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Design and Synthesis of Some 1,3,4-Thiadiazole Amines: Molecular Docking, *in silico* ADMET, *in vitro* Antimicrobials and Antioxidant Studies

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An increase in free radical concentration in the human body due to medications leads to oxidative stress can be counteracted by novel antioxidative agents that lower the concentration of free radical and free radical damages in human body. In present study, 2-thiophene 4-thiadiazole quinoline derivatives (**5a-e**) were designed and synthesized using 2-thiophene quinoline 4-carboxylic acids and thiosemicarbazide. The designed compounds **5a-e** were docked against the protein PDB-ID: 1OC3 and evaluated the antioxidant activity using DPPH assay and also screened for antibacterial and antifungal potential by Agar well diffusion assay followed by *in vitro* antitubercular assay by MABA method. The IC_{50} values for compounds **5a** and **5b** were 415 µg/mL and 396 µg/mL. The binding affinity of docked ligands against the protein 1OC3 ranges from -6.2 to -5.7 kcal/mol. In an antimicrobial investigation, the compounds were found to be active against both bacteria and fungi, as well as sensitive to *M. tuberculosis*.

Keywords: 1,3,4-Thiadiazole amines, Quinoline, 1OC3, DPPH assay, MABA.

INTRODUCTION

Antimicrobial drug discovery is the continuous process to treat the human body from the infections caused by the microbes. Microbes that grow uncontrollably and rapidly in the human body can cause serious health problems which would include death/disorder [1]. As scientists around the world are more concerned about the human lifespan, preventing the harmful effects of microbes is becoming an important aspect. The increase in reactive oxygen species or free radicals concentration is a serious side effect of antimicrobial drugs used to treat infections in human body [2]. These unstable and highly reactive free radicals are involved in the oxidation of lipids, DNA and proteins that could cause degenerative diseases [3] and can be deactivated by antioxidants [4].

Antioxidants serve at a relatively low concentration and so have a different physiological function in human body as a protective component in foods and as an inhibitor during oxidation [5] and thus, protect the body as free radical scavengers by the enzymes; catalase, cytochrome P_{450}, peroxidase and glutathione [6]. Both natural and food additives acting as antioxidants have similar mechanisms, irrespective of their source [7]. Literature survey indicated that the 1,3,4-thiadiazole ring system has played an important role in exhibiting antioxidant and antimicrobial potential [8]. Hence, the discovery and development of antimicrobials with antioxidant properties are necessary so that the discovered drug candidate can be treated for infections with the lower side effect of free radical generation in the human body. Quinoline ring is part of many drugs used in the treatment of infectious diseases, hence in this present study, we have designed and synthesized five derivatives of quinoline bridged thiophene with 1,3,4-thiadiazole ring system and investigated for different antimicrobial properties such as antibacterial, antifungal and antitubercular and simultaneously antioxidant property.

EXPERIMENTAL

Chemicals used for synthesis were from Spectrochem Pvt, Ltd. and Alfa Aesar. The solvents used for synthesis were distilled and of reagent quality. Infrared spectra was obtained by Bruker

spectrophotometer using the KBr pellet method. Agilent spectrometer is used with deuterated DMSO solvent and internal standard TMS to study ^{1}H and ^{13}C NMR spectra. Merck silica gel (100-200 mesh) was used for chromatographic purification.

***In silico* studies:** The *in silico* ADMET parameter of the designed molecules were predicted by the online tool, admetSAR [9] and the drug-likeness and bioactive scores were predicted by the online tool, Molinspiration cheminformatics [10]. The 2D structural models of designed molecules were drawn on ACD/ChemSketch software and SMILES were generated for the molecules along with standard drugs, these smiles notations were used for predicting the individual ADMET, bioactive and drug-likeness scores. AdmetSAR gives data for the evaluation of active molecules and also for the removal of biologically defective major molecules with unwanted functional groups. The overall analysis of the significant molecules involves geometry, surface area and fingerprint properties, which determine the biological significance of the region in a molecule. Caco-2 cell permeability, intestinal absorption, water solubility, hepatotoxicity, blood-brain barrier penetration were the other parameter that helps to understand the metabolic drug mechanism of the designed molecules.

Molecular docking: The 3D X-ray crystal structure of oxidoreductase protein 'HUMAN PEROXIREDOXIN 5' was obtained from the Protein Data Bank (PDB ID: 1OC3) webserver. Preparations of ligands and protein were done by the Chimera docking tool here the energy minimization and merging of non-polar hydrogen were done with this tool. Resolution: 2.64 Å and minimization of energy and application of force field carried out. The ligand and protein were docked using the PyRx software by selection of binding pocket to hit ligands grid was constructed center-x = 15.0415, center-y = 7.6265, center-z = 18.4345, size-x = 32.4215, size-y = 27.0126, size-z = 29.2619. The docked ligand and target protein was analyzed by using Discovery studio 2020 [11,12].

Synthesis of 2-(thiophen-2-yl)quinoline-4-carboxylic acid and its derivatives 3a-e: 2-Acetyl thiophene (0.01 mol) and substituted isatins **2a-e** (0.01 mol) were reacted inside a round bottom flask with ethanol and to this catalytic amount of 33% KOH was added and kept for reflux for 8 h at 75 ºC the progress of the reaction was monitored by TLC technique (Mobile phase: 30% ethyl acetate in *n*-hexane). After complete reflux, allow to cool and pour onto crushed ice slowly and neutralized by HCl (**Scheme-I**). The separated solid was filtered, dried and crystallized by acetonitrile [13].

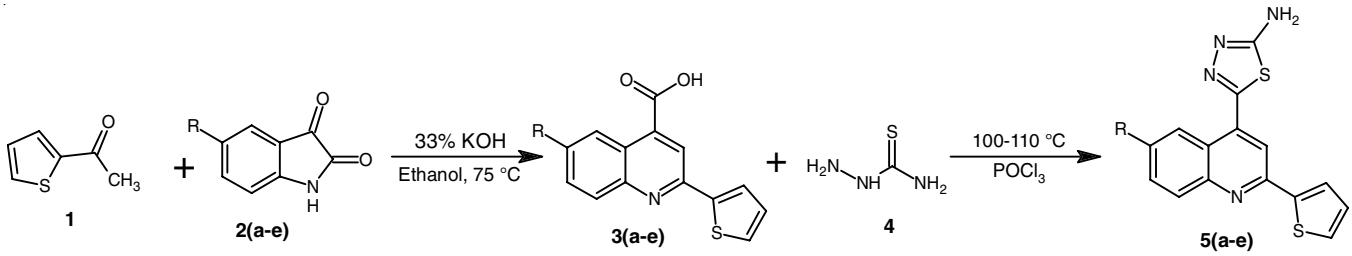
Synthesis of 5-[2-(thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine and its derivatives 5a-e: The obtained derivatives **3a-e** (0.01 mol) and thiosemicarbazide (0.01 mol) were reacted inside a round bottom flask with 5 mL of $POCl_3$ and kept for reflux for 8 h at 100-110 ºC the progress of the reaction was monitored by TLC technique (Mobile phase: 20% ethyl acetate in *n*-hexane). After complete reflux, allow to cool and pour onto crushed ice slowly and neutralize by sodium bicarbonate solution. The separated solid was filtered, dried and crystallized by acetonitrile.

5-[2-(Thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine (5a): m.p.: 142-146 ºC; Yield: 67%; IR (KBr, ν_{max}, cm^{-1}): 3354 (C-NH), 3309 (N-H), 1668 (N=C), 1644 (C=C); ^{1}H NMR (400 MHz) δ (ppm): 5.795 (s, 2H, $-NH_2$), 7.587 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.768 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.921 (d, 1H, *J* = 8 Hz, Ar-H), 8.031 (d, 2H, *J* = 8.4 Hz, Ar-H), 8.652 (s, 1H, Ar-H). ^{13}C NMR (100 MHz) δ ppm: 109.740, 111.859, 112.824, 116.318, 123.868, 124.243, 131.609, 140.440, 145.679, 146.227, 148.239, 152.133, 164.964, 170.623, 173.530; MS: *m/z* = 311.01 (M+1).

5-[6-Chloro-2-(thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine (5b): m.p.: 148-152 ºC; Yield: 72%; IR (KBr, ν_{max}, cm^{-1}): 3362 (C-NH), 3322 (N-H), 1661 (N=C), 1653 (C=C), 785 (C-Cl); ^{1}H NMR (400 MHz) δ (ppm): 5.647 (s, 2H, $-NH_2$), 7.214 (t, 1H, *J* = 7.4 Hz, Ar-H), 7.787 (d, 1H, *J* = 4.4 Hz, Ar-H), 7.846 (d, 2H, *J* = 7.6 Hz, Ar-H), 8.051 (d, 1H, *J* = 4.4 Hz, Ar-H), 8.290 (s, 1H, Ar-H), 8.371 (d, 1H, *J* = 2.4 Hz, Ar-H). ^{13}C NMR (100 MHz) δ ppm: 122.834, 124.556, 124.556, 124.819, 125.402, 127.942, 128.477, 128.798, 129.148, 130.860, 131.094, 131.580, 131.960, 132.086, 135.544; MS: *m/z* = 345.05 (M+1).

5-[6-Fluoro-2-(thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine (5c): m.p.: 149-153 ºC; Yield: 68%; IR (KBr, ν_{max}, cm^{-1}): 3373 (C-NH), 3312 (N-H), 1656 (N=C), 1639 (C=C), 1006 (C-F); ^{1}H NMR (400 MHz) δ (ppm): 5.580 (s, 2H, $-NH_2$), 7.229 (d, 1H, *J* = 4.8 Hz, Ar-H), 7.8079 (t, 1H, *J* = 6.8 Hz, Ar-H), 8.108 (d, 2H, *J* = 3.6 Hz, Ar-H), 8.240 (d, 1H, *J* = 3.6 Hz, Ar-H), 8.475 (s, 1H, Ar-H), 8.555 (d, 1H, *J* = 5.6 Hz, Ar-H). ^{13}C NMR (100 MHz) δ ppm: 118.764, 118.861, 119.902, 120.992, 124.096, 128.513, 128.688, 129.048, 129.203, 130.672, 131.422, 131.927, 132.453, 134.310, 151.755; MS: *m/z* = 328.94 (M+1).

5-[6-Nitro-2-(thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine (5d): m.p.: 120-122 ºC; Yield: 62%; IR (KBr, ν_{max}, cm^{-1}): 3368 (C-NH), 3328 (N-H), 1657 (N=C), 1647 (C=C), 1516 ($-NO_2$); ^{1}H NMR (400 MHz) δ ppm: 5.947 (s, 2H, $-NH_2$), 7.284 (d, 1H, *J* = 4.8 Hz, Ar-H), 7.655 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.914 (s, 1H, Ar-H), 8.247 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.587 (d, 1H, *J* = 8 Hz, Ar-H) 8.753 (s, 1H, Ar-H). ^{13}C NMR (100 MHz) δ ppm: 129.062, 129.364, 131.650, 132.402, 136.340,



Scheme-I: Synthesis of 5-[2-(thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine and its derivatives

138.482, 138.892, 139.317, 139.531, 139.949, 140.153, 140.367, 140.572, 143.341, 149.544; MS: *m/z* = 356.23 (M+1).

5-[6-Bromo-2-(thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine (5e): m.p.: 98-100 ºC; Yield: 70%; IR (KBr, ν_{max}, cm^{-1}): 3355 (C-NH), 3346 (N-H), 1664 (N=C), 1646 (C=C), 658 (C-Br); ^{1}H NMR (400 MHz) δ ppm: 5.786 (s, 2H, -NH_2), 7.225 (d, 2H, *J* = 6.9 Hz, Ar-H), 7.605 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.660 (s, 1H, Ar-H), 8.122 (d, 1H, *J* = 5.7, Ar-H), 8.226 (s, 1H, Ar-H), 8.372 (d, 1H, *J* = 7 Hz, Ar-H). ^{13}C NMR (100 MHz) δ ppm: 103.955, 118.182, 121.992, 123.458, 125.056, 127.613, 127.978, 130.572, 131.453, 132.212, 132.864, 140.155, 143.249, 144.056, 151.928; MS: *m/z* = 390.03 (M+1).

Antibacterial activity: The Agar well diffusion method was used to determine the antibacterial potential of synthesized compounds **5a-e** against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* [14,15]. The test bacteria were aseptically injected into sterile nutrient broth tubes and cultured at 38 ºC for 24 h in this technique. The 24 h old liquid bacterial cultures were swab inoculated on sterile nutrient agar plates, using sterile gel borer; punched the wells of 6 mm diameter in the inoculated plates. Standard antibiotic (chloramphenicol, 5 mg/ML of sterile distilled water (positive control)) prepared. A 100 µL of compound solutions and standard antibiotic solution were transferred aseptically into labeled wells. Sample loaded plates were not disturbed for 30 min and then incubated in the upright position for 24 h at 38 ºC. The inhibited zones developed around the wells were measured using a zone scale or ruler. The size of the zone indicates the effectiveness of the compound towards pathogenic bacteria.

Antifungal activity: The potential of synthesized compounds **5a-e** to inhibit the pathogenic fungi *Aspergillus niger* and *Aspergillus flavus* were determined by Agar well diffusion assay [16,17]. In this assay, the test fungal spore suspension was prepared by inoculating loop full of fungal culture was inoculated into the 5 mL sterile water taken in test tube amended with 2 drops of an emulsifying agent; between 80. Test fungal spore suspensions were swab inoculated on sterile Rose Bengal agar plates, using sterile gel borer; punched the wells of 6mm diameter in the inoculated plates. Standard antifungal (fluconazole, 10 mg/mL of sterile distilled water (positive control)) prepared. A 100 µL of 10 mg/mL concentration compound solutions and standard antifungal solution were transferred aseptically into labeled wells. Sample loaded plates were not disturbed for 30 min and then incubated in the upright position for 72 h at 28 ºC. The inhibited zones developed around the wells were measured using a zone scale or ruler. The size of the zone indicates the effectiveness of the compound towards pathogenic fungi.

Antitubercular activity: The thermally stable and non-toxic microplate Alamar blue assay (MABA) method [18] was adopted to screen the title compounds **5a-e** against *M. tuberculosis* H37RV strain at a concentration from 0.8 µg/mL to 100 µg/mL in a sterile 96 well plate. Sterile deionized water (200 µL) was added to the outer perimeter well to avoid evaporation. 100 µL of Middle brook 7H9 broth and serial dilutions of the compounds were introduced to the 96 wells plate and incubated at 36 ºC for 5 days. The sensitive compounds exhibited blue colour after 24 h incubation against *M. tuberculosis* H37Rv strain and the concentrations were recorded.

Antioxidant activity: Brand William's updated DPPH assay method was used to determine the antioxidant activity of synthesized molecules [19], 4 mg DPPH was dissolved the concentrations of 1 mM stock in 100 mL of methanol, stored at 4 ºC in a dark bottle. The DPPH work concentration was 0.14 mM in the test. Ascorbic acid (2 mg) dissolved in 10 mL of deionized water was used as standard. Adding 140 µL of 1 mM DPPH mixed and incubated at 37 ºC for 30 min, in short to a total of 860 tablets per 50% methanol/ascorbic acid/test samples at different concentrations. Read the absorbance by spectrophotometer at 52 nm against 50% blank methanol, without the addition of the test samples; a control reaction was performed. Colour correction requires the same concentration of methanol free of the test sample. Acceptable ascorbic acid damping values for antioxidant activity have been calculated. The actual absorption is determined by the absorption of the control and the test sample and the IC_{50} values.

RESULTS AND DISCUSSION

***In silico* studies:** Pharmacokinetic ADME descriptor properties were calculated for the molecules with biological importance and are compared with the ranges acquired for standard drugs. The compounds **5a-e** do possess a considerable degree of hydrogen bond donors and acceptors as recorded through *in silico* data. The derivatives have been designed to achieve an increase in binding capacity by hydrogen bonding to the receptors. The synthesized molecules accept Lipinski's rule 5 for oral bioavailability and characters, which enhances their chance to be considered as future drug candidates [20]. To determine the ability to permeate cell membranes of different types, the polar surface area of the derivatives was estimated. The statistics provided by calculating TPSA are well within an acceptable range, even the excretion of the medicines relies on molecular weight and lipophilicity log P. Appropriate Caco-2 permeability and BBB coefficient were used to access the total distribution of substances in the human body. The calculation of BBB values is within an acceptable range. The values generated for synthesized derivatives **5a-e** are shown in Table-1.

Drug likeness and bioactive score: None of the analogues have violated any rules of Lipinski and can be expected to be active orally. The molecular weights of compounds **5a-e** are below 500 and expected to be transported, diffused and absorbed across the membranes pretty easily than the macromolecules. The synthesized compounds **5a-e** correspond to the Lipinski rule. TPSA data of the molecules which are correlated with hydrogen bonding and are an indicator for bioavailability orally as the data is in the range of 64.70 Å to 110.52 Å well below 160 Å the limit (Table-2).

Molinspiration cheminformatics is the software approach to predict the bioactivity score of the synthesized compounds **5a-e** for drug targets and is represented in Table-3. Different pathways, such as interactions with inhibiting protease, nuclear receptor ligands, GPCR ligands and other enzymes, are involved in the physiological functions obtained from the synthesized molecules. The data also show that there is substantial inter-

TABLE-1
PHARMACOLOGICAL PARAMETERS OF THE SYNTHESIZED COMPOUNDS **5a-e** USING admetSAR TOOLBOX

Ligands	logBB	$\log_{HIA}$	Caco	logpGI (substrate)	logPGI (non-inhibitor)	Logs	logpapp
5a	0.9228	1.0000	0.5619	0.7549	0.8570	-2.8633	1.2760
5b	0.9248	1.0000	0.5880	0.7655	0.8540	-3.5561	1.4161
5c	0.9344	1.0000	0.5573	0.7488	0.7362	-3.3226	1.3300
5d	0.8915	0.9962	0.5105	0.7671	0.8695	-3.1988	1.2617
5e	0.9164	1.0000	0.5549	0.7560	0.7751	-3.4659	1.3403
Chloramphenicol	0.9382	0.9871	0.7250	0.7313	0.9019	-2.1694	0.9184
Fluconazole	0.9382	0.9894	0.8867	0.6008	0.8782	-1.8626	1.3598
Isoniazid	0.9895	0.9892	0.6959	0.8315	0.9778	-0.0521	1.2413

TABLE-2
DRUG LIKENESS SCORE FOR THE SYNTHESIZED LIGANDS **5a-e**

Ligands	MW	mi LogP	TPSA	No. atom	nON	nOHNH	nViolation	nRotb
5a	310.41	3.97	64.70	21	4	2	0	2
5b	344.85	4.62	64.70	22	4	2	0	2
5c	328.40	3.13	64.70	22	4	2	0	2
5d	355.39	3.90	110.52	24	7	2	0	3
5e	389.30	4.75	64.70	22	4	2	0	2
Chloramphenicol	323.13	0.73	115.38	20	7	3	0	6
Fluconazole	306.28	-0.12	81.66	22	7	1	0	5
Isoniazid	137.14	-0.97	68.01	10	4	3	0	1

TABLE-3
BIOACTIVE SCORE OF THE SYNTHESIZED LIGANDS **5a-e**

Ligands	Enzyme inhibitor	Kinase inhibitor	Protease inhibitor	Ion channel modulator	GPCR ligand	Nuclear receptor ligand
5a	-0.08	0.27	-0.66	-0.60	-0.36	-0.58
5b	-0.12	0.26	-0.63	-0.59	-0.33	-0.55
5c	-0.08	0.31	-0.63	-0.59	-0.30	-0.49
5d	-0.19	0.15	-0.65	-0.59	-0.43	-0.57
5e	-0.18	0.23	-0.76	-0.71	-0.47	-0.70
Chloramphenicol	-0.22	-0.28	-0.38	-0.41	-0.21	-0.00
Fluconazole	0.04	0.01	-0.09	-0.23	-0.09	0.03
Isoniazid	-1.39	-1.45	-1.05	-2.33	-1.23	-0.66

action between the molecules and the drug targets. Molecules have shown a strong bioactivity score.

Molecular docking: *In silico* molecular docking studies of the synthesized compounds against the oxidoreductase protein PDB Id: 1OC3. The binding affinity of ligands and protein varies from -5.7 to -6.2 kcal/mol, compound **5a** shows a greater binding affinity of -6.2 kcal/mol and others also with good binding affinity varies from -5.7 to -5.9 kcal/mol, compounds **5a**, **5c**, **5d** and **5e** show H-bond interaction with GLY92 and GLU16. The synthesized compounds show electrostatic interactions with ARG95, GLU16, GLY92, ALA90 and ARG86. Binding mode and visual interaction are shown in Table-4 and docking results are discussed in Table-5.

According to the reported method, compounds **3a-e** were synthesized by the mixture of 2- acetyl thiophene (**1**) and substituted isatins **2a-e** in ethanol by a catalytic amount of 33% KOH solution. Compounds **3a-e** and thiosemicarbazide **4**, were taken in 5 mL of phosphorus oxychloride and refluxed at 100-110 ºC to obtain 5-[2-(thiophen-2-yl)quinolin-4-yl]-1,3,4-thiadiazol-2-amine and its derivatives **5a-e** with good yields. The synthesized compounds **5a-e** were purified by crystallization and confirmed by different analytical techniques such as mass, ^{1}H NMR, ^{13}C NMR and infrared spectroscopy. The synthesized compounds **5a-e** showed the absorption bands ranging from 650-645 cm^{-1} for C-Br stretching, 785-780 cm^{-1} for C-Cl stretching, 1006-1001 cm^{-1} for C-F stretching, 1500 cm^{-1} for N=O stretching, 1590-1570 cm^{-1} for C=N stretching, 1670-1610 cm^{-1} for C=C aromatic stretching, 3500-3300 cm^{-1} for N-H stretching, 3370-3355 cm^{-1} for C-N stretching. In ^{1}H NMR spectra, the aliphatic protons appeared in the range between δ 5.5-6 ppm and aromatic protons appeared in the range of δ 6.5-9 ppm, in ^{13}C NMR all the aromatic carbon appeared in a range of 100-170 ppm.

Antibacterial activity: The agar well diffusion method was used to determine the *in vitro* antibacterial activity of the synthesized compounds **5a-e**. In this study, *Escherichia coli* and *Staphylococcus aureus* were selected because of their infectious nature. At a concentration of 10 µg/mL, the test compounds were dissolved in dimethyl sulfoxide (DMSO). The study found that among the compounds tested, all the synthesized compounds **5a-e** were found to be potent against *E. coli* and *S. aureus* (Table-6).

TABLE-4
INTERACTIONS OF SYNTHESIZED COMPOUNDS **5a-e** AGAINST 1OC3

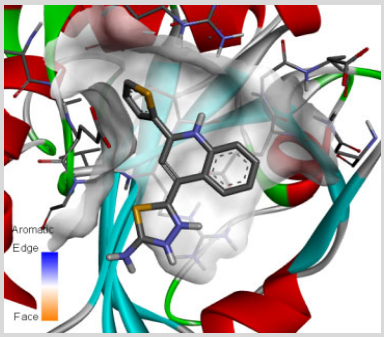
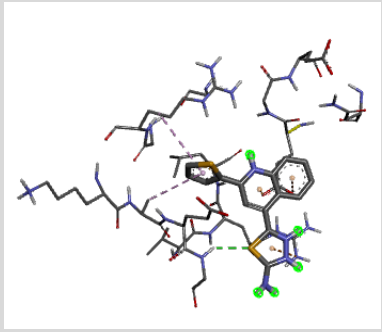
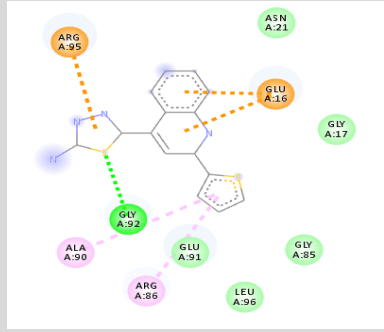
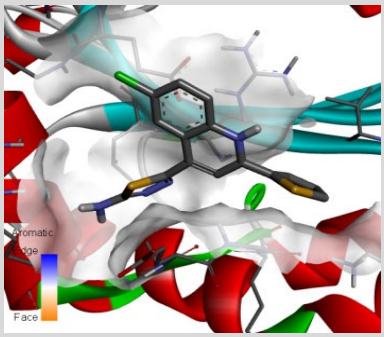
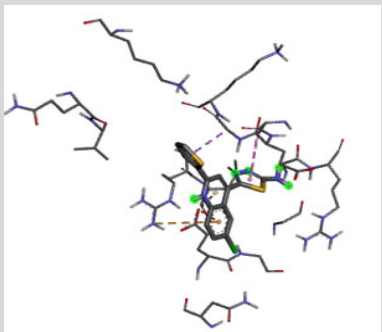
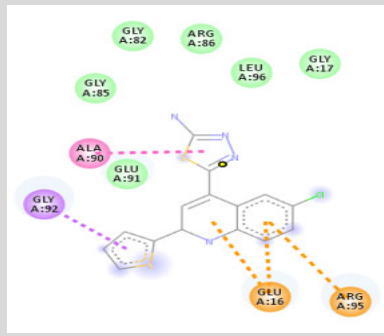
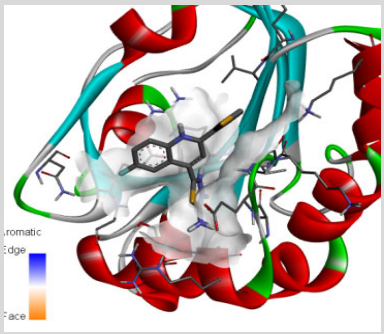
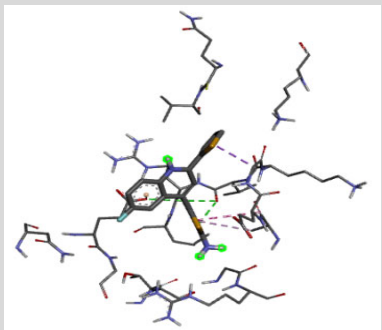
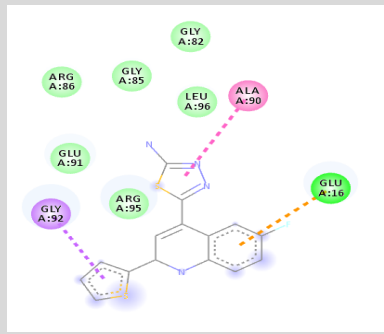
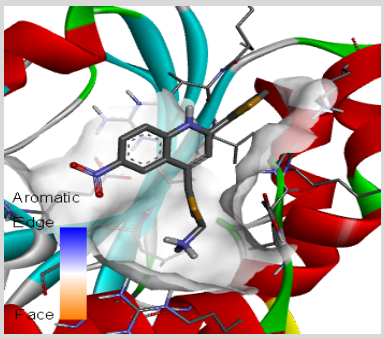
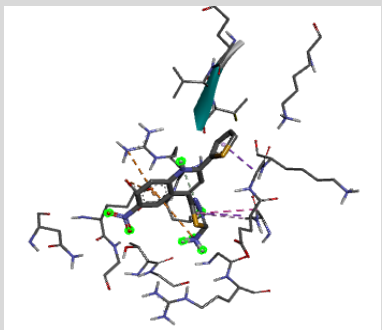
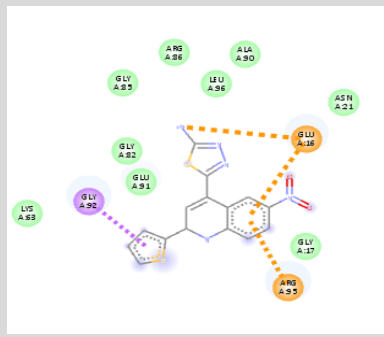
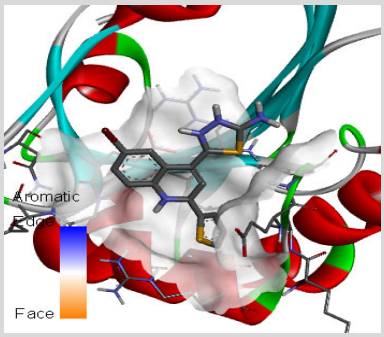
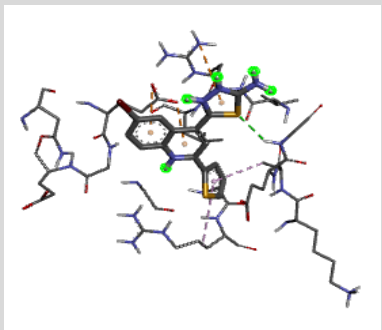
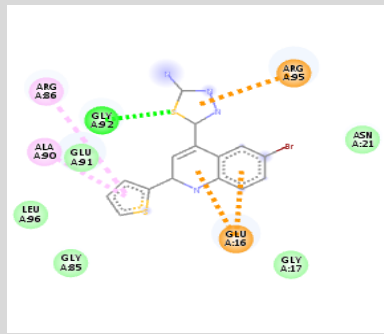
Compd.	Ligand protein interaction	3D Interaction	2D Interaction
5a			
5b			
5c			
5d			
5e			

TABLE-5
DOCKING RESULTS OF SYNTHESIZED COMPOUNDS **5a-e** AGAINST 1OC3

Compound	Hydrogen bond interactions	Hydrogen Bond's length (Å)	Binding affinity (kcal/mol)	Electrostatic interactions	Other interactions
5a	GLY92	2.68	-6.2	ARG95, GLU16	ASN21, GLY17, GLY85, LEU96, GLU91, ARG86, ALA90
5b	–	–	-5.9	ALA90, ARG95, GLU16, GLY92	GLU91, GLY85, ARG86, GLY82, LEU96, GLY17
5c	GLU16	4.05	-5.7	GLY92, ALA90	ARG95, GLU91, ARG86, GLY85, GLY82, LEU96
5d	GLU16	5.32	-5.8	ARG95, GLY92	GLY17, ASN21, ALA90, LEU96 ARG86, GLY85, GLY82, GLU91, LYS63
5e	GLY92	2.32	-5.8	ALA90, ARG86, ARG95, GLU16	LEU96, GLY85, GLU91, ASN21, GLY17
Pramipexole	ARG127, GLY46	6.08, 3.09	-4.1	–	PHE120, PRO45, GLY148, LEU149

TABLE-6
ANTIMICROBIAL ACTIVITY OF COMPOUND **5a-e**

Compounds	Inhibited zone's diameter (mm)			
	Antibacterial activity		Antifungal activity	
	E. coli	*S aureus*	*A. niger*	*A. flavus*
5a	18	17	06	06
5b	10	10.5	06	10
5c	16.5	18.5	12	06
5d	17.5	13.5	20.5	06
5e	18.5	19	09	06
Chloramphenicol	29	26.5	–	–
Fluconazole	–	–	19.5	25
DMSO	–	–	–	–

Antifungal activity: The *in vitro* antifungal activity of synthesized compounds **5a-e** against *A. niger and A. flavus* was examined by agar well diffusion method. The study found that among the compounds tested, compound **5d** shows good activity against the fungi *A. niger* and all other compounds show considering activity against *A. niger* and *A. flavus* (Table-6).

Antitubercular activity: The synthesized compounds **5a-e** were screened against *M. tuberculosis* using microplate Alamar Blue assay (MABA). The study found that among the compounds tested, concerning antitubercular activities, the synthesized compounds **5a-e** were found to be sensitive against *M. tuberculosis* at concentration 100 µg/mL concentration and compound **5b** shows sensitivity at 50 µg/mL concentration (Table-7).

TABLE-7
ANTITUBERCULAR MIC VALUES ARE EXPRESSED

S. No.	Samples	MIC (µg/mL)
i	**5a**	100 ± 0.5
ii	**5b**	50 ± 0.5
iii	**5c**	100 ± 0.5
iv	**5d**	100 ± 0.5
v	**5e**	100 ± 0.5
Std–I Pyrazinamide	–	3.125 ± 0.20
Std–II Ciprofloxacin	–	3.125 ± 0.20
Std–III Streptomycin	–	6.250 ± 0.12

DPPH assay: The radical scavenger effect of synthesized compounds with a free radical stable was studied using DPPH at 520 nm characteristic absorption. The absorption reduction is considered to evaluate radical scavenging levels. The results show that **5b** showed a 359 µg/mL IC_{50} value and **5c** shows a 375 µg/mL IC_{50} value. Both samples are effective in inhibiting DPPH activity. IC_{50} values are 415 µg/mL for **5a** and **5b** and 396 µg/mL. The IC_{50} values of the synthesized compounds are shown in Table-8.

TABLE-8
IC_{50} VALUES OF THE SYNTHESIZED COMPOUNDS ARE REPORTED

Samples	DPPH activities (IC_{50}) (µg/mL)
5a	415
5b	359
5c	375
5d	396
5e	452
Ascorbic acid	72

Conclusion

A series of quinoline coupled bridged thiophene with 1,3,4-thiadiazole ring system derivatives were synthesized in the most efficient multistep route with good yield and screened for *in vitro* antimicrobial activity along with the antioxidant activity. The title compounds *in silico* studies have been determined to comply with ADME studies and Lipinski's five requirements. The antibacterial study shows that all the compounds **5a-e** were found to be potent against *E. coli* and *S. aureus*, the antifungal study found that compound **5d** shows good activity against the fungi *A. niger* and all other compounds show considering activity, antitubercular activity shows that compound **5b** is sensitive against *M. tuberculosis.* Results of DPPH-free radical inhibition experiments show that the IC_{50} value of compound **5b** is 359 µg/mL and the IC_{50} value of compound **5c** is 375 µg/mL. The docked ligands against the oxidoreductase protein PDB Id: 1OC3 vary a binding affinity -5.7 to -6.2 kcal/mol.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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