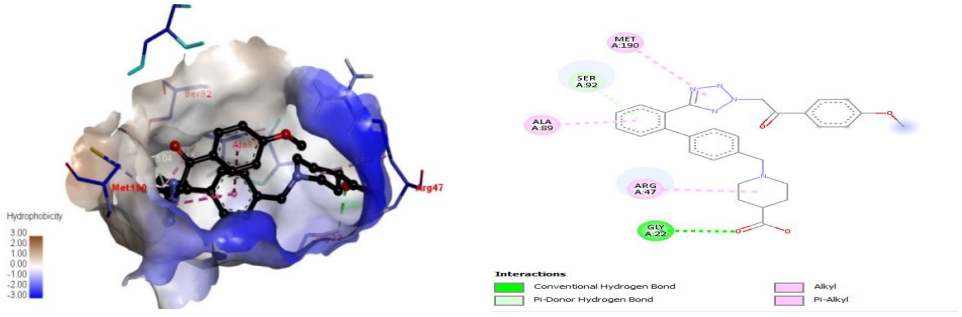
7b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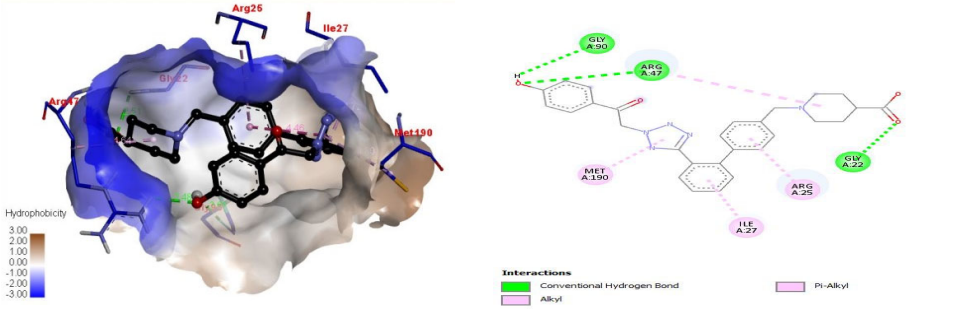
7c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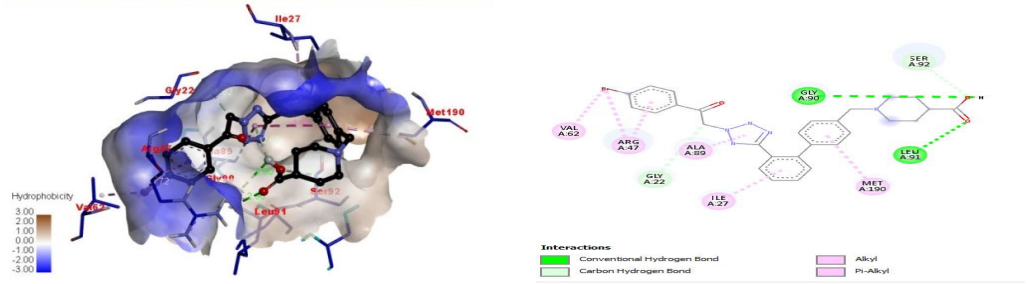

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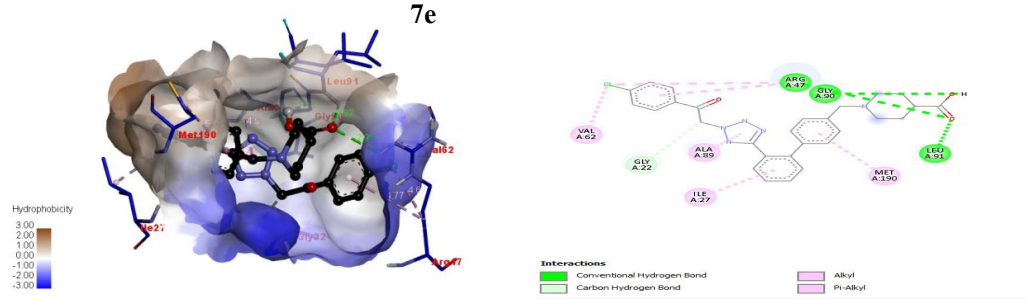

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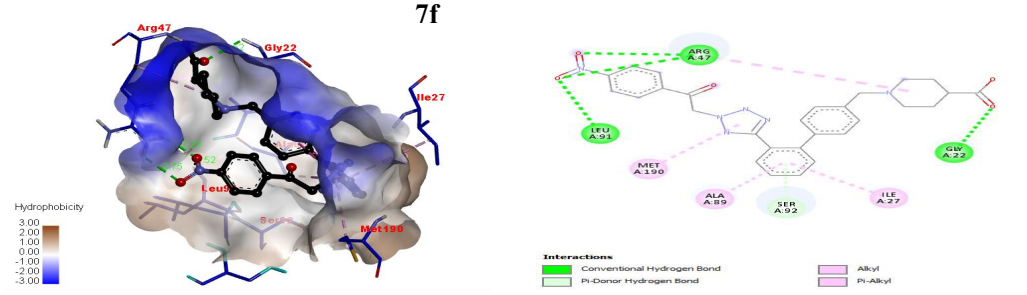

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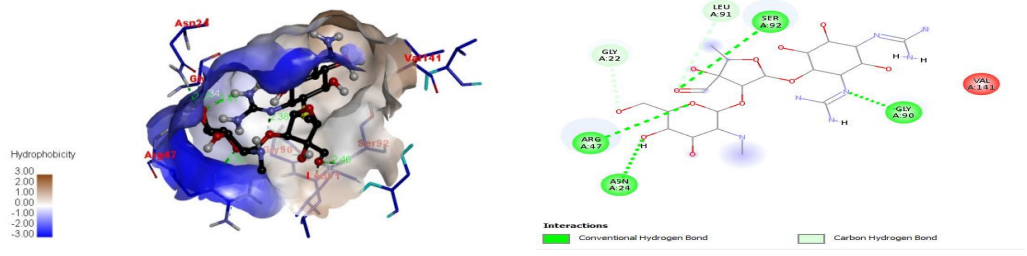
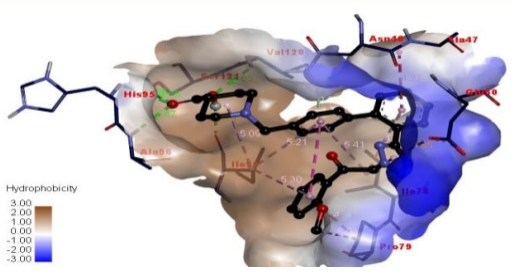


Streptomycin

Fig. 2. Three-dimensional and two-dimensional representations of molecular interactions between **3-oxoacyl-ACP reductase** and synthesized compounds (**7a-g**) and reference drug Streptomycin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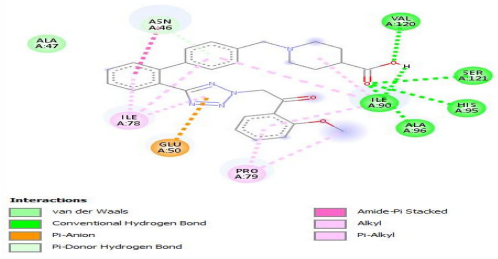
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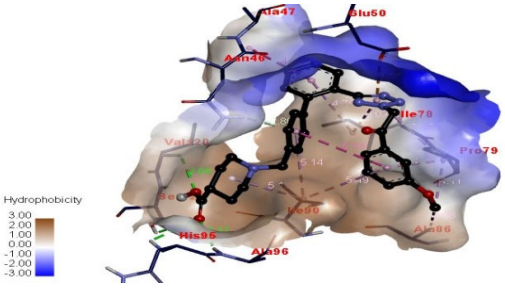
Pi-Alkyl

7a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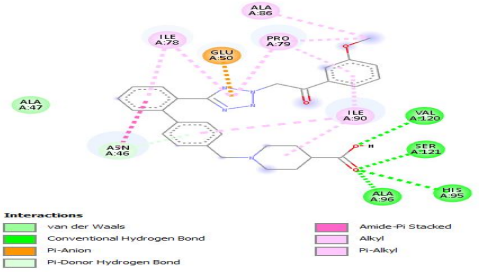
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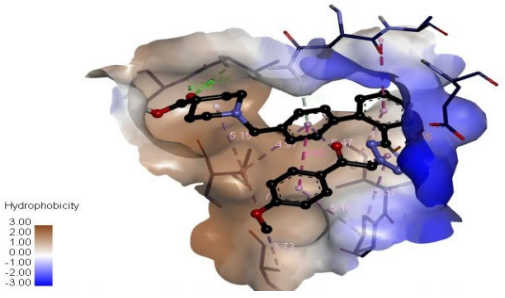
Conventional Hydrogen Bond
Pi-Anion
Pi-Donor Hydrogen Bond
Amide-Pi Stacked
Alkyl
Pi-Alkyl

7b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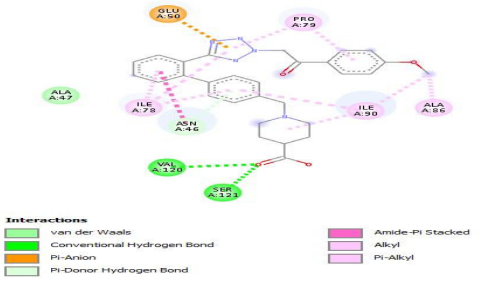
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van der Waals
Conventional Hydrogen Bond
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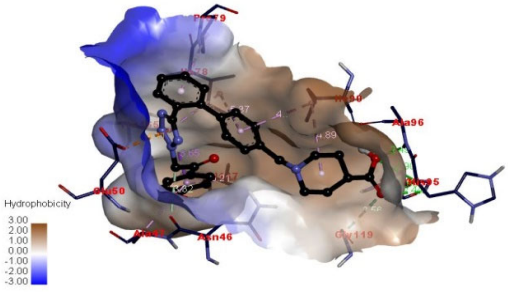
Pi-Donor Hydrogen Bond
Amide-Pi Stacked
Alkyl
Pi-Alkyl

7c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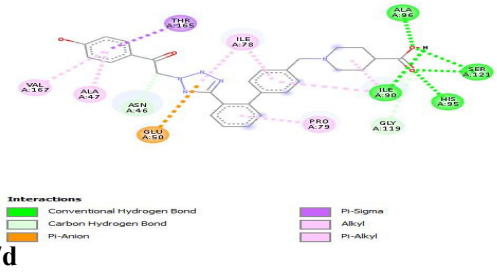
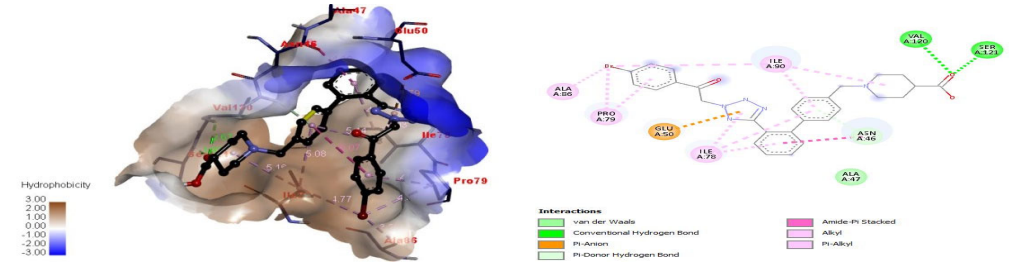
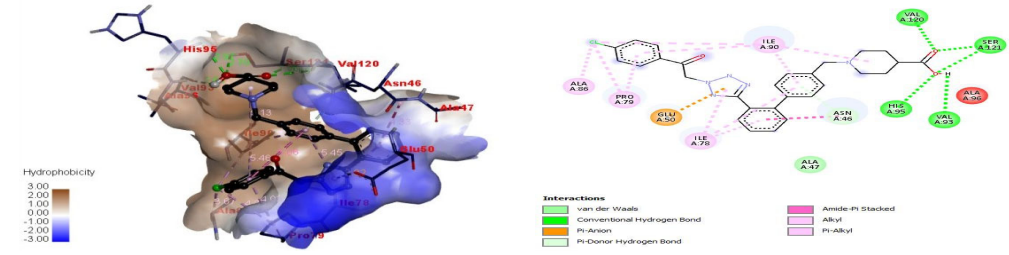

Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Anion
Pi-Sigma
Alkyl
Pi-Alkyl

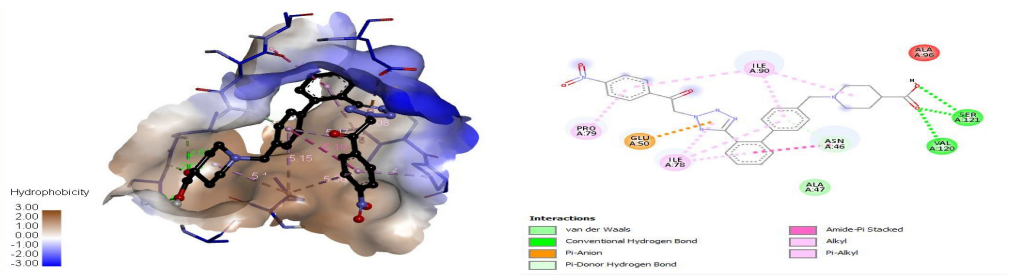
7d



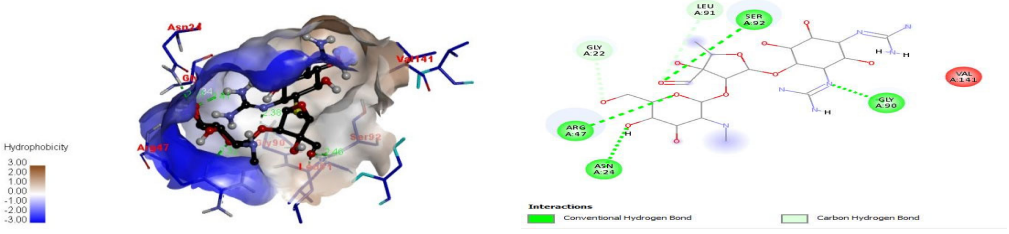
7e



7f



7g



Streptomycin

Fig. 3. Three-dimensional and two-dimensional representations of molecular interactions between DNA gyrase protein with synthesized compounds (**7a-g**) and reference drug Streptomycin

2.4. ADME studies

Physicochemical properties

The study of physicochemical properties is necessary for the design of new pharmacological compounds. A drug-likeness profile can be evaluated through these parameters of the molecule such as molecular weight, the number of heavy atoms, HBA, (hydrogen bond acceptors), HBD (hydrogen bond donors), rotatable bonds, molar refractivity and TPSA (Topological polar surface areas). These parameters were calculated for compounds (**7a-g**) and are tabulated in **Table 6**.

The drug-likeness profiles were calculated based on Lipinski's (MW ≤ 500; HBA ≤ 10 and HBD ≤ 5), Ghose (160 ≤ MW ≤ 480; 40 ≤ MR ≤ 130 and 20 ≤ atoms ≤ 70), Veber's (rotatable bonds ≤ 10 and TPSA ≤ 140), Egan (TPSA ≤ 131.6) and Muegge (200 ≤ MW ≤ 600; the number of aromatic rings ≤ 7; several rotatable bonds ≤ 15; HBA ≤ 10 and HBD ≤ 5).[28,29] The rule-based score defines the compounds into four probability score classes *i.e.* 11%, 17%, 55%, and 85%. The

acceptable probability score is 55% which indicates that it passed the rule of five.[30-31] All the synthesized compounds (**7a-g**) showed a score of 55% (except a **7g** score of 17%) indicating compounds obeyed all the five rules without any violations with good bioavailability. Further, the synthetic accessibility of the compounds was assessed to quantify the complexity of the molecular structure. The results showed that the score was in the range of 4.06-4.21 revealing that the compounds do not have a complex synthetic route (**Table 7**).

Table 6. Physicochemical properties of compounds **7**(**a-g**)

Compd	Formula	M.Wt	No. Heavy atoms	HBA	HBD	Rotatable bonds	Fraction Csp3	Molar Refractivity	TPSA
7a	$C_{29}H_{29}N_5O_4$	511.57	38	8	1	9	0.28	146.44	110.44
7b	$C_{29}H_{29}N_5O_4$	511.57	38	8	1	9	0.28	146.44	110.44
7c	$C_{29}H_{29}N_5O_4$	511.57	38	8	1	9	0.28	146.44	110.44
7d	$C_{28}H_{27}N_5O_4$	497.55	37	8	2	8	0.25	141.97	121.44
7e	$C_{28}H_{26}BrN_5O_3$	560.44	37	7	1	8	0.25	147.64	101.21
7f	$C_{28}H_{26}ClN_5O_3$	515.99	37	7	1	8	0.25	144.95	101.21
7g	$C_{28}H_{26}N_6O_5$	526.54	39	9	1	9	0.25	148.77	147.03

Table 7. Drug likeness, bioactivity and synthetic accessibility score

Compd	Lipinski	Ghose	Veber	Egan	Muegge	bioactivity Score	synthetic accessibility
7a	Yes	No	Yes	Yes	Yes	0.55	4.21
7b	Yes	No	Yes	Yes	Yes	0.55	4.21
7c	Yes	No	Yes	Yes	Yes	0.55	4.17
7d	Yes	No	Yes	Yes	Yes	0.55	4.08
7e	Yes	No	Yes	Yes	Yes	0.55	4.07
7f	Yes	No	Yes	Yes	Yes	0.55	4.06
7g	No	No	No	No	Yes	0.17	4.16

The mean predicted lipophilicity values were evaluated to decide whether the compounds were soluble in aqueous or non-aqueous media and they were calculated by considering the consensus log Po/w.[32] According to this, if a molecule is more soluble then its consensus log Po/w values will be more negative. The results of compounds **(7a-g)** showed that the compounds were not soluble in a non-aqueous medium. Consensus log S (if log S < −10: poorly soluble, < −6: moderately soluble, < −4: soluble, < −2: very soluble, and < 0: highly soluble) values indicated that, the compounds were moderately soluble in an aqueous medium (**Table 8**).

Table 8. Predicted absorption parameters of compounds (**7a-g**)

Compd	Consensus Log Po/w	Consensus	Solubility Class
7a	2.96	-4.42	Moderately soluble
7b	3.08	-4 .42	Moderately soluble
7c	3.20	-4.42	Moderately soluble
7d	2.87	-4.20	Moderately soluble
7e	3.94	-5.26	Moderately soluble
7f	3.88	-4.94	Moderately soluble
7g	2.62	-4.41	Moderately soluble

The pharmacokinetic parameters like absorption, skin permeation, distribution, metabolism and excretion were predicted. According to this predictive model, if a molecule falls in the white region indicates passive gastrointestinal absorption, whereas in the yellow region indicates passive brain permeation. Predicted distribution parameters of the compounds (**7a-g**) suggested that all the synthesized compounds (except **7g**) have high GI absorption. While all the compounds have no blood-brain permeate, hence there was no possibility of causing harmful toxicants in the brain and bloodstream. If the molecules have more negative log Kp value, it is said to be less skin permeate. The compounds (**7a-g**) have more negative log Kp values, therefore these compounds are the least skin permeate (**Table 9**).

Table 9. Predicted distribution parameters of the compounds

Compd	GI absorption	BBB permeant	Log Kp (cm/s)
7a	High	No	-7.67
7b	High	No	-7.67
7c	High	No	-7.67
7d	High	No	-7.82
7e	High	No	-7.45
7f	High	No	-7.23
7g	Low	No	-7.86

Metabolism plays an important role in the bioavailability of drugs as well as drug-drug interactions. Metabolism parameters are important to understanding whether the compounds acted as a substrate (inhibitor) or non-substrate (non-

inhibitor) of certain proteins. Hence, the compounds (**7a-g**) were evaluated for their metabolism parameters and the results revealed that all compounds are found to be substrates of permeability glycoprotein (P-gp). The P-gp is an important protein in assessing active efflux through biological membranes and cytochrome P450 (CYP) enzymes.[33]All the compounds (**7a-g**) were also found to be non-substrates of CYP1A2 inhibitor and substrates of CYP2C19 & CYP2C9 inhibitor. Compounds (**7a-d**) were found to be substrates of CYP2D6 and **7e-g** was found to be non-substrates of CYP2D6. **7a-c** and **7e-g** were found to be substrates of CYP3A4, compound **7d** non-substrates of CYP3A4 inhibitor (**Table 10)**.

Table 10. Predicted metabolism parameters of the compounds **7(a-g)**

Compd	P-gp	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
7a	Yes	No	Yes	Yes	Yes	Yes
7b	Yes	No	Yes	Yes	Yes	Yes
7c	Yes	No	Yes	Yes	Yes	Yes
7d	Yes	No	Yes	Yes	Yes	No
7e	Yes	No	Yes	Yes	No	Yes
7f	Yes	No	Yes	Yes	No	Yes
7g	Yes	No	Yes	Yes	No	Yes

The synthesized compounds (**7a-g**) were subjected to an *in-silico* toxicity evaluation. Toxicological endpoints results suggested that all the compounds were predicted to be non-hepatotoxicity, non-immunotoxicity, and non-cytotoxicity. Compounds **7(a-f)** were non-carcinogenic but the compound **7g** was carcinogenic. Moreover, **7a** and **7f** were non-mutagenic, and **7e** and **7g** were mutagenic in nature. The toxicity class ranges from 1 to 6 are Class I: fatal if swallowed ($LD50 \leq 5$) Class II: fatal if swallowed ($5 < LD50 \leq 50$) Class III: toxic if swallowed ($50 < LD50 \leq 300$) Class IV: harmful if swallowed ($300 < LD50 \leq 2000$) Class V: may be harmful if swallowed ($2000 < LD50 \leq 5000$), Class VI: non-toxic ($LD50 > 5000$). The predicted LD_{50}. [34.35] results suggested that the synthesized compounds were non-harmful if swallowed and belong to class IV (**Table 11**).

Table 11. The predicted activity of the compounds on toxicity endpoints and LD_{50} and the Toxicity class of the compounds

Compd	Hepatotoxicity	Carcinogencity	Immunotoxicity	Mutagencity	Cytotoxicity	Predicted LD_{50} (mg/kg)	Predicted Toxicity Class
7a	Inactive	Inactive	Inactive	Inactive	Inactive	1000	4
7b	Inactive	Inactive	Inactive	Inactive	Inactive	900	4
7c	Inactive	Inactive	Inactive	Inactive	Inactive	500	4
7d	Inactive	Inactive	Inactive	Inactive	Inactive	2000	4
7e	Inactive	Inactive	Inactive	Active	Inactive	1000	4
7f	Inactive	Inactive	Inactive	Inactive	Inactive	2000	4
7g	Inactive	Active	Inactive	Active	Inactive	2000	4

3. Conclusion

In summary, we have synthesised some 1-((2'-(2-(2-(substituted phenyl))-2-oxoethyl)-2*H*-tetrazol-5-yl)-1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid derivatives (**7a-g**) through a multistep reaction. The structures were confirmed by using different spectroscopic techniques. The antibacterial activity result showed that the compound **7g** exhibited the highest zone of inhibition and the remaining compounds exhibited moderate MIC values. Anti-mycobacterial results suggested that the compounds **7b**, **7f**, and **7g** exhibited very good sensitivity as compared to other compounds. *In silico* Molecular docking, results confirmed that all the molecules showed good binding energies in the range of -10.1 to -10.8 kcal/mol for anti-mycobacterial activity and -8.9 to-10.4 kcal/mol for antibacterial activity. The compounds **7g** and **7d** showed the least binding energies of -10.8kcal/mol and -10.4 kcal/mol on the targeted enzymes DNA gyrase and ***3-oxoacyl-ACP reductase*** protein respectively. *In silico* ADME-toxicology studies revealed that the synthesized compounds obeyed all the five rules with good bioavailability sparingly soluble in an aqueous medium. Compounds have no blood-brain permeate; hence there was no possibility of causing harmful toxicants in the brain and bloodstream. The predicted metabolism parameter suggested that all compounds are substrates of P-gp. Toxicological endpoints results suggested that all the compounds were predicted to be non-hepatotoxic, non-immune toxin, and non-cytotoxic in nature. Further, the predicted LD_{50} result suggested that the synthesized compounds were not harmful if swallowed.

Acknowledgments

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4. Experimental

4.1. Materials and methods

High-purity reagents, solvents, and chemicals purchased from Sigma-Aldrich were used for the synthesis without further purification. Alumina TLC plates were used to check the progress of the reaction using ethyl acetate: hexane (1:4) as a

mobile phase. Spots were identified by UV chamber. The melting points were determined by the electro-thermal apparatus using open capillary tubes and are uncorrected. FTIR spectra were recorded on a Bruker spectrophotometer using KBr pellets in the region of 400–4000 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded with the aid of a Bruker spectrometer at 400 MHz and 100 MHz respectively; chemical shifts (δ) were recorded in ppm relative to tetramethylsilane. The mass spectra of the compounds were confirmed by LC-MS 2010, SHIMADZU mass analyzer. Elemental analysis was calculated by using the unique elementary method.

4.2. Synthesis of ethyl 1-((2'-(2-trityl-2H-tetrazole-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylate (3)

In a 100 mL round bottom flask containing 20 mL dimethylformamide were added 2-(N-triphenylmethyltetrazolyl)-4-Bromo methyl biphenyl (TTBB) (**1)** (5 mmol) and ethyl piperidine-4-carboxylate (**2**) (5 mmol). Potassium carbonate (10 mmol) was added as a base to the reaction mixture and the resultant reaction mixture was refluxed for about 45 min. After completion of the reaction, the solvent was evaporated under reduced pressure to afford a solid compound (**3**).
Creamy solid, yield: 70%, M.P 185-187°C; ^1H NMR (400 MHz, DMSO-d6, δ ppm): 1.68 -1.72 (q, 2H, CH_2), 1.92-1.97 (q, 2H, CH_2), 2.88-2.94 (q, 2H, CH_2), 3.02-3.25 (q, 2H, CH_2), 3.303 (s, 3H, $COOCH_3$), 4.67 (s,1H,CH), 6.78-7.02 (dd, 5H, Ar-H), 7.23-7.25 (d, J=8.8,2H, Ar-H) 7.26-7.36 (m, 11H, Ar-H), 7.44-7.45 (d, J=8.8 Hz, 1H, Ar-H), 7.51-7.53 (d, *J*=8.8Hz, 1H, Ar-H), 7.49-7.58 (d, *J*=8.8 Hz, 1H, Ar-H), 7.78-7.80 (d, *J*=8.8 Hz, 1H, Ar-H), 8.20-8.60 (s, 2H, Ar-H), ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 24.45, 34.06, 37.55, 38.87, (C-CH2), 60.23, 61.140,(C-$COOCH_3$), 82.28(C-CH), 125.62, 130.22, 130.47, 130.57, 136.23, 136.24, 140.69, 140.97 (C=C), 163.34, (C-CO), 173.06 (C-C=O); LCMS: m/z 634.66 [M^+]. Anal.Calcd. for $C_{40}H_{37}N_5O_3$: C 75.57%, H 5.87 %, N 11.02%. Found: C71.57%, H4.82% and N 9.39 %.

4.2.1. Synthesis of ethyl 1-((2'-(2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylate (4)

The compound (**3**) (5 mmol) was dissolved in methanol (20 mL) were added then 4N HCl (5mL) and refluxed for about 4 h. The obtained precipitate was dissolved in dichloromethane and separated using a rotary evaporator to get a solid compound (**4**). White solid, yield: 76 %, M.P 155-157 °C; ^1H-NMR (400 MHz, DMSO-*d_6,* δ ppm): 1.17-1.20 (t, 2H, CH_2), 1.66-1.69 (q, 2H, CH_2), 1.95-1.97 (d, *J*=8 Hz, 2H, CH_2), 2.95-2.97 (d, *J*=8 Hz, 2H, CH_2), 3.23 (s, 3H, CH_3), 4.06-4.11 (q, 2H, CH_2), 4.79 (s, 3H, CH_2), 4.85 (s, 1H, CH), 7.55-7.65 (m, 4H, Ar-H), 7.78-7.82 (t, 2H, Ar-H), 7.95-9.97 (d, *J*=8 Hz, 2H, Ar-H), 8.50 (s,1H, OH), and ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 24.41, 33.79, 37.54, 37.79, 38.87 (C-CH_2), 60.21 (C-CH_3), 110.06, 110.09, 118.43, 128.30, 128.94, 128.97, 129.13, 129.58, 130.03, 138.11, 138.52, 137.62, 137.67, 133.80, 133.49, (C=C), 143.88, 143.82, 173.03 (C=O). LCMS: m/z 391.22 [M^+].Anal.Calcd.for$C_{22}H_{25}N_5O_2$: C, 67.50%, H 6.44%; N 17.89. Found: C, 65.45%, H14.20%, N 7.39 %.

4.2.2. Synthesis of 1-((2'-(2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (5)

The intermediate compound (**4**) (5 mmol) was taken in 20 mL methanol and 20% sodium hydroxide (5ml) was added. The resultant reaction mixture was refluxed for about 4h. The resultant product was dissolved in DCM and separated under reduced pressure to get a creamy white-coloured solid compound (**5**). Yellow solid, yield-79%, M.P 203-207°C; ^1H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.54-1.62 (q, 2H, CH_2), 1.79-1.81 (d, *J*=8Hz, 2H, CH_2), 2.12- 2.73 (d, *J*=8Hz, 2H, CH_2), 7.04-7.05 (d, *J*=8Hz, 2H Ar-H), 7.11-7.15 (d, *J*=8Hz, 1H, Ar-H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 27.30, 38.87, 40.13, 51.96 (C-CH_2), 61.24 (C-$COOCH_3$) 126.88, 128.18, 128.63, 128.93, 130.18, 130.57, 140.39, 140.46, (C=C), 159.67 (C-CO), 175.88 (C-C=O). LCMS: m/z 363 [M^+]. Anal.Calcd. for $C_{20}H_{21}N_5O_2$: C66.10%, H5.82%, and N19.27, Found: C 65.45%, H 4.20%, N 10.39%.

4.2.3. Synthesis of 1-((2'-(2-(2-(4-substitutedphenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7a-g)

The intermediate compound (**5**) (5 mmol) was dissolved in 20 mL dimethyl formamide and substituted phenacyl bromides (**6a-g)** (5mmol) was added in the presence of potassium carbonate (10mmol). The resultant reaction mixture was refluxed for about 1 h, and the precipitate separated was filtered and dried using dichloromethane to get a fine-coloured compound (**7a-g**).

4.2.4.1-((2'-(2-(2-(4-Hydroxyphenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7a)

White solid, yield 79%, M.P 176-178^{0}C; FTIR (KBr υ cm^{-1}): 3456 (OH), 2934 (CH), 1676 (C=O), 1574 (C=N), 1514(C=C), 1281(C-O); ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 1.74 (s, 2H, CH_2), 2.00 (s, 2H, CH_2), 2.89-2.91 (d, *J*= 8 Hz, 2H, CH_2), 3.16 (s, 2H, CH_2), 4.24 -4.26 (d, *J*= 8 Hz, 2H, CH_2), 4.80 (s, 2H, CH_2), 5.05 (s, 1H, CH), 6.86 - 6.88 (d, *J*= 8 Hz, 3H, Ar-H), 7.23-7.27 (t, *J*= 16 Hz, 2H, Ar-H),7.44-7.46 (d, *J*= 8 Hz, 2H, Ar-H), 7.53-7.71 (m, 2H, Ar-H), 7.82-7.85 (d, *J*= 12 Hz, 3H, Ar-H), 10.65 (s, 1H, OH), 12.50 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm). 29.13, 29.14, 41.12, 51.75, 60.60, 62.75, 115.42, 115.58, 127.01, 127.88, 128.16, 128.73, 128.93, 129.00, 129.35, 129.50, 129.64, 129.14, 129.94, 130.02, 134.20, 135.19, 135.63, 135.97, 163.22, 163.31, 180.54 and 189.67 LCMS: m/z 497.55[M^+]. Anal.Calcd. For $C_{28}H_{27}N_5O_4$: C 68.28%, H 5.71%, N 13.69%, Found: C 68.23%, H 5.51%, N 12.69%

4.2.5.1-((2'-(2-(2-(3-Methoxyphenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7b)

Pale cream solid, yield 81%, M.P 174-176^{0}C; FTIR (KBr υ cm^{-1}): 3483 OH, 2937(OCH_3), 2841 (CH), 1683 (C=O), 1574 (C=N), 1309(C-O); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.68 (s, 2H, CH_2), 1.88-1.90 (d, *J*= 8 Hz, 2H, CH_2), 2.36 (s, 2H, CH_2), 2.97 (s, 3H, OCH_3), 3.16 (s, 2H, CH_2), 3.81-3.84 (t, *J*= 12 Hz, 4H, CH_2), 5.20 (s, 1H, CH), 7.10-8.13 (m, 12H, Ar-H), 12.32 (s, 1H, OH) and ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm) :29.130, 29.144, 41.712, 51.121, 51.410, 55.841, 60.510, 62.556, 113.421, 118.780, 127.560, 127.637, 128.435, 128.209, 128.751, 129.506, 129.649, 129.828, 135.003, 135.369, 135.666, 137.910, 160.621, 163.544, 181.452, 191.169. LCMS: m/z 511.57 [M$^+$]. Anal.Calcd.for $C_{29}H_{29}N_5O_{45}$: C, 68.09 %; H, 5.71%; N, 13.69%. Found: C, 68.02 %; H, 5.66%; N, 13.56%.

4.2.6.1-((2'-(2-(2-(4-Methoxyphenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7c)

Yellow solid, yield 78%, M.P 179-180^{0}C; FTIR (KBr υ cm^{-1}): 3413 (OH), 2985 (OCH_3), 1694 (C=O), 1627 (C=N), 1573 (C=C), 1260 (C-O). ^{1}H-NMR (400 MHz, DMSO-*d_6*, δ ppm): 1.62 (s, 2H, CH_2), 1.83 (s, 2H, CH_2), 2.25-2.27 (d, *J*= 8 Hz, 2H, CH_2), 2.87-2.89 (d, *J*= 8 Hz, 2H, CH_2), 3.16 (s, 3H, OCH_3), 3.66 (s, 2H, CH_2), 3.85-3.87(d, *J* = 8 Hz, 2H, CH_2), 5.11 (s, 1H, CH), 7.10-7.12 (d, *J* = 8 Hz, 4H, Ar-H), 7.25-7.27 (d, *J* = 8 Hz, 2H, Ar-H),7.45-7.84 (m, 4H, Ar-H), 8.00-8.02 (d, *J*= 8 Hz, 2H, Ar-H), 12.04 (s, 1H, OH).^{13}C-NMR (100 MHz, DMSO-*d_6*, δ ppm). 29.148, 29.231, 41.122, 52.556, 52.921, 55.654, 60.918, 62.524, 114.092, 121.231, 121.867, 126.474, 127.089, 127.685, 128.068, 128.133, 128.867, 129.229, 129.505, 129.728, 134.412, 134.611, 135.428, 235.611, 135.863, 160.610, 164.631, 180.094, 189.128, LCMS: m/z 511.57 [M$^+$]. Anal.Calcd.for $C_{29}H_{29}N_5O_4$: C 68.09 %, H 5.71%, N 13.69%. Found: C 67.99%, H 5.68%, N 13.01%.

4.2.7. 1-((2'-(2-(2-(2-Methoxyphenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7d)

Pale yellow solid; yield 82%; M.P 178-180^{0}C; FTIR (KBr υ cm^{-1}): OH (3456), 2937 (OCH_3), 2811 (CH), 1683 (C=O), 1634 (C=N), 1601 (C=C), 1239 (C-O); ^{1}H NMR (400 MHz, DMSO-*d_6*, δ ppm): 1.60-1.69 (d, *J* = 8 Hz, 2H, CH_2), 1.82-1.84 (d, *J* = 8 Hz, 2H, CH_2), 2.24-2.26 (d, *J*= 8 Hz, 2H, CH_2), 2.85-2.87 (d, *J* = 8 Hz, 2H, CH_2), 3.16 (s, 2H, CH_2), 3.55-3.59(d, *J* = 16 Hz, 3H, OCH_3), 3.91-3.93 (d, *J* = 8 Hz, 2H, CH_2), 4.98 (s, 1H, CH), 7.74-7.01 (m, 12H, Ar-H), 12.24 (s, 1H, OH).^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 29.130, 29.144, 41.122, 51.758, 60.600, 62.756, 115.421, 115.580, 127.018, 127.887, 128.160, 128.737, 128.935, 129.009, 129.351, 129.506, 129.649, 129.949, 130.028, 134.203, 135.199, 135.199, 135.636, 135.636, 135.970, 162.221, 163.314, 180.542, 189.679. LCMS: m/z 511.57 [M$^+$]. Anal.Calcd.for $C_{29}H_{29}N_5O_4$: C 67.59%, H 5.47%, N 14.08%, Found: C, 67.89%, H 5.60%, N 12.98 %.

4.2.8.1-((2'-(2-(2-(4-Fluorophenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7e)

Silky white solid, yield 76%, M.P 176-178^{0}C; FT-IR (KBr υ cm^{-1}): 3431 (OH), 1721 (C=O), 1583 (C=N), 1574 (C=C), 1281 (C-O); ^{1}H NMR (400 MHz, DMSO-*d_6*, δ ppm): 1.65-1.67 (d, *J* = 8 Hz, 2H, CH_2), 1.76 (s, 2H, CH_2), 2.33 (s, 2H, CH_2), 2.94 (s, 2H, CH_2), 3.16 (s, 2H, CH_2), 3.77 (s, 2H, CH_2), 5.29 (s, 1H, CH), 7.11-7.13 (d, *J* = 8 Hz, 2H, Ar-H), 7.29-7.31 (d, *J* = 8 Hz, 2H, Ar-H), 7.49-7.79 (m, 4H, Ar-H), 8.19-8.21 (d, *J* = 8 Hz, 2H, Ar-H), 8.34-8.36 (d, *J* = 16 Hz, 2H, Ar-H), 12.20 (s, 1H, OH) and ^{13}C NMR (100 MHz, DMSO-*d_6*, δ ppm): 27.130, 27.144, 37.878, 39.082, 40.130, 40.122, 51.207, 51.747, 59.600, 62.756, 126.660, 128.137, 128.232, 128.401, 128.651, 129.497, 129.649, 129.827, 130.201, 134.496, 134.634, 135.370, 135.690, 135.970, 138.201, 162.314, 180.541, 189.670. LCMS: m/z 499.54 [M$^+$]. Anal.Calcd.for $C_{28}H_{26}N_5O_3$: C, 69.98%; H, 5.45%; N, 14.57%. Found: C, 65.45% H, 4.20% N, 10.39%.

4.2.9.1-((2'-(2-(2-(4-Chlorophenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7f)

Pale orange solid, yield 81%, M.P. 177-178^{0}C; FTIR (KBr υ cm^{-1}): 1789,1694 (C=O), 1614 (C=N),1574 (C=C), 1247 (C-O), 832 (C-Cl); ^{1}H-NMR (400 MHz, DMSO-*d_6*, δ ppm): 1.65-1.67 (d, *J* = 8 Hz, 2H, CH_2), 1.76 (s, 2H, CH_2), 2.33 (s, 2H, CH_2), 2.89 (s, 2H, CH_2), 3.16 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 5.29 (s, 1H, CH), 7.13-7.15 (d, *J* = 8 Hz, 2H, Ar-H), 7.54-7.57 (t, *J* = 12 Hz, 2H, Ar-H), 7.59-7.79 (m, 2H, Ar-H), 8.08-8.10 (d, *J* = 8 Hz, 2H, Ar-H), 8.19-8.21 (d, *J* = 8 Hz, 2H, Ar-H), 8.25-8.48 (m, 2H, Ar-H), 12.22 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-*d_6*, δ ppm): 29.130, 38.878, 39.082, 40.130, 41.122, 51.211, 51.758, 60.600, 62.756, 127.660, 128.137, 128.235, 128.409, 129.497, 128.651, 128.864, 129.649, 129.828, 131.203, 134.499, 134.636, 135.370, 135.700, 135.973, 139.201, 163.314, 180.542, 189.679. LCMS: m/z 515.99 [M$^+$]. Anal.Calcd.for$C_{28}H_{26}N_5O_3$: C, 65.18%H, 5.08% N, 13.57%. Cl, 87% Found: C, 65.45%H, 4.20% N, 10.39%.

4.2.10. 1-((2'-(2-(2-(4-Nitrophenyl)-2-oxoethyl)-2H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)piperidine-4-carboxylic acid (7g)

Yellow solid, yield 80%, M.P 179-181 ^{0}C; FTIR (KBr υ cm^{-1}): 3456 (OH), 2937 (C=O), 1599 (C=N), 1319 (C-O); ^{1}H NMR (400 MHz, DMSO-*d_6*, δ ppm): 1.80 (s, 2H, CH_2), 2.33 (s, 2H, CH_2), 2.67 (s, 2H, CH_2),2.98-3.00 (d, *J*= 8 Hz, 2H,

CH_2),3.16 (s, 2H, CH_2), 4.30-3.32 (d, *J* = 8 Hz, 2H, CH_2), 5.04 (s, 1H, CH), 7.24-7.26 (d, *J* = 8 Hz, 1H, Ar-H), 7.45-7.61 (m, 3H, Ar-H), 7.69-7.81 (m, 2H, Ar-H), 8.11-8.33 (m, 3H, Ar-H), 8.36-8.38 (d, *J* = 8 Hz, 3H, Ar-H),12.60 (s, 1H, OH). ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 29.111, 29.234, 41.724, 51.151, 51.439, 60.531, 62.576, 127.221, 127.410, 127.560, 128.435, 128.509, 129.351, 129.526, 129.643, 129.732, 129.808, 134.503, 135.369, 135.666, 135.810, 142.910, 152.621, 163.521, 180.132, 190.459. LCMS: m/z 526.54 [M^+]. Anal.Calcd.for $C_{28}H_{26}N_6O_5$: C, 63.87%; H, 4.98%; N, 15.96. Found: C, 65.45%H, 4.20%N, 10.39%.

4.3. Biological studies

4.3.1. Antibacterial activity

The antibacterial activities of the synthesized compounds **7**(**a-g**) were evaluated using against a panel of Gram-positive and Gram-negative bacterial strains namely *Staphylococcus aureus* (MTCC 7443), *Bacillus substiles* (MTCC 121), *Escherichia coli* (MTCC 7410) and *Shigella* (MTCC 1457) at different concentrations. Zone of bacterial growth inhibition and minimum inhibitory concentration (MIC) by compounds were determined against selected bacteria using the Kirby-Bauer disc diffusion method.[36] The stock solution (800µg/mL) was prepared by dissolving the compounds in dimethyl sulphoxide (DMSO). A loop of the bacterial strain was suspended in autoclaved distilled water the turbidity of this solution was set to that of 0.5 McFarland standards.[37,38]About 100 µL of this suspension was applied uniformly on the surface of the nutrient agar plate before placing the antimicrobial assay discs on the plate (5 per plate) and different volumes i.e., 3, 6, 9, and 12 µL of test compounds were loaded on the discs and the last one was loaded with control DMSO. The plates were incubated for 24 h at 37ºC. Then, the average zone of inhibition was measured with a ruler with the least count in mm. For the determination of minimum inhibitory concentration (MIC), all the tubes were incubated at 37ºC for 16 hours and the bacterial test was compared with the standard drug Streptomycin.

4.3.2. Anti-tubercular activity

The anti-mycobacterial activity of compounds was assessed against *M. tuberculosis* using the microplate Alamar Blue assay (MABA) method. This methodology is non-toxic, uses thermally stable reagents, and shows a good correlation with proportional and BACTEC radiometric methods. Briefly, 200µL of sterile deionised water was added to the outer perimeter wells of the sterile 96 well plates to minimize evaporation of medium in the test wells during incubation. The 96 well plates received 100 µL of the Middle brook 7H9 broth and serial dilution of compounds was made directly on a plate.[39] The final drug concentrations tested were 0.2 to 100 µg/mL and plates were covered and sealed with Parafilm and incubated at 37ºC for five days. After this time, 25µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% between 80 was added to the plate and incubated for 24 h. Compounds at eight different concentrations (0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 & 100 µg/mL) were used for the analysis. Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, and Streptomycin were used as standard drugs for comparison.[40] The blue color in the well was interpreted as no bacterial growth, and the pink color was scored as growth. The MIC was defined as the lowest drug concentration which prevented the color change from blue to pink.

4.3.3. in silico Molecular docking study

in-silico molecular docking is a computer technique that is frequently used to predict the binding orientation of small molecule drug candidates to protein targets in order to predict the affinity and activity of the small molecule.[41]

Protein preparation

The crystal structure of E.coil 24k Da domine in complex with clorobiocin - DNA Gyrase Subunit B Protein (PDB ID:1KZN) and The crystal structure of MabA from Mycobacterium tuberculosis 3-oxoacyl-ACP reductase (PDB ID: 1UZL) was downloaded from the protein database (www.rcsb.org.) Before protein preparation, the inhibitors, other ligands, and water molecules were deleted from the protein to obtain clean protein. Polar hydrogen atoms with Gasteiger-Huckel charges were added to the protein before docking. The protein (PDB:1KZN) center of the grid box was set to 45, 45 & 45, and the number of points in x, y & z dimensions to 21.265, 19.166 & 44.255 °A respectively. The protein (PDB: 1UZL) center of the grid box was set to 45, 45 & 45, and the number of points in x, y & z dimensions to 10.053, 12.973 & 11.126 A.

Ligand preparation

For the 2D orientation of the newly synthesized compounds, the Marvin Sketch tool was used and then the compounds were converted to energy-minimizing 3D structures and Gasteiger charges. nonpolar hydrogen atoms and the rotatable bonds were set by using AutoDock 4.2 tools. Auto dock Vina[42] was used for docking purposes and during the docking process, a maximum of 10 conformers were considered for each compound. The ligand with the receptor and binding interactions was visualized by discovery studio.

4.3.4. insilico Oral bioavailability assessment and ADME-toxicology studies

Various physicochemical features and pharmacokinetic descriptors were calculated through the online web tool Swiss ADME.[43] Whereas, *in silico* toxicity, evaluation was carried out using an online server ProTox-II.[44] which gives predicted oral toxicity, cytotoxicity, mutagenicity,carcinogenicity, hepatotoxicity, and immune toxicity values for synthesized compounds (**7a-g**).

Supporting information

This includes spectra of the synthesized compounds (IR, ^{1}H NMR, ^{13}C-NMR, and Mass) as supplementary files S_1-S_{37}.

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Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

Author's contributions

Megha G V: Methodology, Investigation, Writing - original draft. **Yadav D. Bodke:** Investigation, Supervision, Writing - review & editing. **Shanavaz H:** Contributed Docking studies.

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