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Design, Synthesis, Evaluation of Antitubercular and Antioxidant Activity of Isoxazoline Derivatives Derived from Novel Chalcone Intermediates

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Tuberculosis (TB), a bacterial infection caused by *Mycobacterium tuberculosis* (M-TB) is a fatal disease associated with a high degree of mortality. Present work involved synthesis of a series of novel isoxazoline derivatives (**ISOA1-ISOA8**) the new antitubercular agents using newly synthesized chalcones (**CHL1-CHL8**). This was done by subjecting the chalcones to a reaction with hydroxylamine hydrochloride in presence of acetic acid and sodium acetate. The structure of the synthesized compounds was elucidated by IR, ^{1}H NMR and mass spectra. *In silico* studies of isoxazoline were performed for antitubercular activity by using an enoyl acyl carrier protein (EACP) reductase enzyme. Compound **ISOA4** exhibited the best interaction among all these compounds having a docking score of -5.94 compared to standard drug pyrazinamide -6.1. Using *in vitro* models, purified compounds were tested for their antioxidant and antitubercular properties. Compound **ISOA5** showed a significant antioxidant activity compared to ascorbic acid as a standard drug. This study claims new isooxazolines to possess high antioxidant and antitubercular potential, however they must be evaluated for preclinical and clinical significance.

Keywords: Isoxazolines, Chalcones, Antitubercular activity, Antioxidant activity, *Mycobacterium tuberculosis*.

INTRODUCTION

Tuberculosis (TB), the infection caused by *M. tuberculosis* presents a global emergency for new therapeutic options attributed to the rapid drug-resistance. The World Health Organization (WHO) Global Tuberculosis 2022 report highlighted that in 2021, the estimated number of deaths caused by TB was more than double the number caused by HIV/AIDS [1]. Continuous development of new therapeutic moieties against TB infection is critical need in the current scenario. Evidence suggests isoxazolines as an important synthons and pharmacophores attributed to their broad range of pharmacological and chemotherapeutic applications. Due to their low cytotoxicity, derivatives of isoxazole are used as scaffolds for generating new drugs with diverse biological activities like antibacterial [2], anticonvulsant [3], anti-tubercular [4], anticancer [5], antioxidant [6], anti-inflammatory [7], adenosine antagonist [8], fungicidal [9], herbicidal [10], antidiabetic [11], muscle relaxant [12] and antimicrobial [13] activities.

Many substituted isoxazole derivatives are found in variety of marketed drugs, for example: cycloserine, acivicin, broxaterol, sulfamethoxazole, sulfisoxazole, oxacillin, isoxaflutole, danazol, zonisamide, risperidone, drazoxolon, valdecoxib, leflunomide, danazol, isocarboxamide and drazoxolon [14]. Hence isoxazole is considered an essential pharmacophore in modern drug discovery especially in antibacterial. In drug discovery, the chalcones also attains high importance attributed to their anticancer [15], antioxidants [16], analgesic [17], antimalarial [18], antimicrobial [19] activities. Because of the presence of active keto ethylenic group in isoxazoles they are considered as highly reactive intermediates and also useful in elucidating the structure of natural products [20]. The significant bioactivities of isoxazolines and chalcones render them as useful pharmacophore in medicinal chemistry and drug research. Hence based on good affinity and binding exhibited by isoxazoline towards EACP receptor, the present study was designed to study a new series of isoxazoline derivatives and screen them for their antioxidant and anti-tubercular activity.

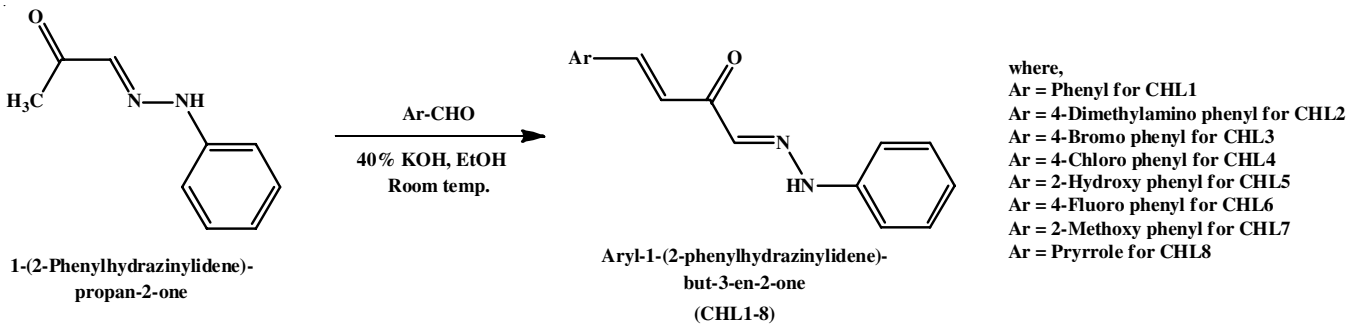
EXPERIMENTAL

All the chemicals and reagents were either procured from Merck (India) or Sigma-Aldrich (USA) in this study. Melting points were recorded by Open capillary tube method using SMP11 Analogue apparatus. Characterization of newly synthesized compounds was done using IR spectra recorded on Alpha Bruker FTIR spectrometer at wavelength ranging from 400 to 4000 cm^{-1}. Nuclear magnetic resonance spectra recorded on ^1H spectrometer on Bruker DPX 300 using DMSO as solvent, on δ value scale in ppm with tetramethylsilane as standard and mass spectra was recorded on FABMASS. Purity of the synthesized compounds and reactions monitoring were done by TLC over aluminum sheets with silica gel 60 F_{254} (0.2 mm) (Merck, India) using methanol:acetone (9:1) solvent mixture in a UV chamber using a SPRECTROLINE® CM-26 UV viewing chamber [21,22].

Synthesis of novel chalcone intermediates (CHL1-8) using 1-(2-phenyl hydrazinylidene)propan-2-one: Novel chalcone derivatives were synthesized as per the standard protocol with minor modification [23], briefly a mixture of aromatic aldehyde (0.01 mol) and various substituted 1-(2-phenylhydrazinylidene) propan-2-one (0.01 mol) were stirred together in the presence of 20% KOH (4 mL). Stirring was continued till the completion of reaction, which was determined by taking few drops of the solution, diluting with water and acidifying it with dilute HCl. Precipitation indicates the completion of reaction. Next, the reaction mixture was poured into crushed ice and acidified with 5% HCl (**Scheme-I**). Product was filtered, washed and recrystallized using ethanol. The characterization data of the synthesized chalcone intermediates (CHL1-8) are given in Tables 1 & 2.

Synthesis of novel isoxazoline derivatives (ISOA1-ISOA8): The novel isoxazoline derivatives were also synthesized as per the standard protocol with minor modifications [24]. Briefly, ethanolic solution of the selected chalcone (0.01 M) containing 0.01 M of anhydrous sodium acetate dissolved in a minimum amount of acetic acid was reacted with hydroxylamine hydrochloride solution. The reaction mixture was refluxed for 8 h on the heating mantel. After the completion of reaction, the solution was cooled to obtain the final products, which were purified by recrystallization from the absolute ethanol (**Scheme-II**). The purity of the intermediate and final compounds was checked by HPLC methods. All the synthesized compounds were characterized by IR, ^1H NMR and mass spectral studies.

***In vitro* antioxidant activity using DPPH assay:** The antioxidant activity of the synthesized compounds was done as per the standard protocol of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with minor modifications [25]. Briefly, a solution of DPPH in methanol (0.2 mM) was prepared and different concentrations of the samples (ranging from 10 to 50 µg/mL)



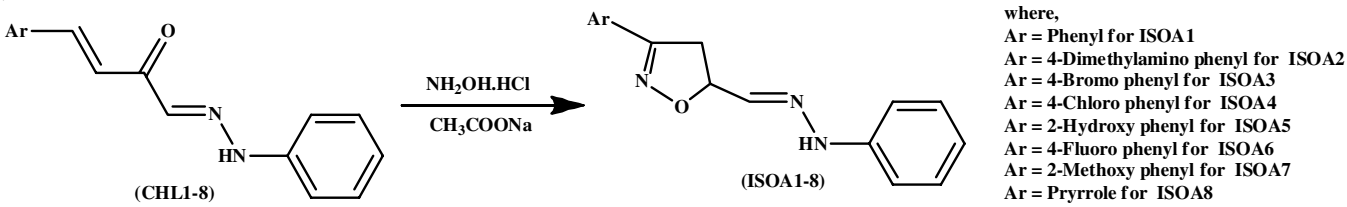
Scheme-I: Synthesis of novel chalcone intermediates (**CHL1-8**)

TABLE-1
PHYSICAL DATA OF COMPOUND **CHL1-8** AND **ISOA1-8**

Compd.	IUPAC name	m.f.	m.p. (°C)	m.w.	Yield (%)
CHL-1	4-Phenyl-1-(2-phenylhydrazono)but-3-en-2-one	$C_{16}H_{14}N_2O$	126-128	250.29	72
CHL-2	4-(4-(Dimethylamino)phenyl)-1-(2-phenylphydrazono)but-3-en-2-one	$C_{18}H_{19}N_3O$	165-167	293.85	55
CHL-3	4-(4-Bromophenyl)-1-(2-phenyl hydrazono)but-3-en-2-one	$C_{16}H_{13}N_2OBr$	212-214	329.18	68
CHL-4	4-(4-Chlorophenyl)-1-(2-phenylhydrazono)but-3-en-2-one	$C_{16}H_{13}N_2OCl$	140-142	283.7	81
CHL-5	4-(2-Hydroxyphenyl)-1-(2-phenylhydrazone)but-3-en-2-one	$C_{16}H_{14}N_2O_2$	165-167	266.29	58
CHL-6	4-(4-Flurophenyl)-1-(2-phenylhydrazone)but-3-en-2-one	$C_{16}H_{13}N_2O_2F$	147-149	268.28	68
CHL-7	4-(2-Methoxyphenyl)-1-(2-phenylhydrazone)but-3-en-2-one	$C_{17}H_{16}N_2O_2$	175-177	280.37	62
CHL-8	1-(2-Phenylhydrazone)-5(1*H*-pyrrol-2-yl)but-3-en-2-one	$C_{14}H_{13}N_3O$	136-138	239.27	64
ISOA-1	3-Phenyl-5-((2-phenylhydrazono) methyl)-4,5-dihydro isoxazole	$C_{16}H_{15}N_3O$	134-136	265.30	54
ISOA-2	N,N-Dimethyl-4-(5-((2-phenylhydrazono)methyl)-4,5-dihydro isoxazol-3-yl)aniline	$C_{18}H_{20}N_4O$	144-146	308.37	48
ISOA-3	3-(4-Bromophenyl)-5-((2-phenylhydrazono)methyl)-4,5-dihydro isoxazole	$C_{16}H_{14}N_3OBr$	161-163	344.19	74
ISOA-4	3-(4-Chlorophenyl)-5-((2-phenylhydrazono)methyl)-4,5-dihydro isoxazole	$C_{16}H_{14}N_3OCl$	158-160	299.74	67
ISOA-5	2-(5-(2-Phenylhydrazono)methyl)-4,5-dihydro isoxazole-3-yl)phenol	$C_{16}H_{15}N_3O_2$	161-163	281.30	38
ISOA-6	3-(4-Fluorophenyl)-5-((2-phenylhydrazono)methyl-4,5-dihydroisoxazole	$C_{16}H_{14}N_3OF$	133-135	283.29	66
ISOA-7	3-(2-Methoxyphenyl)-5-((2-phenylhydrazono)methyl)-4,5-dihydroisoxazole	$C_{17}H_{17}N_3O_2$	165-167	295.33	58
ISOA-8	5-((2-Phenylhydrazono)methyl-3-(1*H*-pyrol-2-yl)-4,5-dihydroisoxazole	$C_{14}H_{14}N_4O$	126-128	254.28	71

TABLE-2
SPECTROMETRIC CHARACTERIZATION DATA OF COMPOUND **CHL1-8** AND **ISOA1-8**

Compd.	FTIR (cm^{-1})	^{1}HNMR (DMSO-d_6, ppm) δ	Mass (*m/z*)
CHL1	3342 (N-H), 3062 (C-H), 1545 (C=N), 1512 (C=C), 1643 (C=O)	6.4, 6.6 (s, Ali-H, 2H), 6.9 (s, N=C-H, 1H), 7.4-7.8 (m, Ar-H, 9H), 9.8 (s, NH, 1H)	250.2 (M+), 251.1 (M+1)
CHL2	3368 (N-H), 3065 (C-H), 1654 (C=O), 1518 (C=N), 1484 (C=C)	3.5 (s, N (CH_3),, 6H), 6.3, 6.4 (s, Ali-H, 2H), 6.5 (s, N=C-H, 1H), 7.2-7.6 (m, Ar-H, 9H), 10.2 (s, NH, 1H)	293.1 (M+)
CHL3	3359 (N-H), 3042 (C-H), 1648 (C=O), 1523 (C=N), 1469 (C=C), 810 (C-Br)	6.2-6.3 (s, Ali-H, 2H), 6.6 (S, N=C-H, 1H), 7.1-7.6 (m, Ar-H, 9H), 9.9 (S, NH, 1H)	329.1 (M+)
CHL4	3382 (N-H), 3058 (C-H), 1653 (C=O), 1528 (C=N), 1449 (C=C), 853 (C-Cl)	6.1, 6.2 (s, Ali-H, 2H), 6.7 (s, N=C-H, 1H), 7.2-7.5 (m, Ar-H, 9H), 9.7 (s, NH, 1H)	283.2 (M+), 285.1 (M+2)
CHL5	3359 (N-H), 3079 (C-H), 1672 (C=O), 1506 (C=N), 1479 (C=C), 3410 (O-H)	6.7, 6.5 (s, Ali-H, 2H), 6.9 (s, N=C-H, 1H), 7.2-7.7 (m, Ar-H, 9H), 10.3 (s, NH, 1H)	266.2 (M+)
CHL6	3365 (N-H), 3056 (C-H), 1688 (C=O), 1514 (C=N), 1465 (C=C), 810 (C-F)	6.2, 6.4 (s, Ali-H, 2H), 6.8 (s, N=C-H, 1H), 7.1-7.6 (m, Ar-H, 9H), 10.4 (s, NH, 1H)	268.2 (M+)
CHL7	3379 (N-H), 3042 (C-H), 1694 (C=O), 1508 (C=N), 1472 (C=C)	6.1, 6.3 (s, Ali-H, 2H), 6.9 (s, N=C-H, 1H), 7.3-7.8 (m, Ar-H, 9H), 9.9 (s, NH, 1H), 3.8 (s, OCH_3, 3H)	280.2 (M+)
CHL8	3326 (N-H), 3062 (C-H), 1686 (C=O), 1561 (C=N), 1524 (C=C)	6.1, 6.2 (s, Ali-H, 2H), 6.8 (s, N=C-H, 1H), 7.2-7.8 (m, Ar-H, 7H), 9.8 (s, NH, 1H), 10.4 (m, pyrrole, 1H)	239.2 (M+)
ISOA1	3327 (N-H), 3059 (C-H), 1564 (C=N), 1511 (C=C), 1251 (C-O)	2.3 (s, CH_2, 2H), 7.1 (s, N=C-H, 1H), 7.3-8.2 (m, Ar-H, 10H), 11.5 (s, NH, 1H)	265.30 (M+)
ISOA2	3397 (N-H), 3060 (C-H), 1557 (C=N), 1508 (C=C), 1223 (C-O)	2.3 (s, CH_2, 2H), 7.4 (s, N=C-H, 1H), 2.7 (s, 2 x CH_3, 6H), 7.5-8.1 (m, Ar-H, 8H), 13.1 (s, NH, 1H)	308.37 (M+)
ISOA3	3294 (N-H), 3004 (C-H), 1508 (C=N), 1555 (C=C), 1224 (C-O), 915 (C-Br)	2.0 (s, CH_2, 2H), 7.0 (s, N=C-H, 1H), 7.2-8.3 (m, Ar-H, 9H), 13.0 (s, NH, 1H)	344.20 (M+)
ISOA4	3203 (N-H), 3063 (CH), 1603 (C=N), 1549 (C=C), 1235 (C-O), 835 (C-Cl)	2.4 (s, CH_2, 2H), 7.2 (s, N=C-H, 1H), 7.3-8.1 (m, Ar-H, 9H), 8.3 (s, NH, 1H)	299.7 (M+), 301.75 (M+2)
ISOA5	3388 (O-H), 3001 (C-H), 1601 (C=N), 1548 (C=C), 1255 (C-O)	7.4-8.3 (m, Ar-H, 8H), 2.4 (s, CH_2, 2H), 7.3 (s, N=C-H, 1H), 8.1 (s, NH, 1H)	282.5 (M+1)
ISOA6	3324 (N-H), 3065 (C-H), 1562 (C=N), 1508 (C=C), 1230 (C-O), 790 (C-F)	2.4 (s, CH_2, 2H), 7.2 (s, N=C-H, 1H), 7.3-8.1 (m, Ar-H, 8H), 8.4 (s, NH, 1H)	283 (M+)
ISOA7	3319 (N-H), 3056 (C-H), 1557 (C=N), 1510 (C=C), 1226 (C-O)	7.1-7.9 (m, Ar-H, 8H), 2.3 (s, CH_2, 2H), 7.1 (s, N=C-H, 1H), 8.7 (s, NH, 1H), 3.6 (s, OCH_3, 3H)	295 (M+)
ISOA8	3318 (N-H), 3058 (C-H), 1558 (C=N), 1516 (C=C), 1227 (C-O), 3341 (N-H of pyrrole)	7.1-7.8 (s, Ar-H, 8H), 2.3 (s, CH, 2H), 7.2 (s, N=C-H, 1H), 8.5 (s, NH, 1H), 8.7 (s, NH of pyrrole, 1H)	254 (M+)



Scheme-II: Synthesis of isoxazoline derivatives (**ISOA1-8**)

were mixed with 100 µL of this solution. For 30 min, the reaction mixture was incubated at room temperature, after which at 517 nm, the absorbance was determined. All studies were carried out in triplicate, with ascorbic acid serving as reference drug. By measuring the absorbance of the control and test, the percentage of inhibition was estimated. The percentage of inhibition was determined as per the formula given in eqn. 1:

$$\text{Inhibition } (\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_c is the absorbance of control and A_s is the absorbance of the test.

***In vitro* antioxidant activity using nitric oxide radical scavenging method:** The synthesized compounds were further subjected to evaluate of their antioxidant activity using standard protocol of nitric oxide radical scavenging assay with minor modifications [26]. Briefly, at a physiological pH, nitric oxide (NO) radicals were produced through the use of a sodium nitroprusside solution. Specifically, 50 µL of 10 mM sodium nitroprusside solution was mixed with 50 µL of samples containing concentrations ranging from 10-50 µg/mL as well as 50 µL of phosphate buffer (pH 7.4). This mixture was then incubated for 150 min at 25 ºC. After which, 50 µL of Griess's reagent (composed of orthophosphoric acid (2%), naphthyl ethylene diamine-dihydrochloride (0.1%) and sulphanilamide (1%) was added to 150 µL of incubated solution. The optical density of the mixture was determined at 546 nm and ascorbic acid was considered as a standard. The percentage of inhibition was determined as per the formula given in eqn. 1.

***In vitro* antioxidant activity using superoxide radical scavenging method:** The synthesized compounds were also subjected to evaluate their antioxidant potential using standard

protocol of superoxide radical scavenging assay with minor modifications [27]. To initiate the reaction, a mixture of nitro blue tetrazolium (NBT) solution (NBT (156 µM) in phosphate buffer (100 mM) at pH 7.4), NADH solution (NADH (468 µM) in phosphate buffer (100 mM) at pH 7.4) and solution of the sample in water was prepared in a ratio of 50 µL each. The reaction was initiated by the addition of 50 µL of phenazine methosulphate (PMS) solution (PMS (60 µM) in phosphate buffer (100 mM) at pH 7.4) to the above-mentioned mixture. The reaction mixture was incubated for 5 min at 25 ºC. The absorbance was measured at 560 nm in comparison to a control. Ascorbic acid was employed as the standard. The percentage of inhibition was determined as per formula given in eqn. 1.

Anti-TB activity using Alamar blue dye: The synthesized compounds were further evaluated for their anti-TB potential against *Mycobacterium tuberculosis* (H37 RV strain, ATCC 27294) using standard protocol of microplate Alamar blue assay (MABA) with minor modifications [28]. Sterilized distilled water (200 µL) was added to the outer perimeter wells of the 96-well plate to prevent medium evaporation in the test wells during incubation. The Middlebrook 7H9 broth (100 µL) and serial dilutions of the molecules were added to the plate. The ultimate concentrations of the compounds were analyzed against the microorganism ranged from 100 to 0.2 µg/mL. After parafilm sealed, the plates were incubated at 37 ºC for 5 days. After the incubation period, a mixture of Almar Blue reagent (25 µL) and Rween 80 (10%) was added to the plate in a 1:1 ratio and the plate was incubated for an additional 24 h. A pink colour in the well showed growth whereas as blue colour indicated no bacterial growth. The minimum concentration of the compounds that showed the colour changing from blue to pink is termed as minimal inhibitory concentration (MIC).

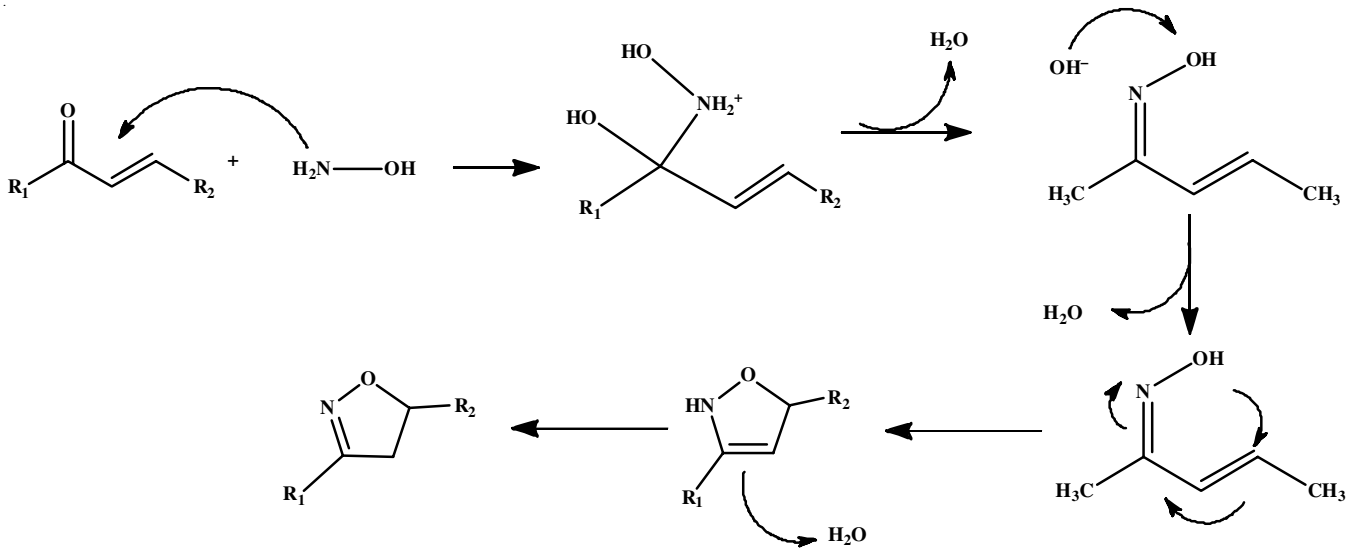
***In silico* anti-tubercular activity:** The synthesized isoxazolines were also subjected to *in silico* studies against enoyl-acyl carrier protein (EACP) reductase enzyme. Selected target is the enzyme which is responsible for fatty acid elongation. It catalyzes the reduction of alpha beta unsaturated fatty acids in complex with the enzyme and it is an important target for the development of potential synthetic heterocyclic antitubercular agents [29].

RESULTS AND DISCUSSION

Present study was aimed to synthesize the substituted 3-phenyl-5-((2-phenyl hydrazono)methyl)4,5-dihydro isoxazole derivatives using hydroxylamine hydrochloride in the presence of acetic acid and sodium acetate from chalcone derivatives. The exact mechanism followed by this reaction is yet to be fully understood but latest report reveals that formation of 5-arylisoxazoles could be achieved by the reaction (**Scheme-III**) [24]. One molecule of dimethylamine is eliminated when Michael addition of chalcone derivatives and hydroxylamine gives an unstable intermediate. Finally, the intramolecular cyclization and dehydration gives rise to the final product.

This work was supported with the molecular docking studies in order to evaluate antitubercular and antioxidant activities of new synthesized compounds. The IR, ^{1}H NMR and mass spectral data of thesynthesized compounds were used to confirm the structure their chemical structures. The bending and stretching frequencies of the functional groups of various chalcone intermediates and isoxazoline derivatives were ascertained by IR spectroscopy for the intermediates and final compounds. The intensity of stretching bands of amine group (N-H) were observed at 3380-3341 cm^{-1} for all the compounds. Low intensity band in the region 1570-1535 cm^{-1} was observed for (C=N) group in all compounds. The carbonyl group stretching was appeared at 1695-1640 cm^{-1} region whereas the aromatic C=C stretching was appeared in 1565-1510 cm^{-1} region. The C-H aromatic stretching was observed at 3010-2975 cm^{-1}. Medium stretching of carbon and chlorine, carbon and fluorine, carbon and bromine containing phenyl group was appeared in the region of 850-770 cm^{-1} for compounds **CHL3**, **CHL4**, **CHL6**, **ISOA3**, **ISOA4** and **ISOA6**.

All the intermediates (**CHL1-CHL8**) and final compounds (**ISOA1-ISOA8**) were also characterized by nuclear magnetic resonance spectroscopy (^{1}H NMR). All aromatic protons appeared



Scheme-III: Mechanism of reaction for the synthesis of isoxazoline derivatives

in the form of multiplets in the region δ 7.1-7.7 ppm in all the compounds. Two aliphatic protons were observed as singlet at δ 6.2-6.6 ppm in all chalcone intermediates. Protons of amine group appeared as singlet at δ 8.3-10.3 ppm in all the compounds. The N=C-H protons were observed as singlet at δ 6.6-7 ppm, whereas two methyl group protons were observed as singlet at δ 2.2-2.7 ppm accounting for six protons in compounds **CHL-2** and **ISOA-2**. Active methylene group (–CH_2) two protons were appeared at δ 2.0-2.5 ppm in all isoxazoline derivatives. NH protons of pyrrole was observed at δ 10.4 ppm in compound **ISOA8** and δ 8.7 ppm in compound **CHL8**. Three protons of methoxy group appeared as a singlet at δ 3.8 ppm in compound **CHL7** and δ 3.6 ppm in compound **ISOA7**, which are all in conformity with the theoretical values. The molecular ion peak observed for various chalcones at 250.2, 293.1, 329.1, 283.2, 266.2, 268.2, 280.2 and 239.2 respectively for compounds **CHL1**, **CHL2**, **CHL3**, **CHL4**, **CHL5**, **CHL6**, **CHL7** and **CHL8**, which are in conformity with the calculated mass. Only in compounds **CHL4** and **ISOA4** because of chlorine isotope the M+2 peak was observed. The molecular ion peak of various isoxazoline derivatives were appeared at 265.3, 308.37, 344.2, 299.7, 282.5, 283.0, 295.0 and 254.0 respectively for compounds **ISOA1**, **ISOA2**, **ISOA3**, **ISOA4**, **ISOA5**, **ISOA6**, **ISOA7** and **ISOA8**, which are in agreement with calculated mass of individual compounds. The characterization of all molecules was supported with standard literature [30,31].

The *in silico* molecular docking was performed on eight analogues of isoxazoline derivatives using Schrödinger software [32]. The compounds were docked with drug target (EACP) enoyl-acyl carrier protein reduction enzyme and respective docking score of compounds **ISOA1-8** and interaction of compound **ISOA4** with EACP are presented in Table-3 and Fig. 1. Compound **ISOA-4** showed good docking score of -5.94, which is considered as good antitubercular agent.

TABLE-3
G-SCORE OF ISOXAZOLINE DERIVATIVES

Compound	G-Score	Compound	G-Score
ISOA-4	-5.94	**ISOA-3**	-5.45
ISOA-8	-5.78	**ISOA-5**	-5.38
ISOA-7	-5.67	**ISOA-2**	-5.10
ISOA-1	-5.64	**ISOA-6**	-5.05

Among synthesized isoxazoline derivatives, two compounds ISOA4 and ISOA5 exhibited good antitubercular activity against pyrazinamide as standard drug. Presence of electron-withdrawing groups was responsible for showing good antitubercular activity, which are represented in Table-4.

Antioxidant studies of isoxazoline derivatives (**ISOA1-ISOA8**) revealed that hydroxyl substitutions at second position of the benzene ring in compound **ISOA-5** induced significant antioxidant activity with IC_{50} value of 19.46 and 17.88 by DPPH method and CUPRAC method, respectively. Unsubstituted compound **ISOA-1** showed good results in nitric oxide method with IC_{50} value of 27.86, whereas halogen substituted derivatives **ISOA-3** at fourth position produced good activity with an IC_{50} value of 4.18 by superoxide method against ascorbic acid as standard drug which are given in Table-5.

Conclusion

In present study, a novel series of isoxazoline derivatives (**ISOA1-ISOA8**) were synthesized and docked for antitubercular activity using drug target (EACP) enoyl-acyl carrier protein

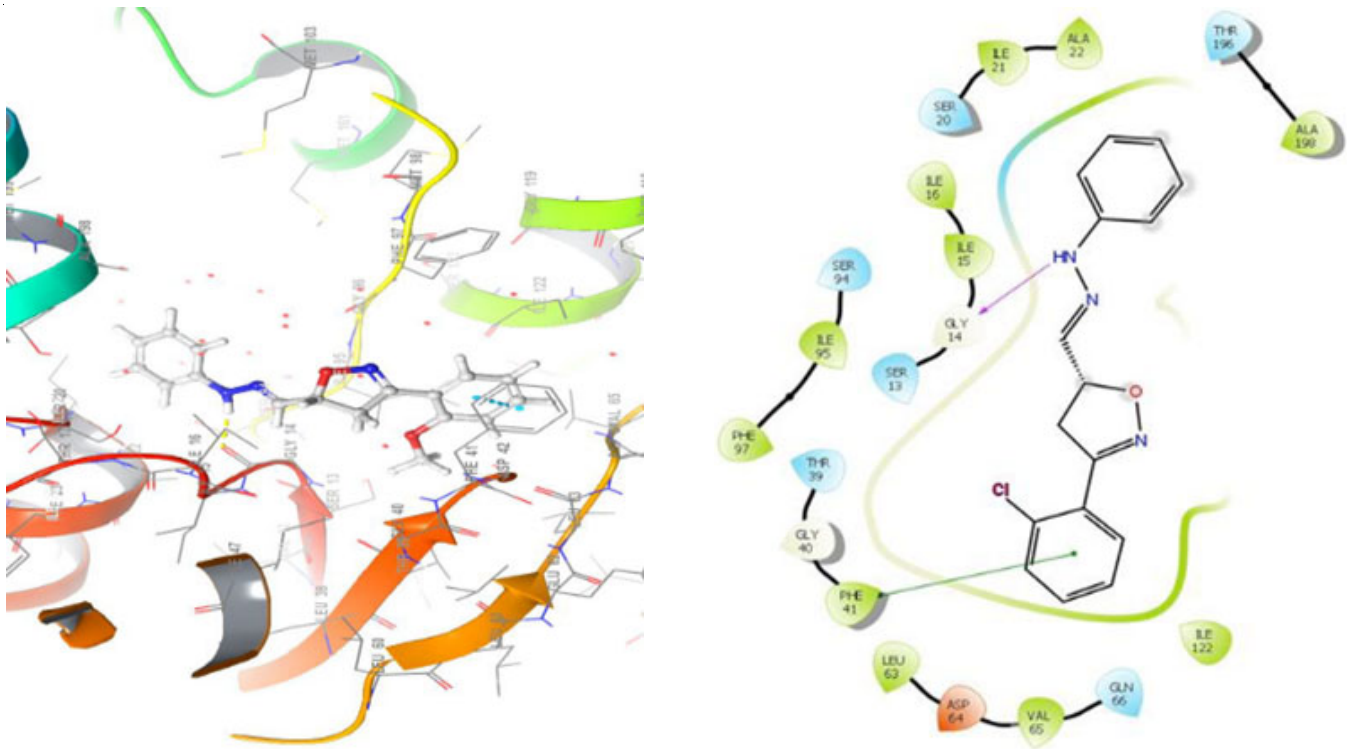


Fig. 1. 2D and 3D interaction of compound **ISOA4** with EACP

TABLE-4
ANTI-TUBERCULAR ACTIVITY OF ISOXAZOLINE DERIVATIVES BY ALAMAR BLUE DYE TECHNIQUE

Sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.60 µg/mL	0.8 µg/mL
ISOA-1	R	R	R	R	R	R	R	R
ISOA-2	S	S	R	R	R	R	R	R
ISOA-3	R	R	R	R	R	R	R	R
ISOA-4	S	S	S	S	S	R	R	R
ISOA-5	S	S	S	S	S	R	R	R
ISOA-6	R	R	R	R	R	R	R	R
ISOA-7	S	S	R	R	R	R	R	R
ISOA-8	R	R	R	R	R	R	R	R
Pyrazinamide	S	S	S	S	S	S	R	R

TABLE-5
ANTIOXIDANT ACTIVITY OF ISOXAZOLINE DERIVATIVES (IC_{50} VALUE)

Compound	DPPH	Nitric oxide	CUPRAC	Superoxide
ISOA-1	107.31 ± 4.98	27.86 ± 3.07	142.34 ± 6.66	81.08 ± 2.41
ISOA-2	158.29 ± 2.57	625.72 ± 7.81	110.83 ± 7.5	82.4 ± 5.76
ISOA-3	21.58 ± 7.73	86.72 ± 7.87	196.41 ± 5.38	4.18 ± 2.63
ISOA-4	27.25 ± 2.47	71.39 ± 2.91	34.47 ± 4.99	36.73 ± 2.59
ISOA-5	19.46 ± 4.8	54.82 ± 4.11	17.88 ± 5.58	68.17 ± 2.78
ISOA-6	190.17 ± 2.25	184.17 ± 6.31	7.07 ± 5.84	124.08 ± 4.19
ISOA-7	87.89 ± 4.88	306.57 ± 6.02	23.97 ± 5.95	51 ± 2.83
ISOA-8	26.62 ± 2.78	104.52 ± 3.95	972.5 ± 6.14	21.37 ± 4.57
Ascorbic acid	7.16 ± 1.21	16.63 ± 2.23	24.61 ± 5.74	3.72 ± 0.87

reductase enzyme. All the synthesized compounds were resulted in good yields. Synthesized compounds were evaluated for antitubercular activity, antioxidant activity and characterized by spectral data. Docking results of compound **ISOA-4** has revealed that this moiety can be considered as promising lead molecule in the development of antitubercular agent. Compounds **ISOA-1**, **ISOA-3** and **ISOA-5** can be used as good antioxidants. However, the synthesized compounds must be further evaluated for their clinical and preclinical significance.

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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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