

Synthesis, Characterization and Biological Activity of Some New 1,2,4 Triazole And Isoxazoline Derivatives

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Abstract:

A new series of 1, 2, 4 triazole and Isoxazoline derivatives were synthesized to meet structural requirements essential for Antioxidant, antimicrobial, Antidiabetic and Anti-tubercular activity. 3-(2-(3, 5-diphenyl-4, 5-dihydroisoxazol-4-yl) ethyl)-4H-1,2, 4-triazol-4-amine) SS series of 1, 2, 4 triazole and 4-methyl-3,5-diphenyl-4,5-dihydroisoxazole AS series of Isoxazoline compound were synthesized. IR, ¹H-NMR, Mass spectra and elemental analysis were recorded to confirm the structure of target compounds. Selected compound of SS and AS series were screened for in vitro antimicrobial activity against *S.Aureus*, *S. Epidermis*, *E.coli*, *P. Klebsiella*, *A. Flavus*, *A. Niger*. MIC values were determined by well diffusion method. Chloro, hydroxyl, methoxy substituted derivatives exhibited significant Antibacterial and Fungicidal potential. The in vitro Antioxidant activity of the compound SS and AS series were evaluated by DPPH free radical scavenging assay. Maximum DPPH radical scavenging activity was observed in compound SS6 as 87.83 % at 32 ug/ml and compound AS1 as 14.56 at IC₅₀. All the above compound screened for Antidiabetic and antitubercular activity. Among the series, compounds SS1, SS11, AS2, AS11 displayed potent α glucosidase inhibitory activity with IC₅₀ values of 179, 72.74, 101.8 and 104.8 μ g/ml and compound SS 12 and AS 12 showed equivalent antitubercular activity at 3.125 μ g/ml conc. and were the potent antitubercular derivatives.

Keywords: 1, 2, 4 triazole, Isoxazole, Antimicrobial, Antidiabetic, Antitubercular

Introduction

Heterocyclic Compounds containing three nitrogen atoms, especially Heterocyclic ring with three nitrogen atoms, like 1,2,4 triazole ring, are one of the most important active Pharmaceutical scaffolds. These scaffolds are able to form hydrogen bonds with different targets, which leads to the improvement of Pharmacokinetics, Pharmacological and toxicological Properties of Compounds.¹ 1, 2, 4- triazole has received substantial attention due to their effective biological importance like anticancer¹, antibacterial², anticonvulsant³, anti-inflammatory⁴, analgesic⁴, antifungal⁵, antidepressant⁶, antitubercular⁷, antimarial⁸ and hypoglycemic activities⁹. The isoxazole nucleous is well known for its medicinal importance and a number of related compounds are known to exhibit antifungal¹⁰, antimicrobial¹¹, anticancer¹², analgesic¹³, anti-inflammatory¹³, antituberculin¹⁴, antiviral¹⁵, antipsychotic¹⁶ and hypoglycemic activities.¹⁷

In appraisal of the above mentioned facts we describe herein the synthesis of some new 1, 2, 4 triazole and isoxazoline derivatives and evaluation of their in vitro antimicrobial, antioxidant, antidiabetic and antitubercular activities.

Experimental:

Melting points were determined by open capillary method on Veego (Model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded Shimadzu 8400-SFT-IR Spectrophotometer using ATRA sampling Technique ¹H NMR spectra was obtained on Bruker AV III 500 MHz spectrometer spectra in CDCl₃ and chemical shifts are given in parts per million, downfield from Tetramethylsilane (TMS) as an internal standard Mass spectra were obtained from Bruker Impact HD 3050 system instrument at the SPPU, Pune to monitor the reaction as well as to established the identity and purity of reactant and Products, thin layer chromatography was performed on microscopic slides (2 x 7.5 cm) coated with silica gel G F254, using Benzene:Methanol (7:3) solvent system and the spot were visualized under ultra-violet light (254 nm) or exposure to iodine vapours.

Synthesis and characterization of 3-(2-(3, 5-diphenyl-4, 5-dihydroisoxazol-4-yl)ethyl)-4H-1,2,4-triazol-4-amine (Scheme-1)

Step I) Synthesis of Chalcones (I):

Proper acetophenone (0.01 mol) in ethanol and aromatic aldehyde (0.01 mol) in ethanol were mixed and 10 ml of 40% sodium hydroxide solution was added with stirring. The subsequent solution was reserved overnight at room temperature. The mixture was then poured over crushed ice and acidified with dil. HCl. The solid gained was filtered, dried and recrystallized from ethanol.

Step II) Synthesis of isoxazolines (3,5-diphenyl-4,5-dihydroisoxazole) (II):

The isoxazolines were prepared by reacting a mixture of purified chalcones (0.01 mol), hydroxylamine hydrochloride (0.03 mol) and a solution of NaOH (0.01 mol) in dry distilled ethanol by refluxing for 6 h. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product was filtered, washed with sufficient coldwater, dried and purified by recrystallization from ethanol.

Step III) General procedure for the synthesis of 3-(3,5-diphenyl-4,5-dihydroisoxazol-4-yl)propanehydrazide (III):

To isolation of compound II in ethanol (50 ml, formaldehyde (0.6 ml, 0.02 mol) and ethyl acetate (1.76 ml, 0.02 mol) was added. The reaction mixture was refluxed for 2-6 h. The solvent was distilled off and poured into ice water resulting solids were filtered off, dried and recrystallized using appropriate solvent.

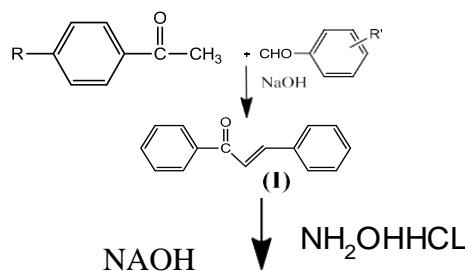
Step IV) Synthesis of monopotassium(II)mono(2-(3-(3,5-diphenyl-4,5-dihydroisoxazol-4-yl)propanoyl) hydrazine carbodithioate) (IV):

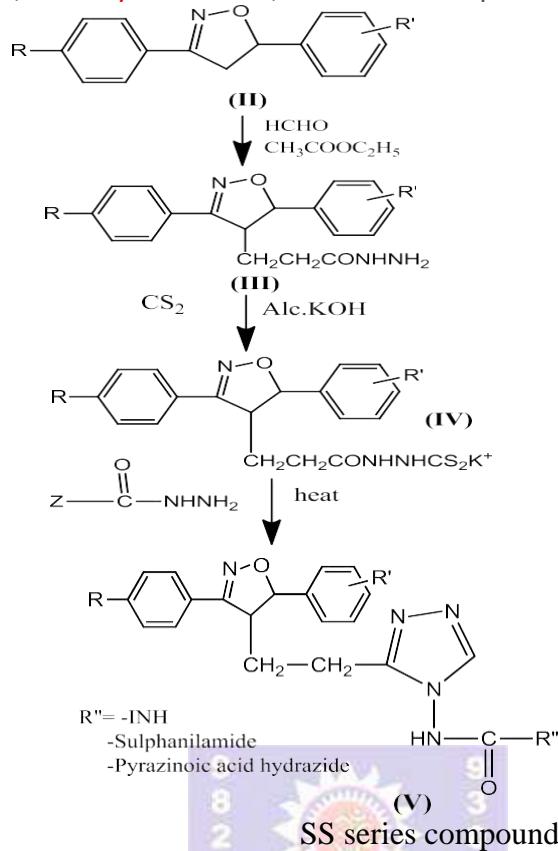
Compound III (0.01 mol) and potassium hydroxide (0.067 g, 0.012 mol) in absolute alcohol (20 ml) was refluxed with carbon disulfide (1.14 g, 0.015 mol) for 6-7 h in RBF. (The mixture used directly for step (V)

Step V) Synthesis of 3-(2-(3, 5-diphenyl-4, 5-dihydroisoxazol-4-yl) ethyl)-4H-1, 2,4-triazol-4-amine (V):

Compound IV potassium dithiacarbazinate (0.01 mol) and substituted hydrazides (0.01 mol) were dissolved in alcohol and refluxed in RBF for 6-10 h. When profuse evolution of hydrogen disulfide was observed. The reaction mixture was cooled and poured in cold water or crushed ice. On acidification with 10 ml HCl, 4-(substituted carboxamide) 3-substituted-5-mercapto-1,2,4-triazole was obtained in good yield as white to brown colour precipitate which was washed with cold water and recrystallized from aqueous ethanol. Following the same procedure, all the derivatives were synthesized. Percentage yield, Melting point and elemental data were recorded in table 1. IR spectral data, ¹HNMR and Mass spectral data stated in table 2.

Figure 1. Synthesis of Compound SS series





(3-(2-(3, 5-diphenyl-4, 5-dihydroisoxazol-4-yl) ethyl)-4H-1,2, 4-triazol-4-amine)

Table 1. Physical and analytical data of synthesized selected 1,2,4 triazole derivatives (SS-series)

Sr. No	R	R'	R''	Compound Code	Molecular Formula	Mol.Wt.	MP($^{\circ}\text{C}$)	RfV value	% Yield
1	-H			SS1	C25H24N6O3S	488.56	97-100	0.54	45
2	-H			SS2	C26H23N5O5S2	549.62	108-110	0.72	40
3	-H			SS3	C25H21N7O4S	515.54	105-107	0.62	55
4	-H			SS6	C35H32N6O4S	632.73	175-178	0.82	56
5				SS7	C34H28Cl2N6O4S	686.13	156-159	0.71	53
6				SS11	C28H27N7O3S	541.62	156-158	0.80	58
7				SS12	C28H28N6O4S2	576.69	85-87	0.63	63
8				SS16	C36H32Cl3N7O3S	749.11	75-78	0.62	67

Synthesis and characterization of substituted isoxazolines (Mannichreaction)

General procedure for the synthesis of substituted 4-methyl-3,5-diphenyl-4,5-dihydroisoxazole (Mannich reaction) : (Chalcone AS1-AS12)

An equimolar quantity (0.004 mol) of substituted amine in 10 ml of ethanol was added and aq. formaldehyde solution dissolve in 10 ml of ethanol. The reaction mixture was stirred for 1h at room temperature and refrigerated for 48 h the product was separated by suction filtration and recrystallize from ethanol.

To a solution of compounds (II) (0.01mol) in methanol (30 ml), formaldehyde (0.02 mol) and corresponding acid hydrazides(0.02 mol) were added. The reaction-mixture was refluxed for 6 - 10 h. The solvent was distilled off and the residue was poured into ice water. The precipitate solid was filtered off, dried and recrystallized from ethanol. Following the same procedure, all the derivatives were synthesized. Percentage yield, Melting point and elemental data were recorded in table 3. IR spectral data, ¹HNMR and Mass spectral data stated in table 4.

Figure 2. Synthesis of Compound SA series

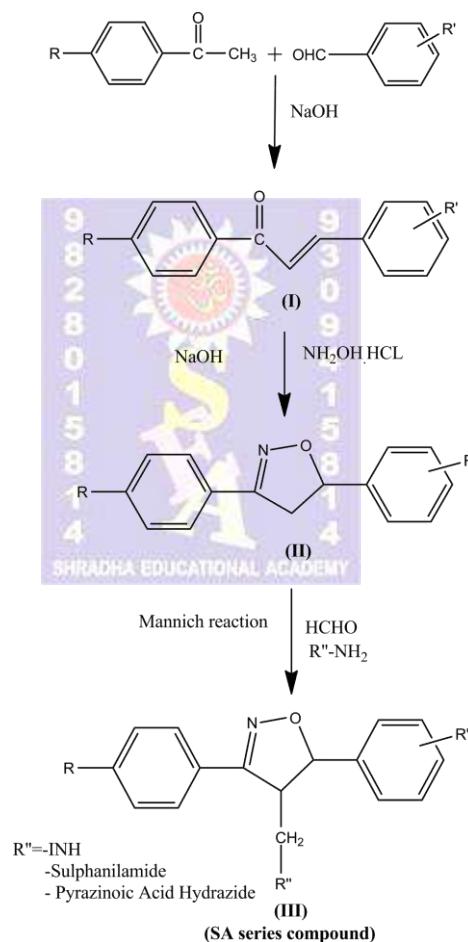


Table 2. Physical and analytical data of synthesized selected Isoxazole derivatives (AS-series)

Sr. No.	R	R'	R''	Compound Code	Molecular Formula	Mol. Wt.	MP (°C)	Rf Value	% Yield
1	-H			AS1	C ₂₂ H ₂₀ N ₄ O ₃	388.42	85-92	0.61	52
2	-H			AS2	C ₂₂ N ₁₁ O ₃ S ₄	423.48	123-128	0.81	68

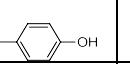
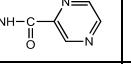
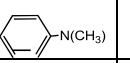
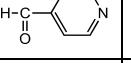
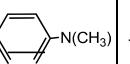
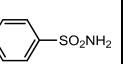
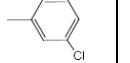
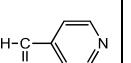
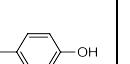
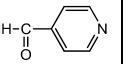
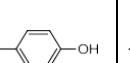
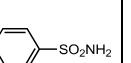
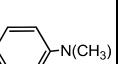
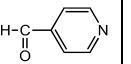
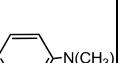
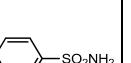
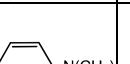
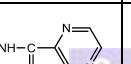
3	-H			AS3	C21H19N5O3	389.41	145-149	0.65	51
4	-H			AS4	C24H25N5O2	415.49	88-90	0.54	40
5	-H			AS5	C24H26N4O3S	450.55	161-163	0.72	65
6	-H			AS6	C24H26N4O3S	450.55	161-163	0.72	65
7				AS7	C23H22N4O4	418.45	270-275	0.62	38
8				AS9	C23H23N3O5S	453.51	175-178	0.82	56
9				AS10	C25H27N5O3	445.51	155-159	0.71	53
10				AS11	C25H28N4O4S	480.58	155-159	0.71	53
11				AS12	C24H26N6O3	446.50	156-158	0.80	58

Table 3. IR Spectral, ¹H NMR and Mass spectral Data of Selected Compound SS and AS series

Compound	IR (KBr) cm ⁻¹	¹ H-NMR (δ ppm)	MS (FAB positive ion mode) m/z
SS1	3407.17 (N-H amine), 3058.24 (Ar-CH), 2916.47, 2890.43 (CH alkane) 1612.54 (c=o amide), 1464.02 (CH al) 898.86 (CH Ar) 1611.58 (C=C Ar)	3.033 (6H Ar-N(CH ₃) ₂), 5.66 (1H isoxazole -O-CH-CH-C=) 2.38 (1H iso CH-H) 3.704 (2H iso CH ₂) 6.73-6.76 (2H , al CH ₂) 6.617 (1H , H) 6.708-6.758 (4H Ar-H), 7.274-7.466 (5H Ar-H) , 7.695-7.866 (4H Ar-H)	481.55
SS12	3214.41 (NH amine), 3058.24 (Ar-CH), 1916.47, 2851.85 (CH alkane), 1662.69 (C=O amide), 1491.02 (CH al), 1364.38 (CH al), 827.49 (CH Ar), 1613.51 (C=C Ar), 787.95 (C-Cl)	3.32 (1H isoxazole ring OCH-CHC=N), 3.65 (1H iso CH-CH) , 3.5 (1H al-CH), 2.1 (2 H al-CH ₂), 6.617 (1H CH), 5-9 (1H NH-C=O), 7.25-7.45(4H Ar-H) 7.51-7-64 (2H Ar-H)	576.69
SS16	3545.28 (NH amine), 3056.31 (Ar-CH), 2919.36, 2850.88 (CH alkane), 1678.13 (C=O amide), 1490.06 (CH al), 1398.44 (S=O), 1154.43 (S=O sym stretch), 927.79 (Ar-CH), 1592.29 (C=C), 825.45(C-Cl)	3.32 (1H isoxazoline ring OCH-CHC=N), 3.65 (1H CH-CH iso), 3.5 (1H CH-al), 2.1 (2H CH-al), 6.906 (1H CH), 2.25 (1H, SO ₂ NH ₂), 7.23-7.48 (4H Ar-CH), 7.49-7.79 (2H Ar.CH)	549.11
AS4	3373.71 (Ar-H), 1381(C=O=N), 1230.63 (CN amine), 1159.26 (S=O Sy.stretch), 1361.79 (S=O Asy. Stretch) 3242.45, 3373.61 (NH sulphonamide) 1546.60 (NH sulphur) 950 (N=O isoxa.) 1309.71 (C-N amine)	2.97 (6H CH ₃ al) , 3.67 (1H isoxz-OCH-CHC=), 3.35 (1H isoxa CH=CH), 6.73 (2H CH-al), 5.65 (1H amine-N), 9.76 (2H SO ₂ NH ₂), 7.28 (4H Ar-H), 7.42 (5H Ar-H), 7.71 & 7.48 (2H Ar-H)	415.49
AS9	3068.85 (Ar-H), 3408.33 (NH hydrazide), 2845.10(CH al), 1664.62 (CONH), 1597.11 (C≡N), 1519.96 (C=C), 750.33 (CH), 1058.96 & 1249.91 (CO Phenylalkyl), 1180.47 (2.93 (6H CH ₃ al), 3.65 (1H isoxOCH-CHC=), 3.06 (1 H isoxz CH-CH), 3.88 (3 H OCH ₃), 6.73 (2H CH-al), 5.62 (2 HNH N-amine), 6.60 & 6.90 (4H Ar-H) , 7.28 & 7.60 (2H Ar-H)	453.51

	N-N hydrazide), 1311.64 (C-O-N), 1365.65 (C-N Ar amine), 1232.55 (CN-amine		
AS 10	3066.92 (Ar-CH), 2839.31 (CH-al), 3375.54 (NH str.), 1244.13 (C-O-N), 1155.40 (S=O), 1350.22 (S=O asym.), 1301.99 (CN amine), 1030.02 & 1244.13 (C-O phenylether)	2.93 (6H al-CH ₃), 3.62 (1H isoxa.ring OCH-CHC=), 3.28 (1H isoxa. CH-CH), 3.842 (3H OCH ₃), 6.71 (2H alCH ₂), 5.59 (1H N-amine), 6.69 & 6.9 (4H Ar-H), 7.226 & 7.63 (2H Ar-H)	445.51

Biological Activity:

Antimicrobial Activity:

Determination of Zone of Inhibititon:

The zone of inhibition of selected test compound of AS and SA series against gram positive bacteria *S. Aureus*, *S. epidermis* and gram negative bacteria *Klebsiella*, *E.Coli* was determined by well diffusion method. In this assay measure diameter inhibition of zone using measuring device to each test substance required to inhibit the growth of micro-organism was determined. Standard Antifungal drug Fluconazole and standard Antibacterial drug Ciprofloxacin was tested at concentration of 10 ug/ml and 30 ug/ml. The plate were inspected visually to determine the growth of the organism as indicated by turbidity. Zone of Inhibition value of each tested compound were recorded in table. 4, 5

Table 4 :Data of Zone of Inhibition of Gram positive and negative bacteria for Isoxazolines

CompoundCode	Meanzoneof inhibition(inmm)																			
	Gram+vebacteria								Gram-vebacteria											
	S.Aureus				S.Epidermis				Klebsiella				E.coli							
Conc.□g/ml	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5
AS1	17	14	11	9	-	28	25	23	15	13	18	15	-	-	-	14	12	10	8	-
AS2	19	15	13	10	8	25	23	18	13	10	15	12	-	-	-	15	12	-	-	-
AS3	18	15	13	11	9	26	24	20	18	15	12	10	-	-	-	18	15	12	-	-
AS4	16	14	10	8	-	27	25	23	21	18	20	18	16	13	-	14	12	-	-	-
AS5	18	16	14	10	-	28	25	23	20	18	18	15	13	-	-	20	17	15	10	-
AS7	18	15	13	10	8	23	20	17	15	13	12	10	-	-	-	15	10	-	-	-
AS9	29	26	22	18	9	28	25	23	22	18	28	21	18	15	12	24	21	19	14	10
AS10	15	10	-	-	-	21	16	12	10	8	21	13	10	-	-	16	13	-	-	-
AS11	17	15	13	10	-	22	20	17	14	10	20	16	14	12	-	15	12	10	-	-
AS12	12	10	8	-	-	28	26	24	20	18	16	14	10	-	-	18	15	12	-	-
Ciprofloxacin(10 μ g)	26				26				30				32							

Table 5 :Data of zone of inhibition of fungi for 1,2, 4-triazoles:

CompoundCode	Meanzoneof inhibition(inmm)									
	A.niger					A.flavus				
Conc.in□g/ml	75	50	25	10	5	75	50	25	10	5
SS1	23	20	18	-	-	23	20	18	-	-
SS3	18	16	15	10	-	19	18	15	14	-
SS4	22	20	19	10	-	23	20	18	15	-
SS6	23	20	18	16	-	24	20	18	-	-

SS7	16	15	13	-	-	17	16	14	-	-
SS11	21	20	18	16	-	23	22	20	-	-
SS12	23	20	18	10	-	24	22	24	26	-
SS16	18	17	16	-	-	23	22	23	25	
Fluconazole(30μg)	26					26				

Table 6 : Data of Zone of Inhibition of Fungi of Isoxazolines

CompoundCode	Meanzoneof inhibition(inmm)									
	Gram+vebacteria									
	<i>A.niger</i>					<i>A.flavus</i>				
Conc.in \square g/ml	75	50	25	10	5	75	50	25	10	5
AS1	30	20	18	-	-	30	28	25	12	10
AS2	32	28	25	20	-	32	28	24	12	10
AS3	30	20	15	-	-	20	15	10	-	-
AS4	26	20	-	-	-	30	25	23	22	20
AS5	28	20	-	-	-	30	28	26	25	15
AS7	26	20	-	-	-	15	10	-	-	-
AS9	30	20	18	15	9	30	28	25	12	10
AS10	18	15	10	-	-	15	13	12	-	-
AS11	20	14	8	-	-	14	12	-	-	-
AS12	18	15	-	-	-	13	10	8	-	-
Fluconazole(30μg)	26					26				

Table 7 : Results of zone of inhibition of bacteria for 1, 2,4-triazoles:

Compoun dCode	Meanzoneof inhibition(inmm)																			
	Gm+vebacteria										Gm-vebacteria									
	<i>S.Aures</i>					<i>S.Epidermis</i>					<i>P.Klebsiella</i>				<i>E.coli</i>					
Conc.in \square g /ml	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5
SS1	23	20	16	10	-	22	20	15	-	-	17	16	14	10	8	23	22	20	18	-
SS3	19	18	16	-	-	22	21	-	-	-	19	15	14	10	-	22	20	18	-	-
SS4	20	18	17	10	-	10	-	-	-	-	22	21	20	18	10	19	16	10	-	-
SS6	24	22	20	10	-	16	10	-	-	-	12	-	-	-	-	24	22	20	-	-
SS7	17	15	10	-	-	20	18	16	-	-	11	10	-	-	-	16	15	13	-	-
SS11	23	20	-	-	-	16	14	-	-	-	18	16	14	-	-	14	12	10	-	-
SS12	24	23	20	-	-	8	-	-	-	-	21	20	18	-	-	24	22	20	18	-

SS16	23	20	17	-	-	12	-	-	-	-	19	18	16	-	-	13	12	10	-	-
Ciprofloxacin(10 μ g)				26			26				30					32				

Antioxidant Activity:

In the DPPH free radical scavenging activity, compounds (AS1-AS12) were evaluated for their free radical scavenging activity with ascorbic acid as standard compounds. The IC₅₀ was calculated for each compound as well as ascorbic acid as standard and summarised in table 6.

When DPPH reacts with antioxidant compounds, which can donate hydrogen, it is reduced. Succeeding the reduction, its deep violet color in methanol bleached to yellow, showing a significant absorption decrease at 517 nm. Then 3ml of various concentrations (2, 4, 8, 16 and 32 μ g/ml) of the compounds (SS61-SS42) dissolved in ethanol were added to 1ml of ethanol solution of DPPH (40 μ g/ml). After a 30 minute incubation period at room temperature, the absorbance was read against a blank at 517nm (Shimadzu UV-Vis spectrophotometer) Ascorbic acid was used as the reference compound. All tests and analyses were done in three replicates and the results were averaged. Free radical DPPH inhibition in percentage (AA %) was calculated as follows:

$$\text{Scavenging Effect (\%)} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{Control}}} \times 100$$

Table 8 : Results of % Scavenging Activity of selected 1,2,4 Triazoles:

Sr.No.	Sample	%ScavengingActivityAtDifferentConcentrations(Mean \pm SD)				
		2 μ g/mL	4 μ g/mL	8 μ g/mL	16 μ g/mL	32 μ g/mL
1.	Ascorbic Acid	19.70 \pm 0.36	25.92 \pm 1.19	37.96 \pm 0.38	55.77 \pm 0.26	89.61 \pm 0.36
2.	SS1	19.84 \pm 0.13	33.44*** \pm 1.01	54.02*** \pm 0.87	62.77*** \pm 0.52	87.83** \pm 0.73
3.	SS3	15.5*** \pm 0.50	28.11*** \pm 0.81	36.75 \pm 0.86	51.11*** \pm 0.34	69.46** \pm 0.53
4.	SS4	17.18*** \pm 0.98	24.68 \pm 0.71	36.5 \pm 1.06	46.27*** \pm 0.55	58.8** \pm 0.87
5.	SS6	13.46*** \pm 0.66	23.86** \pm 0.84	37.29 \pm 0.60	41.21*** \pm 0.56	51.98** \pm 0.63
6.	SS7	16.08*** \pm 0.58	25.32 \pm 0.79	45.71*** \pm 0.48	54.16 \pm 1.06	64.57** \pm 0.21
7.	SS11	14.01*** \pm 0.70	21.06*** \pm 0.66	36.97 \pm 0.89	62.29*** \pm 0.28	74.48** \pm 0.89
8.	SS12	12.35*** \pm 0.54	29.04*** \pm 0.54	34.53*** \pm 0.74	43.87*** \pm 2.11	66.11** \pm 0.97
9.	SS16	13.53** \pm 0.48	24.78 \pm 0.71	32.91*** \pm 0.64	47.38*** \pm 0.66	58.82** \pm 0.88

Table 9 : Results of IC₅₀ antioxidant activity of 1, 2,4-triazoles (DPPH Assay):

Sample	IC ₅₀ (μ g/ml)(n=6)						Mean \pm SD
AA	13.3	14.35	14.22	14.27	14.02	14.23	14.23 \pm 0.11
SS1	11.62	11.54	11.78	11.68	11.37	11.75	11.62*** \pm 0.15
SS3	18.19	18.53	18.2	18.2	18.31	18.29	18.28** \pm 0.12
SS4	22.28	22.23	22.54	22.6	23.29	23.07	22.66** \pm 0.42

SS6	27.26	27.16	27.6	27.1	26.55	27.38	27.17** \pm 0.35
SS7	18.45	18.1	18.61	18.62	18.36	18.14	18.38** \pm 0.22
SS11	16.31	16.47	16.5	16.4	16.62	16.63	16.48** \pm 0.12
SS12	20.77	21.05	20.87	19.98	20.5	20.52	20.61** \pm 0.37
SS16	22.26	22.22	22.76	23.07	23.31	23.3	22.82** \pm 0.49

Table 10. Results of % Scavenging Activity of antioxidant activity of selected Isoxazolines

Sample	% Scavenging Activity At Different Concentrations (Mean \pm SD)				
	2ug/mL	4ug/mL	8ug/mL	16ug/mL	32ug/mL
Ascorbic Acid	19.70 \pm 0.36	25.92 \pm 1.19	37.96 \pm 0.38	55.77 \pm 0.26	89.61 \pm 0.36
AS1	17.13*** \pm 0.39	30.88*** \pm 0.65	42.06*** \pm 0.78	52.44*** \pm 0.89	65.68*** \pm 0.80
AS2	21.82*** \pm 0.58	34.61*** \pm 0.276	48.37*** \pm 0.38	56.17 \pm 0.74	67.02*** \pm 0.77
AS3	17.08*** \pm 0.66	24.20** \pm 0.67	43.20*** \pm 0.56	56.88 \pm 0.88	62.28*** \pm 0.46
AS4	15.26*** \pm 0.79	30.73*** \pm 0.40	38.28 \pm 0.43	45.37*** \pm 0.53	62.90*** \pm 0.47
AS5	15.26*** \pm 0.46	30.73*** \pm 0.77	33.12*** \pm 0.60	47.38*** \pm 1.01	54.86*** \pm 0.69
AS7	16.70*** \pm 0.72	24.92 \pm 0.70	31.07*** \pm 0.74	46.22*** \pm 0.63	61.19*** \pm 1.23
AS9	18.49 \pm 0.64	23.66*** \pm 0.73	29.98*** \pm 0.56	48.76*** \pm 0.66	52.84*** \pm 0.62
AS10	17.48*** \pm 0.66	26.53 \pm 0.66	30.94*** \pm 0.73	39.21*** \pm 0.72	50.52*** \pm 0.67
AS11	18.41*** \pm 0.73	29.53*** \pm 0.82	45.60*** \pm 0.84	47.45*** \pm 0.90	59.00*** \pm 0.97
AS12	27.2*** \pm 0.61	36.83*** \pm 0.55	38.16 \pm 0.87	48.53*** \pm 0.79	57.19*** \pm 0.76

Table 11: Results of IC50 of antioxidant activity of substituted Isoxazoline

SAMPLE	IC50 ug/mL(n=6)						Mean \pmSD
AA	14.3	14.35	14.22	14.27	14.02	14.23	14.23 \pm 0.11
AS1	18.76	17.2347	19.324	18.52	17.32	18.24	18.23*** \pm 0.82
AS2	15.82	14.9989	15.896	16.43	15.87	15.43	15.74* \pm 0.48
AS3	19.28	18.23	18.98	17.99	18.87	19.87	18.87*** \pm 0.68
AS4	21.12	21.23	20.61	20.77	20.25	21.07	20.84*** \pm 0.37
AS5	14.26	15.55	14.67	13.77	14.76	14.35	14.56 \pm 0.59
AS7	23.45	22.38	21.66	22.45	21.55	21.99	22.24*** \pm 0.69

AS9	21.65	20.65	20.21	21.23	22.75	21.52	21.33*** \pm 0.87
AS10	21.65	20.65	20.21	21.43	22.34	20.11	21.06*** \pm 0.88
AS11	21.65	20.48	20.21	21.43	22.34	20.11	21.04*** \pm 0.90
AS12	21.65	21.99	20.77	21.53	22.88	21.78	21.77*** \pm 0.68

Antidiabetic Activity:

In Vitro α glucosidase inhibition activity for the newly synthesized compound was determined by spectrophotometric assay using acarbose as the reference compound. To 50 μ l of enzyme, add 250 μ l of buffer or test sample and incubate at 37°C for 30 minutes. Add 500 μ l of sucrose solution and incubate at 37°C for 20 minutes, heat on boiling water bath for 2 minutes to arrest the reaction and cool. Measure glucose concentration by Glucose Oxidase method. Glucose estimation done by mixing 100 μ l of sample with 500 μ l of glucose reagent (Glucose reagent kit) the incubate at room temperature for 10 minutes. Measure the absorbance at 510 nm. The % inhibition of α -glucosidase is calculated as follows.

$$\% \text{ inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance(test)}}{\text{Absorbance(control)}} \times 100$$

Table 12 : α -Glucosidase inhibition by given test Samples

Sample	Conc.(μ g/ml)	OD@ 405nm	% Inhibition	IC50(μ g/ml)
Control	0	0.998	0.0	101.8
AS2	6.125	0.915	8.3	
	12.5	0.834	16.4	
	25	0.775	22.3	
	50	0.610	38.9	
	100	0.522	47.7	
	200	0.311	68.8	
AS11	6.125	0.958	4.0	IC50 was not calculated due to lesser inhibition
	12.5	0.836	16.2	
	25	0.772	22.6	
	50	0.671	32.7	
	100	0.590	40.9	
	200	0.514	48.5	
SS6	6.125	0.956	4.3	179
	12.5	0.925	7.3	
	25	0.846	15.2	
	50	0.695	30.4	
	100	0.595	40.4	
	200	0.346	65.3	
AS5	6.125	0.924	7.4	104.8
	12.5	0.811	18.8	
	25	0.703	29.5	
	50	0.663	33.6	
	100	0.502	49.7	
	200	0.312	68.7	

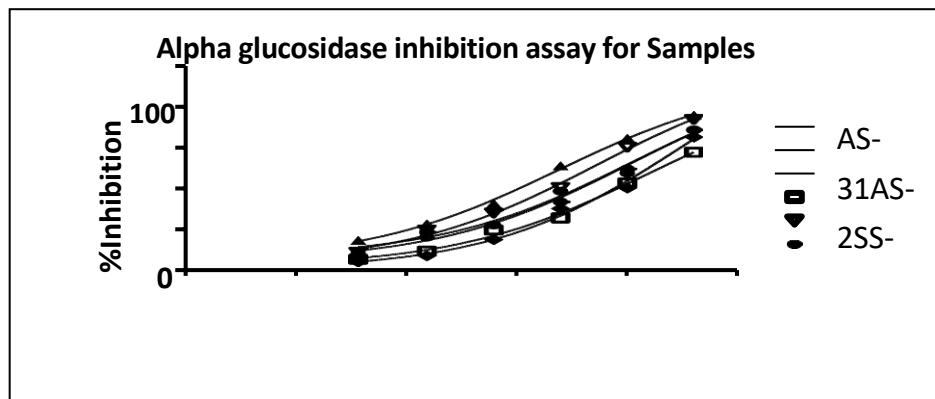


Figure 3 : IC50 plots for α -Glucosidase inhibition by given test Samples

	AS-2	AS-31	SS-6	SS-26	AS-11
LogIC50	2.008	1.690	2.253	1.862	2.021
IC50	101.8	48.99	179.0	72.74	104.8

Anti-Tubercular Activity:

Compound AS1-SS12 were evaluated for their in vitro antitubercular activity against *M. tuberculosis*. In Vitro antimycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA). 200 μ l of sterile deionized water was added to all outside perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells throughout incubation. The 96 wells plate got 100 μ l of the Middle brook 7H9 broth and sequential dilution of compounds were made straight on plate. The tested final drug concentrations were 100 to 0.2 μ g/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 μ l of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24h. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink. The result of Anti-tubercular activity were recorded in table 6.

Table 13 : Results of Antitubercular Activity

Sr. No.	Sampl es	100 μ g/m l	50 μ g/ ml	25 μ g/ ml	12.5 μ g/m l	6.25 μ g/m l	3.12 μ g/m l	1.6 μ g/ ml	0.8 μ g/ ml
1	AS1	S	S	R	R	R	R	R	R
2	AS2	S	S	R	R	R	R	R	R
3	AS3	S	S	R	R	R	R	R	R
4	AS4	S	S	R	R	R	R	R	R
5	AS5	S	S	R	R	R	R	R	R
6	AS7	S	S	S	R	R	R	R	R
7	AS9	S	S	S	S	R	R	R	R
8	AS10	S	S	S	S	R	R	R	R
9	AS11	S	S	S	R	R	R	R	R

10	AS12	S	S	S	R	R	R	R	R
11	GS3	S	S	R	R	R	R	R	R
12	GS12	S	S	S	S	R	R	R	
13	SS1	S	R	R	R	R	R	R	R
14	SS4	S	R	R	R	R	R	R	R
15	SS6	S	R						

Result and Discussion:

Initial chalcones were synthesized by using differently substituted acetophenone and aromatic aldehyde following Claisen-Schmidt's condensation reaction. Further the chalcones were treated with nucleophilic reagent hydroxyl amine hydrochloride under reflux to get isoxazoline compounds. The target compounds, 3-(2-(3, 5-diphenyl-4, 5-dihydroisoxazol-4-yl) ethyl)-4H-1, 2, 4-triazol-4-amine (SS1,SS2,SS3,SS6,SS7,SS11, SS12&SS16) are synthesized from mono potassium (II) mono (2-(3-(3,5-diphenyl-4,5-dihydroisoxazol-4-yl) propanoyl) hydrazine carbodithioate) (IV) by using methanol as solvent and using isonicotinic acid hydrazide, pyrazinoic acid hydrazide and sulphanilamide. Structures of all derivatives have been elucidated by ¹H-NMR, HR-MS and IR spectral measurements. The solid state IR (ATR,cm⁻¹) spectra of these compounds reveal a characteristic N-H Stretch secondary amine 3500-3100 of hydrazide and aromatic Stretch between 3150-3050cm⁻¹. The C-H stretch alkane group present in the at 3000-2850 cm⁻¹. The C-H bend (aliphatic -CH₂bend) group reveal peaks at 1375-1465 cm⁻¹. The C=C group of Aromatic ring showed stretching vibrations at around 1600-1475 cm⁻¹. The ¹H NMR spectra of all target derivatives (SS1,SS2,SS3,SS6,SS7,SS11,SS12&SS16) were recorded in CDCl₃. ¹HNMR has revealed signal around at δ3.32-3.70 accounting for isoxazoline nucleus. Signal for the aromatic protons were present in between δ 8 and 7. Thus, all the protons were accounted for the respective structures. Mass spectra of the compounds SS series showed molecular ion peaks with high abundance at m/z in agreement with their molecular formula.

Similar procedure involved for Isoxazoline derivative by using Mannich base. The target compounds, Mannich bases of isoxazolines (**AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS9,AS10,AS11,AS12**) are synthesized from isoxazoline by using methanol as solvent and using isonicotinic acid hydrazide and sulphanilamide with formaldehyde. Structures of all derivatives have been elucidated by ¹H-NMR, HRMS and IR spectral measurements. The solid state IR(ATR,cm⁻¹) spectra of these compounds reveal a characteristic N-H Stretch 3200-3408 of hydrazide and aromatic Stretch between 3000-3200 cm⁻¹. The imine group of Isoxazoline ring (C=N) group present in the isoxazoline ring reveal peaks at 1600-1550 cm⁻¹. The C=C group of Aromatic ring showed stretching vibrations at around 1400-1500 cm⁻¹. C-O-N group reveal peaks at 1250-1396 cm⁻¹. The ¹H NMR spectra of all target derivatives (**AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS9,AS10,AS11,AS12**) were recorded in CDCl₃. ¹HNMR has revealed signal around at δ3.62-3.74 accounting for isoxazoline nucleus. Signal for the aromatic protons were present in between δ 8 and 7. Thus, all the protons were accounted for the respective structures.

Newly synthesized derivatives of selected AS and SS series were tested for invitro Antimicrobial activity. From the structure of potent antimicrobial compounds amongst the synthesized series it can be conclude that groups like -Cl, -OH and OCH₃ at substituent on phenyl ring as well as isonicotinic acid hydrazide / sulphanilamide on isoxazoline positively contributes for antimicrobial potential. In compounds (SS11,SS12andSS42) chlorophenyl substituent on Isoxazoline ring are present at triazole ring which different contributed in the radical scavenging ability. Majority of the tested compounds in these series showed good interaction with the DPPH radical at 32±g/ml concentration. Maximum DPPH radical

scavenging activity was observed in compound SS6 (87.83 % with an IC₅₀ of 11.62±0.06) which is highest among the synthesized derivatives or comparable with standard antioxidant ascorbic acid (89.61 % with an IC₅₀ of 14.23±0.04) at the same concentration. It appears that compounds SS7, SS16, SS26 and SS41 are also significant scavengers of the DPPH radical with % inhibition (69.46% (18.28±0.059), 64.57% (18.38±0.09), 74.48% (16.48±0.05), 66.11% (20.61±0.15) respectively). In Antioxidant results showed that good efficacy. This increased activity may be due to existence of the -NH groups. In Selected Isoxazoline series (AS1-AS12) Compound AS11 have shown promising antioxidant activity at IC₅₀ (14.56±0.59) while AS2 (15.74*±0.48) showed a moderate antioxidant activity when compared with standard drug ascorbic acid (14.23±0.11). In compound of 1, 2, 4 triazole and Isoxazoline series. The majority of compounds exhibited a varying degree of α -glucosidase inhibitory activity with IC₅₀ values between 20.7 and 61.1 μ M when compared with standard acarbose (IC₅₀ = 1.131 μ g/ml). Among the series, compounds (SS1, SS11, AS2, AS11) displayed potent inhibitory activity with IC₅₀ values of 123.7, 179, 72.74, 101.8 and 104.8 μ g/ml. The Sample AS-31 has shown better inhibitory activity against α -Glucosidase activity with 75.9% inhibition with IC₅₀ of 48.99 μ g/ml compared to other Samples. Compound of AS and SS series Derivatives AS42 and SS12 showed equivalent antitubercular activity as ciprofloxacin and pyrazinamide at 3.125 μ g/ml conc. and were the potent antitubercular derivatives. Derivatives AS21, AS22, AS36, AS42, AS21 and SS12 showed equivalent antitubercular activity as streptomycin at 6.25 μ g/ml concentration.

Conclusion:

In summary, We have designed and synthesized new 1, 2, 4 triazole and Isoxazoline derivative. There chemical structures were fully confirmed by IR, H-NMR, Mass spectra and elemental analysis. The obtained result indicated that the synthesized compound possessed relatively high to moderate Antioxidant, Antimicrobial, Antidiabetic and Anti-tubercular Activity. The DPPH radical scavenging activity of 1,2,4 triazole derivatives containing -SH and -NH groups (SS6) have shown comparable antioxidant potential with the Ascorbic acid. Maximum DPPH radical scavenging activity was observed in compound SS6 which is highest among the synthesized derivatives or comparable with standard antioxidant ascorbic acid. The compounds SS12, SS5, SS11, SS16, SS1 and SS3 exhibited promising activity against fungi. Compound SA2 and SA5 shown that the isoxazoline derivative exhibits better antioxidant activity. The compounds SA1, SA2, SA9, SA5, SA4 and SA7 exhibited promising activity against fungi. Isoxazoline derivative containing -Cl, -OH and OCH₃ at substituent on phenyl ring as well as isonicotinic acid hydrazide / sulphanilamide on isoxazoline positively contributes for antimicrobial potential. Those compound shown promising antioxidant activity were screened for *invitro* α -glucosidase activity. Compounds SS1, SS11, AS5, AS2, AS11 displayed potent inhibitory activity with IC₅₀ value. Derivatives SA 2 and SS12 showed equivalent antitubercular activity as ciprofloxacin and pyrazinamide and were the potent antitubercular derivatives.

References:

1. Leila Emami, Sara Sadeghian, Ayyub Mojaddami et al., Design, synthesis and evaluation of novel 1,2,4-triazole derivatives as promising anticancer agents, Emami et al. *BMC chemistry* (2020) 16:19, pp. 1-18.
2. Navin B. Patel, Faiyazalam M. Sheikh, Synthesis and antimicrobial activity of new thiazolidine derivatives containing 2 amino-6-methoxybenzothiazole, *Saudi pharmaceutical journal* (2010) 18, pp-129-136
3. Aysel Gursoy, NalanTerzioglu, Synthesis and isolation of new Regiosomeric-4-thiazolidinones and their anticonvulsant activity, *Turk J Chem*, 29, 2005, pp. 247- 254.
4. Hunashal RD, Ronad PM, Maddi VS, Satyanarayana D et al., Synthesis, anti-inflammatory and analgesic activity of 2-[4-(substituted benzylideneamino)-5-(substitutedphenoxyethyl)-4H-1,2,4-triazol-3-yl-thio] acetic acid derivatives. *Arabian J Chem* 2011; pp. 1-9.

5. C. Tratat, M. Haroun, A. Paparisva et al., Design, synthesis and biological evaluation of new substituted 5-benzylideno-2-adamantylthiazo (3,2-b) 1, 2, 4 triazole-6(5H) ones. Pharmacophore models for antifungal activity. *Arabian Journal of Chemistry* (2018) 11 pp. 573-590
6. Kane MJ, Dudley MW, Sorensen MS et al., Synthesis of 1,2,4-Dihydro-3H-1,2,4 triazole-3-thiones as potential antidepressant agents. *J Med Chem* 1988; 31: 1253-8.
7. Nareshvarma seelam, S. P. Shrivastava, Prasanthi et al., Synthesis and in vitro study of some fused 1, 2, 4 triazole derivatives as antimycobacterial agents. *Journal of Saudi chemical society* (2016) 20 pp. 411-418
8. Xiao Z, Waters NC, Woodard CL, Li PK. Design and synthesis of pfmrk inhibitors as potential antimalarial agents. *Bioorg Med Chem Lett* 2001; 11: 2875-8.
9. Yadav D. Bodke, Arun Sinde, Shankarappa Biradar et al., Synthesis, invitro and invivo anti-hyperglycemic activity of 1,2,4 triazolebenzylidene and 1,3,4 thiadiazole derivatives. *IJPR* (2014) 4 pp. 1245-1255.
10. Santos MM, Faria N, Iley J et al., Reaction of naphthoquinones with substituted nitromethanes. Facile synthesis and antifungal activity of naphtho[2,3-d]isoxazole-4,9-diones. *Bioorg Med Chem Lett* 2010; 20: 193-5.
11. Hamed Ead, Nadia H metwalli, Nagwa M Morsi, Synthesis and antimicrobial activity of 5-(2-thienylmethylene) derivatives of thiazolidin-4-one by cyclo-addition reactions, *ArchPharm Res*, 13(1), 1990, pp. 5-8.
12. Zhengming Li, Yuxin Li, Wei Chen et al., Synthesis and anti-tumor activity of series of regioselective3-thiazolidine acetic acid derivatives, *ChemBiol Drug Des*, 78, 2011, pp. 969-978.
13. Jayashankar B, Rai KM, Baskaran N et al., Synthesis and pharmacological evaluation of 1,3,4-oxadiazole bearing bis(heterocycle) derivatives as anti-inflammatory and analgesic agents. *Eur J Med Chem* 2009; 44 pp. 3898-3902.
14. Monforte P, Grasso S, Chimirri A, et al., antitumor activity of series of 2-alkyl-3-[2-(1, 3, 4-thiadiazolyl)]-4-thiazolidinones, *FarmacoSci*, 43(10), 1988, pp. 851-856.
15. Mazzei M, Balbil A, Sotofattoril E et al., Synthesis of new 3,5disubstituted isoxazoles with specific anti-group B rhinovirus activity in vitro. *Eur J Med Chem* 1993; 28: 669-74.
16. Barcelo' M, Ravina E, Masaguer CF et al., Synthesis and binding affinity of new pyrazole and isoxazole derivatives as potential atypical antipsychotics. *Bio org Med Chem Lett* 2007; 17: pp. 4873-77.
17. Kumar A, Maurya RA, Sharma S et al., Design and synthesis of 3,5-diarylisoaxazole derivatives as novel class of antihyperglycemic and lipid lowering agents. *Bio org Med Chem* 2009; 17: 5285-92.