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Synthesis, characterisation and in vitro Evaluation of some novel 1h-1, 2, 4-Triazole-3-Thiol derivatives

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Abstract
Conventional synthesis of 1H-1,2,4-triazole-3-thiol derivatives have been synthesized at laboratory scale by condensation of 5-oxazolones prepared from various aldehydes with thiosemicarbazide. The compounds structures were characterized by IR, H^1 NMR and C^{13} NMR. All the compounds have been evaluated for antimicrobial and antioxidant activity by in vitro methods and were compared with their corresponding standards. Of all the synthesised derivatives compounds **3a, 5a, 7a, 12a** exhibited good antimicrobial properties.

Keywords*:* 1,2,4-triazole-3-thiol, antimicrobial, antifungal and antioxidant activity.

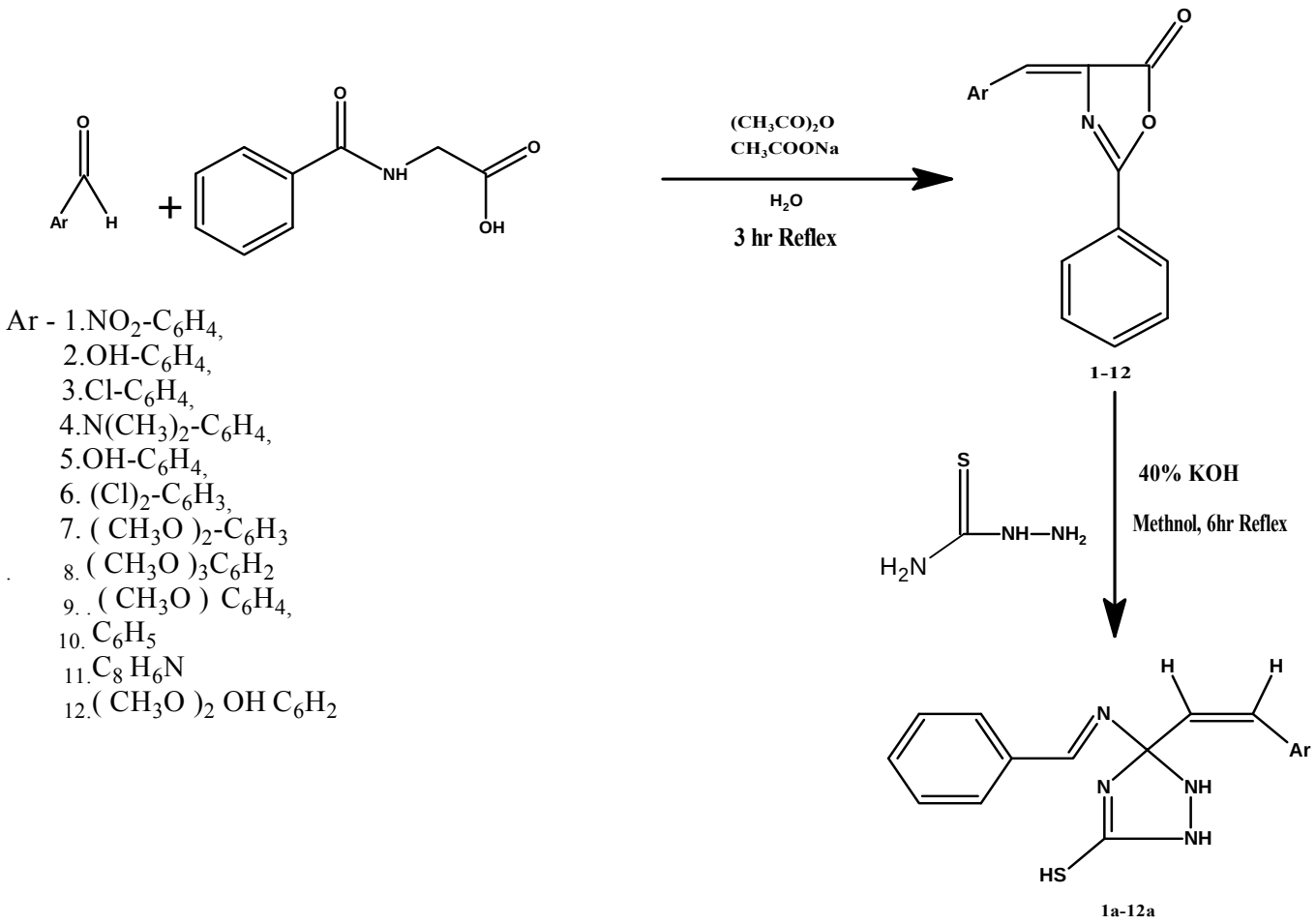
1. Introduction
1, 2, 4-Triazole rings are relatively stable heterocyclic rings and its derivatives exhibited a wide range of applications. They exhibit significant biological and pharmacological activities such as antibacterial [1], antifungal [2], antiviral [3], anticonvulsant [4], anti-inflammatory [5], analgesic [6], antitumour [7], antitubercular, antimalarial, antimigraine, potassium channel activators [8], anti-convulsant [9], anticancer[10], hypoglycaemic [11-12], antidepressant [13], anti-proliferative [14],and antioxidant [15]. The triazole moieties are also used for treating non-Hodgkin's lymphoma [16]. Moreover, substituted 1,2,4-triazoles are an important common structural motifs with wide applications in coordination chemistry [17-18] and material chemistry [19-21]. 3,4,5-Trisubstituted 1,2,4-triazoles also act as intermediates in the synthesis of many drugs available in the market such as maraviroc (UK-427,857) [22], triazolam [23] and sitagliptin (MK-0431) [24].There are several methods for the synthesis 1,2,4-triazoles from amidines and nitriles [25], thioamides and hydrazides [26], acyl hydrazines [27], diacylhydrazide, acyl thiocyanates, esters, hydrazides, thiosemicarbazone, aminoguanidine,1,2,4-oxadiazole.[28] However, some of these methods have disadvantages such as long reaction times, low yields, difficulty in preparation of starting materials and tedious workup, due to which, there is still scope to find new methods for the synthesis of 1,2,4-triazoles.
In the present work, we reported an efficient method for the synthesis for 1,2,4-triazole derivatives by conventional method and evaluated antimicrobial, antioxidant activities by i*n vitro* assays.

2. Materials and Methods
Melting points were determined in open glass capillaries using Gallen Kamp (MFB-600) melting point apparatus and were uncorrected. IR spectra (KBr discs) Bruker analyzers were confirmed by Shimadzu FT-IR Spectrophotometer using KBr pellets technique, ModelNo.8400S (Japan). 1H NMR spectra were recorded on Bruker 400 MHz NMR spectrometer (Switzerland) using DMSO as solvent. T.L.C. was run on silica gel G plates using ethyl acetate: n-Hexane (7:3) as developing solvent. All other chemicals used in the present study were either of A.R or G.R quality.

2.1. Drugs and Chemicals
Hippuric acid – (LOBA-B.NO-G228507), Acetic anhydride – (FISHER'S SCIENTIFICB.NO-92757004-2), Sodium Acetate – (FINAR-B.NO-19095780), Methanol (SD FINE-CHEMLIMITED- B.NO-IOZA-0502-0409-13),Glacial acetic acid – (LOBA-B.NO-LL13871205), Charcoal – (QUALINGENS-BNO-17335406-S), Ethanol – (CSS-B.NO-110605), N,N-Dimethylformamide (DMF) – (LOBAB.NO-LIO1571306), Thiosemicarbazide– (LOBA-B.NO-G51851303).

2.2 General method for the Synthesis of1H-1,2,4-triazole-3-thiol derivatives(1a-12a)

The synthesis was carried out in two steps which includes synthesis of 4-benzylidene-2-phenyloxazol-5(4H)-ones (1-12) as step-1 product obtained by condensation of 0.01 moles of hippuric acid with 0.02 moles of different types of aromatic aldehydes in presence of 0.075 moles of acetic anhydride and 0.025 moles of sodium acetate with 2ml of water refluxed for 3hr. The reaction mixture was cooled; precipitate was filtered, dried, recrystallized from methanol. The product from step1 condensed with equimoles (0.001moles)of thiosemicarbazide in the presence of methanol and a few drops of 40% KOH refluxed for 6hrs, cooled; the product formed was filtered, dried and recrystallized from methanol. The progress and the purity of the reaction were confirmed by thin layer chromatography and melting point. The procedure was illustrated under **Scheme 1** and the physical data were tabulated in **Table 1**.



Scheme 1

Table 1: Physical Data

Code	Compound	M.F	M.W	MP(°c)	%Yield	C%	H%	O%	N%	S%	Cl%
1a	2-((1Z)-2-(3E)-3-(benzylideneamino)-2,3-dihydro-5-mercapto-1H-1,2,4-triazol-3-yl)vinyl)phenol	$C_{17}H_{16}N_4OS$	324	204	61	62.94	4.97	17.27	4.93	9.88	-
2a	(5E)-5-(4-nitrostyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol	$C_{17}H_{15}N_5O_2S$	353	201	63	57.78	4.28	19.82	9.05	9.07	-
3a	(5E)-5-(4-(dimethylamino)styryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3thiol	$C_{19}H_{21}N_5S$	351	168	65	64.93	6.02	-	19.93	9.12	-
4a	(5E)-5-((Z)-2-(1H-indol-3-yl)vinyl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-thiol	$C_{19}H_{17}N_5S$	347	223	67	65.68	4.93	-	20.16	9.23	-
5a	4-((1Z)-2-((3E)-3-(benzylideneamino)-2,3-dihydro-5mercapto-1H-1,2,4-triazol-3-yl)vinyl)2,6-dimethoxyphenol	$C_{19}H_{20}N_4O_3S$	384	184	69	59.36	5.24	12.48	14.57	8.34	-
6a	(5E)-5-(benzylideneamino)-2,5-dihydro-5-sryryl-1H-1,2,4-triazole-3-thiol	$C_{17}H_{16}N_4S$	308	180	68	66.21	5.23	-	18.17	10.40	-

7a	(5E)-5-(3,4,5-trimethoxystyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol	$C_{20}H_{22}N_4O_3S$	398	168	66	60.28	5.56	12.05	14.06	8.05	-
8a	(5E)-5-(4-tmethoxystyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol	$C_{18}H_{18}N_4OS$	338	186	65	63.88	5.36	4.73	16.56	9.47	-
9a	(5E)-5-(2,3-dichlorostyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol	$C_{17}H_{14}Cl_2N_4S$	377	191	62	54.12	3.74	-	14.85	8.50	18.79
10a	(5E)-5-(4-chlorostyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol	$C_{17}H_{15}ClN_4S$	342	190	60	59.56	4.41	-	16.34	9.35	10.34
11a	(5E)-5-(3,5-dimethoxystyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol	$C_{19}H_{20}N_4O_2S$	368	167	65	61.94	5.47	8.68	15.21	8.70	-
12a	4-((1Z)-2-(3E)-3-(benzylideneamino)-2,3-dihydro-5-mercapto-1H-1,2,4-triazol-3-yl)vinyl)phenol	$C_{17}H_{16}N_40S$	324	213	67	62.94	4.97	4.93	17.27	9.88	-

Compound 1a: 2-((1Z)-2-(3E)-3-(benzylideneamino)-2,3-dihydro-5-mercapto-1H-1,2,4-triazol-3-yl)vinyl)phenol: Yield 61 %, mp. 204 °C;FTIR (γ max, cm^{-1})1521 (-***C*=*N*** stretch), 3164 (=***C*-*H*** stretch), 989 (=***C*-*H*** bend), 2318 (-**SH**), 1613 (-**C=C**), 3445 (-**NH** 2^0 amine stretch), 3361 (-OH stretch), 1113(-C-O); ^{1}H NMR(400MHZ,DMSO): ***δ*** 8.640(s, ***J*** 1.00 N=C**H**),***δ***7.381, 7.192 (C**H**=C), ***δ*** 7.987, 7.970, 7.966, 7.822, 7.805, 7.789, 7.786, 7.668, 7.650 (d, ***J*** 0.12, m, ***J*** 2.44 Ar –**H**), ***δ*** 1.839 (s, ***J*** 0.08 C-S-**H**)***δ*** 4.497 (s, ***J*** 2.00 C-O**H**)***δ*** 2.515, 2.510, (C-N**H**) ; ^{13}CNMR (400MHZ,DMSO): ***δ*** 130.26, 128.66, 127.59, 126.77, 125.05, 124.14, 119.30, 115.96, 114.83 (Ar-**C**), ***δ*** 157.87 (**C=N**), ***δ*** 150.24 (**C-OH**), ***δ*** 123.3, 129.5 (**C=C**), ***δ*** 165.88(**C-SH**).

Compound 2a:(5E)-5-(4-nitrostyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol: Yield 63 %, mp. 353 °C; FTIR (γ max, cm^{-1})1615 (-***C*=*C*** stretch), 3170(=***C*-*H*** stretch), 993 (=***C*-*H*** bend), 2064 (**-SH**), 1525 (**-C=N**), 3366 (-**NH** 2^0 amine stretch),1525 (Asymmetric stretch-**NO_2**),1409 (Symm etric stretch- **NO_2**);^{1}H NMR (400MHZ,DMSO):***δ*** 8.11 (s, ***J*** 1.00 N=C**H**),***δ***6.06, 6.69 (C**H**=C), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 7.56, 7.56, 8.14, 8.14 (d, ***J*** 0.13, Ar **-H**), ***δ*** 1.7 (C-S-**H**) ***δ*** 2.5, 2.5 (C-N**H**) ; ^{13}CNMR (400MHZ,DMSO): ***δ*** 129.2, 128.2, 131.1, 128.9, 129.2, 127.3, 127.3, 123.8, 123.8 (Ar-**C**), ***δ*** 160.9 (**C=N**), ***δ*** 123.3, 129.5 (**C=C**), ***δ*** 165 (**C-SH**), ***δ*** 147.2 (**C**-NO).

Compound3a:(5E)-5-(4-(dimethylamino)styryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3thiol: Yield 65 %, mp. 351 °C;FTIR (γ max, cm^{-1}) 1614 (-***C*=*C*** stretch), 3174 (=***C*-*H*** stretch), 993 (=***C*-*H*** bend), 2318 (C-**SH**), 1540 (-**C=N**), 3380 (-**NH** 2^0 amine stretch), 1540 (-C-N), 1407(-CH_3 bend); ^{1}H NMR (400MHZ,DMSO):***δ*** 8.11 (s, ***J*** 0.99 N=C**H**),***δ***5.76, 6.55 (C**H**=C), ***δ*** 7.62, 7.62, 7.29, 7.29, 7.29, 7.12, 6.54, 7.12 (m, ***J*** 0.22, Ar **-H**), ***δ*** 1.50 (C-S-**H**) ***δ*** 2.0, 2.0 (C-N**H**), ***δ*** 2.85, 2.85 (N-C**H**$_3$) ; ^{13}CNMR (400MHZ,DMSO): ***δ*** 129.2, 128.9, 131.1, 128.9, 129.2, 127.3, 114.2, 127.3, 114.2 (Ar-**C**), ***δ*** 160.9 (**C=N**), ***δ*** 123.3, 129.5 (**C=C**), ***δ*** 163 (**C**-SH) ***δ*** 40.3, 40.3 (N-**C**H$_3$).

Compound 4a:(5E)-5-((Z)-2-(1H-indol-3-yl)vinyl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-thiol: Yield 67 %, mp. 347 °C;FTIR (γ max, cm^{-1}) 1611 (-***C*=*C*** stretch), 3159 (=***C*-*H*** stretch), 991 (=***C*-*H*** bend), 2318 (-SH), 1526 (**-C=N**), 3359 (-**NH** 2^0 amine stretch);); ^{1}H NMR (400MHZ,DMSO):***δ*** 8.11 (s, ***J*** 0.99 N=C**H**),***δ***5.76, 6.55 (C**H**=C), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 7.00, 7.00, 7.00 (m, ***J*** 0.35 Ar **-H**), ***δ*** 1.50 (C-S-**H**) ***δ*** 2.0, 2.0, 10.1 (C-N**H**) ; ^{13}CNMR (400MHZ,DMSO): ***δ*** 129.2, 128.9, 131.1, 128.9, 129.2, 111.1, 120.1, 122.2, 119.0 (Ar-**C**), ***δ*** 130.8, 135.5 (**C-NH**), ***δ*** 129.3, 129.5 (**C=C**), ***δ*** 163 (**C-SH**).

Compound 5a:4-((1Z)-2-((3E)-3-(benzylideneamino)-2,3-dihydro-5-mercapto-1H-1,2,4-triazol-3-yl)vinyl)2,6-dimethoxyphenol: Yield 69 %, mp.384 °C;FTIR (γ max, cm^{-1})1615 (-***C*=*C*** stretch), 3173 (=***C*-*H*** stretch), 994 (=***C*-*H*** bend), 2065 (-SH-), 1539 (-**C=N**), 3355 (-**NH** 2^0 amine stretch),3255 (**-OH** stretch), 1289 **(-C-O)**;^{1}H NMR (400MHZ,DMSO): ***δ*** 3.73, 3.73 (O-C**H**$_3$),***δ*** 5.0 (C-OH), ***δ*** 8.11 (s, ***J*** 1.00 N=C**H**),***δ***5.76, 6.55 (C**H**=C), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 6.20, 6.20 (d, ***J*** 0.12, Ar **-H**), ***δ*** 1.50 (C-S-**H**) ***δ*** 2.0, 2.0 (C-N**H**); ^{13}CNMR (400MHZ,DMSO): ***δ*** 129.2, 128.9, 131.1, 128.9, 129.2, 104.3, 104.3, (Ar-**C**), ***δ*** 160.9 (**C=N**), ***δ*** 129.3, 129.5 (**C=C**), ***δ*** 163 (**C-SH**) ***δ*** 56.2, 56.2 (O-**C**H$_3$) ***δ*** 132.0 (**C**-OH).

Compound 6a:(5E)-5-(benzylideneamino)-2,5-dihydro-5-sryryl-1H-1,2,4-triazole-3-thiol : Yield 68 %, mp. 308 °C;FTIR (γ max, cm^{-1}) 1610(-***C*=*C*** stretch), 3163 (=***C*-*H*** stretch), 990 (=***C*-*H*** bend), 2319 (**-SH**), 1524 (**-C=N**), 3355 (-**NH** 2^0 amine stretch); ^{1}H NMR (400MHZ,DMSO): ***δ*** 8.11 (s, ***J*** 1.01 N=C**H**),***δ***5.76, 6.55 (C**H**=C), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 7.30, 7.30, 7.21, 7.14, 7.21 (m, ***J*** 0.47 Ar **-H**), ***δ*** 1.50 (C-S-**H**) ***δ*** 2.0, 2.0 (C-N**H**) ; ^{13}CNMR (400MHZ,DMSO): **δ** 129.2, 128.9, 131.1, 128.9, 129.2, 126.4, 128.7, 128.0, 128.7, 126.8, 135.2 (Ar-**C**), ***δ*** 160.9 (**C=N**), ***δ*** 129.3, 129.5 (**C=C**), ***δ*** 163 (**C-SH**).

Compound7a:(5E)5(3,4,5trimethoxystyryl)-5(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol: Yield 66 %, mp. 398 °C;FTIR (γ max, cm^{-1}) 1617 (-***C*=*C*** stretch), 3169 (=***C*-*H*** stretch), 993 (=***C*-*H*** bend), 3000 (**-SH**), 1529 (**-C=N**), 3366 (-**NH** 2^0 amine stretch),1282 (-C-O); ^{1}H NMR(400MHZ,DMSO): ***δ*** 1.682 (s, ***J*** 3.00 C-S**H**), ***δ***8.630 (s, ***J*** 2.02 N=C**H**), ***δ*** 7.531, 7.62, 7.210, 7.29,7.29, 6.26, 6.26 (s, ***J*** 4.84, Ar-**H**), *δ* 3.828, 3.742, 3.684 (m, ***J*** 14.26, O-C**H**$_3$), *δ* 2.510, 2.00 (C-N**H**),***δ***4.282(C**H**=C),;^{13}CNMR(400MHZ,DMSO): **δ** 129.2, 128.9, 131.1, 128.9, 103.9, 103.9 (Ar-**C**),***δ*** 181.14 (**C**-SH), ***δ*** 164.21(**C**-N), ***δ*** 174.46 (N=**CH**),***δ*** 123.3, 129.5 (**C=C**), ***δ*** 40.13, 39.92, 39.71 (O-**C**H$_3$).

Compound 8a: (5E)-5-(4-tmethoxystyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol: Yield 65 %, mp. 338 °C;FTIR (γ max, cm^{-1}), 1607 (-

C*=*Cstretch), 3173 (=***C-H*** stretch), 992 (=***C-H*** bend), 2318 (-**SH**), 1560 (-**C=N**), 3351 (-**NH** 2^0 amine stretch),1250 (-C-O);^{1}H NMR(400MHZ,DMSO): ***δ*** 1.5 (C-S**H**), ***δ***8.11 (s, ***J*** 2.03 N=C**H**), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 7.19, 6.72, 6.72, 7.19 (s,***J*** 4.84, Ar-**H**),***δ***5.76, 6.55 (C**H**=C), *δ* 3.73 (-C**H**$_3$), *δ* 2.0, 2.0 (C-N**H**);^{13}CNMR(400MHZ,DMSO): **δ** 129.2, 128.9, 131.1, 128.9, 129.2, 127.4, 114.2, 114.2, 127.4, (Ar-**C**),***δ*** 160.9 (**C=NH**), ***δ*** 123.3, 129.5 (**C=C**), ***δ*** 163 (**C**-SH) ***δ*** 55.9 (**C**H$_3$).

Compound 9a: (5E)-5-(2,3-dichlorostyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol: Yield 62 %, mp. 377 °C;FTIR (γ max, cm^{-1}) 1612 (-***C=C*** stretch), 3170 (=***C-H*** stretch), 991 (=***C-H*** bend), 2510 (C-**SH**), 1520 (-**C=N**), 3252 (-**NH** 2^0 amine stretch),789 (-C-Cl);^{1}H NMR(400MHZ,DMSO): ***δ*** 8.619 (s, ***J*** 1.03 N=C**H**), ***δ*** 7.538, 7.187, 7.62, 7.29, 7.29, 7.12, 7.03, 7.09 (d, ***J*** 2.06 Ar-**H**),***δ***5.92, 6.82 (C**H**=C), ***δ*** 2.510 (S-**H**)***δ*** 2.0, 2.0 (C-N**H**);^{13}CNMR (400MHZ,DMSO): ***δ*** 128.9, 129.92, 131.1, 128.9, 129.2,129.5 (Ar-**C**), ***δ*** 123.13,129.5 (C=**C**), ***δ*** 181.19 (**C**-SH), ***δ*** 160.9 (**C**=N), ***δ*** 128.1, 133.3(**C**-Cl).

Compound 10a: (5E)-5-(4-chlorostyryl)-5-(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol: Yield 60 %, mp. 342 °C;FTIR (γ max, cm^{-1}) 1611 (-***C=C*** stretch), 3177 (=***C-H*** stretch), 988 (=***C-H*** bend), 2310 (-**SH**), 1518 (-**C=N**), 3250 (-**NH** 2^0 amine stretch),788 (-C-Cl);^{1}H NMR(400MHZ,DMSO): ***δ*** 8.11 (N=C**H**), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 7.24, 7.22, 7.22, 7.24 (d, ***J*** 2.08 Ar-**H**),***δ***2.0,2.0(CN**H**)***δ***1.5(C-S**H**);^{13}CNMR(400MHZ,DMSO):***δ***129.2,128.9, 131.1, 128.9, 129.2, 127.8, 128.8, 127.8, 128.8 (Ar-**C**), ***δ***123.13, 129.5 (**C=C**), ***δ*** 163 (**C**-SH), ***δ*** 160.9 (**C**=N), ***δ*** 127 (**C**-Cl).

Compound11a:(5E)5(3,5dimethoxystyryl)5(benzylideneamino)-2,5-dihydro-1H-1,2,4-triazole-3-thiol: Yield 65 %, mp. 368 °C;FTIR (γ max, cm^{-1}) 1641(-***C=C*** stretch), 3258 (=***C-H*** stretch), 997 (=***C-H*** bend), 2065 (-**SH**), 1542(-**C=N**), 3356 (-**NH** 2^0 amine stretch),1268(-C-O);^{1}H NMR (400MHZ,DMSO): ***δ*** 8.11 (N=C**H**),***δ***5.76, 6.55 (C**H**=C), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 6.37, 6.37, 6.16 (s, ***J*** 4.82, Ar -**H**), ***δ*** 1.50 (s, ***J*** 3.01 C-S**H**) ***δ*** 2.0, 2.0 (C-N**H**)***δ*** 3.73, 3.73 (m, ***J*** 14.26, O-C**H**$_3$); ^{13}CNMR (400MHZ,DMSO): **δ** 129.2, 128.9, 131.1, 128.9, 129.2, 102.9, 102.9, 99.6(Ar-**C**), ***δ*** 160.9 (**C=N**), ***δ*** 123.3, 129.5 (**C=C**), ***δ*** 163 (**C-SH**)***δ*** 55.9, 55.9 (O-**C**H$_3$).

Compound 12a:4-((1Z)-2-(3E)-3-(benzylideneamino)-2,3-dihydro-5-mercapto-1H-1,2,4-triazol-3-yl)vinyl)phenol: Yield 67 %, mp. 324 °C;FTIR (γ max, cm^{-1}) 1613(-***C=C*** stretch), 3168 (=***C-H*** stretch), 992 (=***C-H*** bend), 2316 (**-SH**), 1528 (-**C=N**), 3363 (-**NH** 2^0 amine stretch),3255(-OH stretch), 1268 (-C-O);^{1}H NMR (400MHZ,DMSO): ***δ*** 8.11 (s, ***J*** 1.00 N=C**H**),***δ***5.76, 6.55 (C**H**=C), ***δ*** 7.62, 7.29, 7.29, 7.29, 7.62, 7.13, 6.68, 6.68, 7.13 (d, ***J*** 0.12, m, ***J*** 1.07 Ar -**H**), ***δ*** 1.50 (C-S**H**) ***δ*** 2.0, 2.0 (C-N**H**)***δ*** 5.0 (s, ***J*** 2.00 C-O**H**); ^{13}CNMR (400MHZ,DMSO): **δ** 129.2, 128.9, 131.1, 128.9, 129.2, 127.8, 115.8, 127.8(Ar-**C**), ***δ*** 160.9 (**C=N**), ***δ*** 123.3, 129.5 (**C=C**), ***δ*** 163 (**C-SH**)***δ*** 157.7 (**C-O**H).

4. Assay procedures

4.1 Procedure for ferric reducing antioxidant power (FRAP Assay) [29]:

In ferric reducing antioxidant power assay,1ml of a test sample of DMF(N,N Dimethylformamide) extract in different concentrations were mixed with 1 ml of 0.2M sodium phosphate buffer (pH-6.6) and 1 ml of 1% potassium ferricyanide (FINAR- B.NO-18042046) in separate test tubes. The reaction mixtures were incubated at a temperature controlled water bath at 50 ^{0}C for 20 min followed by the addition of 1 ml of 10% trichloroacetic acid (MERCK-B.NOMD9M590220). The mixtures were then centrifuged for 10 min at room temperature. To the supernatant 1ml of deionized water 200 µl of 0.1%$FeCl_3$ (LOBA-B.NO-SL26831111) was added. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced with distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A700 after blank subtraction.

Standard drug: Ascorbic acid (LOBA- B.NO-SL44911205) was taken as a reference standard and the concentration of the standard drugs were prepared by making the concentration of 2, 4, 6, 8, 10 µg/ml with DMF. The results were tabulated in Table 2

Table 2: IC_{50} VALUES

Compound	IC_{50} µg/ml	%Inhibition
1a	13.7	19
2a	14.7	43
3a	8.3	9
4a	13.2	22
5a	13.3	46
6a	13.9	89
7a	18.5	87
8a	15	23
9a	11	10
10a	14.9	31
11a	15.2	40
12a	13	74
Ascorbic acid	7	100

4.2 Antibacterial activity [30]

All the synthesized compounds 1a-12a were examined for antibacterial activity against an assortment of two gram-positive bacteria *Staphylococcus aureus NCIM2901, Bacillus subtilis MTCC 441* and two Gram-negative bacteria *Pseudomonas aeruginosa* and *Proteus vulgaris MTCC 1771* by diffusion method. Tetracycline and Chloramphenicol were used as an internal standard.

Nutrient agar (High media) was dissolved and distributed in 25ml quantities in boiling tubes and were sterilized in an autoclave at 121 ^{0}C (15 lbs /sq.in) for 20minutes. The medium was inoculated at one percent level using 18 hrs old cultures of the test organism mentioned above aseptically into sterile petridishes and allowed to set at room temperature for above 30min. In a size of 4 inches petridishes, five cups of 8mm diameter at equal distance were made in each plate. In the cups the test solutions of different concentrations were added and in another plate cups were made for standard and control. The plates, thus prepared were left for 90 minutes in a refrigerator for diffusion. After incubation for 24 hours at 37 ^{0}C the plates were examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition measured and recorded. The results were represented in Table 3.

4.3. Antifungal activity [31]

The antifungal activity of compounds was assayed against three different fungal strains *Aspergillus niger MTCC 282, Penicillium chrysogenum MTCC5108* and *Penicillium notatum NCIM 742.*

Potato dextrose agar[27] (Hi- media) was dissolved and distributed in 25 ml quantities in 100ml conical flasks and were sterilized in an autoclave at 121 °C (15lbs/sq.in) for 20 minutes. The medium was inoculated with 1% 18hr old cultures of organisms aseptically in to sterile petridish and allowed to set at room temperature for about 30 minutes. At a size of 4 inches petridish 5 cups of 8mm diameter at equal distance were made in a petriplate with a sterile borer. The solutions of test concentrations (250µg/ml, 200µg/ml, 150µg/ml and 100µg/ml) and standard were added to respective cups aseptically and labelled accordingly. DMF as control did not show any inhibition. The plates were left for 90 minutes in refrigerator for diffusion and incubated for 72 hrs at $37^0 \pm 1\ ^0$c. The plates were examined for inhibition zones. Fluconazole was used as standard. The experiments were performed in duplicate and the average diameters of the zones of inhibitions were summarized in **Table 4**.

Table 3: Antibacterial Activity

Compound	Zone of inhibition (Diameter in Cm)															
	Bacillus subtilis				Staphylococcus aureus				Pseudomonas aeruginosa				Proteus vulgaris			
	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml
1a	1.3	1.3	1.4	2	1.2	1.1	1.2	1.4	0.9	1.1	1.2	1.3	1.1	1.2	1.2	1.3
2a	1.3	1.3	1.4	1.5	1.1	1.3	1.3	1.5	1.1	1.1	1.2	1.3	1.1	1.2	12	1.4
3a	1.2	1.3	2	1.5	1.1	1.2	1.2	1.2	1	1.1	1.2	1.3	1	1.1	1.1	1.2
4a	1	1.1	1.2	1.2	1.1	1.2	1.2	1.3	0.9	1	1.1	1.1	0.9	1	1.1	1.1
5a	1	1.1	1.1	1.1	1.1	1.1	1.2	1.2	0.9	1.1	1.1	1.2	1.1	1.2	1.2	1.3
6a	1.3	1.4	1.5	1.5	1.1	1.2	1.2	1.3	1.1	1.2	1.2	1.3	1	1.1	1.1	1.2
7a	1.2	1.2	1.3	1.3	1	1.1	1.1	1.2	1.1	1.2	1.4	1.4	0.9	1	1.1	1.2
8a	1.1	1.2	1.2	1.3	1	1.1	1.2	1.3	1.1	1.2	1.3	1.5	1.1	1.2	1.2	1.3
9a	1.1	1.1	1.2	1.2	1.1	1.2	1.3	1.3	1.2	1.4	1.4	1.5	1.1	1.2	1.3	1.4
10a	1.2	1.3	1.3	1.4	1.1	1.1	1.1	1.2	1.1	1.2	1.2	1.3	1	1.2	1.2	1.3
11a	1.1	1.2	1.2	1.3	1.1	1.1	1.1	1.2	1	1.2	1.3	2	1.2	1.3	1.3	1.4
12a	1.1	1.2	1.3	1.4	1.1	1.1	1.2	1.3	1.2	1.3	1.4	1.5	1	1.1	1.1	1.2
Tetracycline	2	2	2.1	2.2	2.1	2.2	2.3	2.3	2	2.1	2.1	2.2	2	2.1	2.2	2.3
Chloramphenicol	2.1	2.2	2.3	2.4	2	2.1	2.2	2.3	2.1	2.2	2.3	2.4	2	2.1	2.2	2.3

Table 4: Anti-Fungal activity

Compound	Zone of inhibition (Diameter In Cm)											
	Aspergillus niger				Penicillium chrysogenum				Penicillium notatam			
	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml
1a	0.8	0.9	1	1.1	1	1.2	1.8	1.9	1.5	1.6	1.7	2
2a	0.9	1.1	1.2	1.3	0.9	1	1.1	1.2	1.1	1.2	1.3	1.4
3a	0.9	1	1.1	1.2	0.8	0.9	1	1.1	0.9	1.1	1.3	1.4
4a	0.9	1	1.1	1.2	1	1.1	1.2	1.3	1.1	1.2	1.3	1.4
5a	0.9	1	1.2	1.3	1.2	1.5	1.6	1.8	0.9	1	1.5	1.6
6a	0.8	0.9	1	1.1	1	1.1	1.2	1.3	1.1	1.2	1.3	1.4
7a	0.8	0.8	1	1.1	1.1	1.2	1.3	1.4	1.2	1.3	1.4	1.5
8a	0.8	0.9	1.1	1.2	0.9	1.5	1.6	1.8	0.9	0.9	1	1.1
9a	0.9	1	1.2	1.3	0.8	1	1.5	2	1.2	1.3	1.4	2
10a	0.7	0.8	1	1.1	1.2	1.5	1.7	2	1.2	1.3	1.5	1.7
11a	0.8	0.9	1	1.1	1	1.2	1.8	1.9	1.5	1.6	1.7	2
12a	0.7	0.9	1	1.1	0.9	1.1	1.3	1.5	1.3	1.5	1.7	1.9
Fluconazole	1.6	1.7	1.8	1.9	1.7	1.8	1.9	2	1.8	1.9	2	2.1

5. Results and Discussion

The starting compounds 4-benzylidene-2-phenyloxazol-5(4H)-one derivatives (1-12) react with corresponding thiosemicarbazide in presence of potassium methoxide refluxed for 6 hrs to give1H-1,2,4-triazole-3-thiol derivatives (1a-12a) respectively. The structures of the compounds were established through IR and 1H and 13C NMR spectral data. The IR spectra of (1a-12a) exhibited absorption bands for secondary amine (-NH) at 3380-3350, cm-1, (-N-N) at 1240-1280 cm-1 and (-C-N) at 1155-1158 cm-1, imines (–C=N–) at 1521-1540 cm-1, alkenes (–C=C–) 1620-1640 cm-1. The 1H NMR spectra of these compounds revealed signals at δ = 7.65-7.98 ppm a multiplet for aromatic protons, δ = 1.83 ppm a singlet for thiol or SH, δ = 2.0 ppm a doublet for(C-NH), δ = 8.11 ppm a singlet for(N=CH). The 13C NMR spectra of these compounds revealed signals at δ = 114.2-130 ppm peaks for aromatic carbons, δ = 1.83 ppm a singlet for thiol or SH, δ = 130-134 ppm for(C-N), δ = 160.9 ppm for(N=C),), δ = 123.3,129.5 ppm for alkene carbons (–C=C-), δ = 181.14ppm for thiol carbon (-C-SH)

All the compounds (1a-12a) were tested for antibacterial activity against an assortment of two gram-positive bacteria *Staphylococcus aureus NCIM 2901*, *Bacillus subtilis MTCC 441* and two gram-negative bacteria *Pseudomonas aeruginosa*, *Proteus vulgaris MTCC 1771.*Tetracycline and Chloramphenicol were used as standards. For antifungal activity against three fungal strains *Aspergillus niger MTCC 282, Penicillium chrysogenum MTCC5108* and *Penicillium notatum NCIM 742*. Fluconazole was used as standard.

The results of antimicrobial activities of synthesized compounds were shown in **Table 3 and 4**.All the compounds showed significant antibacterial and antifungal activity, but

more active towards gram positive bacteria. of all the derivatives synthesized compounds **3a, 5a, 7a, 12a** exhibited good antimicrobial properties.

The structure-activity relationship studies based on the above *in vitro* results clearly indicate that compounds with moderate electron donating groups mainly mono and dialkyl substituted amino group, methoxy on the aromatic ring showed increased potency when compared to the strong electron donating groups such as hydroxy. The intense activity of the compounds is also greatly influenced by the position of the groups on the ring. The para substitution showed higher significant activity when compared to the electron donating groups at ortho position which clearly indicates that para substitution is responsible for increased activity. The results also indicate the influence of rise in activity with increase in the number of alkyl and alkoxy groups mainly methyl or methoxy substituents.

5.1. Conclusion

A series of new 1,2,4-triazole-3-thiolderivativesweреprepared by conventional method and evaluated for their *In-vitro* antimicrobial, ferric oxide reducing properties for which the mechanisms underlying this process remain to be fully elucidated. It is intended that the results from these studies will assist in elucidating their precise mechanism of action and provide an approach for further optimization and development to get new leads in the treatment of microbial infections.

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