Research Article

Design, Synthesis and Evaluation of Biological activity of certain Novel Triazole schiff bases

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ABSTRACT

4- Formyl phenoxy acetic acid (**1**) obtained from 4-hydroxy benzaldehyde yielded the corresponding Schiff bases (**2a$_l$-a$_{10}$**) bearing free carboxyl group on treatment with primary amines/acid hydrazides under anhydrous condition. Several 4-amino-3-substituted-5-mercapto-1,2,4-triazoles (**3a$_l$-a$_{10}$**) were prepared in excellent yields by the condensation of Schiff bases bearing free carboxyl group with thiocarbohydrazide through a single step reaction. Elemental analysis, IR and ^{1}HNMR data confirmed the structure of the newly synthesized compounds. Synthesized triazole derivatives were investigated for their antibacterial, antifungal, anti-inflammatory and analgesic activities. The tested compounds showed significant anti-inflammatory and antibacterial activities. However these compounds exhibited moderate analgesic and antifungal activities.

Keywords: 4-amino triazoles, bioactivity, Schiff bases, spectral characterization, anti-inflammatory and analgesic activities.

INTRODUCTION

Synthesis of compounds for potent and diverse biological activity has always drawn considerable interest. Several five membered aromatic systems having three heteroatoms at symmetrical positions have been studied because of their interesting physiological properties[1,2]. 4-amino-3-substituted-5-mercapto-1,2,4-triazoles as a bifunctional agent, can react with various electophillic reagents and their derivatives have been reported to possess broad spectrum of biological activities[3-6] which have stimulated much interest in the chemistry of triazoles. Similarly Schiff bases are also known for their anti-inflammatory, anticancer and antitubercular activity. So it was though that triazoles bearing Schiff base moiety could be more potent bioactive compounds.

The above observations coupled with our continued interest in the synthesis of biologically active compounds, prompted us to undertake the synthesis of heterocycles wherein the two biologically active moieties like triazoles and Schiff bases are present together and also to explore the activities associated with these nuclei.

The required 4-amino-3-substituted-5-mercapto-1,2,4-triazoles (**3a$_l$-a$_{10}$**) were prepared in excellent yield through multistep reactions. 4-hydroxy benzaldehyde was reacted in alkaline condition with aqueous chloroacetic acid to obtain 4-formyl phenoxy acetic acid (**1**) which is on treatment with different amines/hydrazides under anhydrous conditions furnished Schiff bases (**2a$_l$-a$_{10}$**) bearing a free carboxyl group. The synthesis of 4-amino-3-substituted-5-mercapto-1,2,4-triazoles was accomplished in a single step by condensing Schiff bases with thiocarbohydrazide following the literature method[7] and the synthesised derivatives were investigated for their biological activities like antibacterial, antifungal, anti-inflammatory and analgesic activities.

The reaction sequence leading to the formation of desired compound are outlined in scheme-**I**. As depicted in the scheme the Schiff bases (**$2a_1$-a_{10}**) bearing free carboxyl group cyclised to 4-amino-3-substituted-5-mercapto-1,2,4-triazoles (**$3a_1$-a_{10}**) by reacting with thiocarbohydrazide. The hydrazide used for preparing Schiff bases (**$2a_7$-a_{10}**) were obtained from hydrazinolysis of esters of substituted aryl\ aryloxy acetic acid.

The Schiff bases (**$2a_1$-a_{10}**) were obtained by condensing amines/hydrazides with 4-formyl phenoxy acetic acid which in turn obtained by reacting 4-hydroxy benzaldehyde with chloro acetic acid in alkaline condition. The IR spectrum of compound 4-formyl phenoxy acetic acid showed characteristic absorption bands in the region 3060 (-OH of COOH), 2852 & 2918 (aromatic C-H stretching, strong broad bands), 1755 (C=O of CHO) and 1714 (C=O of COOH), 844 (1,4-disubstituted benzene ring). The compound gave positive tests for carboxyl and aldehyde groups. The disappearance of absorption bands at 1755 for C=O of CHO in the IR spectrum and appearance of a new peak C=N in the range 1600-1615 cm^{-1} indicated the formation of the Schiff bases. Chemical tests showed negative test for aldehyde group. The ^{1}HNMR spectrum of **$2a_1$**, showed characteristic signals at δ 3.5 (1H, s, CH=N), 4.8 (2H, s, OCH_2) 7.0-7.8 (9H, m, Ar-H), 9.8 (1H, s, OH). The spectra of other Schiff bases also confirm their formation.

The reaction of Schiff bases with thiocarbohydrazide furnished in the single step the required triazole derivatives. IR spectra of compound **3a** showed characteristic absorption bands at 3439 (NH_2), 3065 (aromatic C-H stretching), 2926 (CH stretch of OCH_2), 1651 (NH bending), 1599 (C=N), 1512 (C=C), 1246 (C=S), 840 (1,4-disubstituted benzene ring). The disappearance of strong bands at 2852 and 2918 for OH of COOH of Schiff base and appearance of new broad peak at 3439 for NH_2 of triazole confirms the formation of triazole, ^{1}HNMR spectrum of **3a** showed characteristic signals at δ 8.5 (1H, s, CH=N), 4.8 (2H, s, -OCH_2), 5.9 (2H, s, NH_2), 7.0-7.8 (9H, m, Ar-H). The peak for OH of COOH at 9.8 is disappeared and the new peak for –SH of triazole appeared at 13.8 confirming the formation of triazole. The spectra of other Schiff bases also confirm their formation.

Biological activity

In the present investigation, certain synthesized derivatives were screened for their following biological activity.

a) Antibacterial and antifungal activity[8]

The triazole derivatives (**$3a_2$, $3a_4$, $3a_6$, $3a_7$, $3a_8$, & $3a_9$**) were screened for in-vitro antibacterial activity against the standard strains of *Staphylococcus aureus, Bacillus subtilis* (**MMTC-441**) (Gram positive), *Escherichia coli* (**MMTC-4315**) and *Pseudomonas auriginosa* (**MMTC-424**) (Gram negative) by cup-plate method at 100 and 200 µg/ml concentration. DMSO was used as solvent control. Streptomycin and Ampicillin were used as standard drugs.

The synthesized compounds were also tested for antifungal activity against fungal organisms *Aspergillus oryzae* (**MMTC-1122**) and *Candida albicans* (**MMTC-183**) by Cup-plate method in potato dextrose agar (**PDA**) medium at the concentration levels of 100 and 200 µg/ml. DMSO was used as solvent control and Griseofulvin, Clotrimazole were used as standard.

The antibacterial and antifungal screening of the compounds revealed that triazole derivatives exhibited weak to moderate activity. Among these triazole derivatives, it was observed that the compounds derived from acid hydrazides like **$3a_8$-a_{10}** were found to possess much higher activity than

triazole derivatives derived from aromatic amines (**$3a_1$-a_7**). This may be due to the presence of CONH group. The activity of the compounds was found to be 50 % of Streptomycin and almost 70 % of Ampicillin. However no conclusions could be drawn for probable mechanism of action or structural activity relationship (**SAR**) among the compounds of the above series.

b) Anti-inflammatory and analgesic activity[9,10]

The triazole derivatives (**$3a_2$, $3a_4$, $3a_6$, $3a_7$, $3a_8$, $3a_9$**) were screened for anti-inflammatory activity using rat hind paw method of Winter et al, modified by Dhawan and Srimal. The compounds were also screened for analgesic activity using Eddy's hot plate technique.

Anti-inflammatory activity carried out by carrageenan induced rat paw oedema method revealed that triazole derivatives of both mono substituted amines and acid hydrazides showed significant anti-inflammatory activity comparable to standard drug (Ibuprofen). The activity was found to be 86 % of the standard and it continued till the 4th hr under test.

Table 3A: Anti-inflammatory activity of triazole derivatives

Treatment group	Anti-inflammatory activity decrease in paw oedema volume and percentage of reduction							
	1st Hour		2nd Hour		3rd Hour		4th Hour	
	Oedema Vol. (ml)	% red.	Oedema Vol. (ml)	% red.	Oedema Vol. (ml)	% red.	Oedema Vol. (ml)	% red.
Control	0.6916 ±0.2712	-	0.895 ±0.018	-	1.091 ±0.481	-	1.425 ±0.613	-
Ibuprofen	0.415 ±0.0214	39	0.343 ±0.029	61	0.320 ±0.0275	70	0.2966 ±0.024	80
$3a_2$	0.5466 ±0.0290	20	0.5166 ±0.032	42	0.450 ±0.0288	58	0.435 ±0.026	68
$3a_4$	0.5583 ±0.0247	19	0.505 ±0.029	43	0.445 ±0.0325	59	0.426 ±0.032	69
$3a_6$	0.516 ±0.0247	25	0.48 ±0.031	46	0.415 ±0.037	61	0.4033 ± 0.031	71
$3a_7$	0.5166 ± 0.037	25	0.466 ± 0.039	47	0.401 ± 0.0399	63	0.398 ± 0.038	72
$3a_8$	0.483 ± 0.027	30	0.436 ± 0.024	51	0.380 ± 0.023	65	0.358 ± 0.023	74
$3a_9$	0.4766 ± 0.022	31	0.428 ± 0.020	52	0.371 ± 0.022	66	0.358 ± 0.016	74

Analgesic activity of the compounds was carried out in albino mice of either sex by noting the reaction time to the painful stimuli. Eddy's Hot plate technique was used for the study. The reaction time for each mouse was recorded at a time interval of 0, 15, 30, 60, 120, 240 and 480 minutes after the administration of test substance.

Though at the initial hours the analgesic activity of the compounds were slightly higher in comparison to standard drugs, the detailed study revealed that compounds exhibited only moderate analgesic activity**.**

Table 3B: Analgesic activity of triazole derivatives

Treatment group	Average reaction time (Mean ± SEM) and percentage analgesic activity.						
	0 Hour	½ Hour	1 Hour	2 Hour	4 Hour	6 Hour	% analgesic activity (PAA)
Control	2.75 ± 0.30	3.25 ± 0.30	3.66 ± 0.30	4.08 ± 0.23	4.25 ± 0.21	4.45 ± 0.58	-
Phenazone	3.50 ± 0.18	5.75 ± 0.21	7.08 ± 0.23	8.41 ± 0.27	9.83 ± 0.21	12.5 ± 0.22	357.14
Diclofenac	3.41 ± 0.20	4.75 ± 0.21	5.83 ± 0.21	7.16 ± 0.27	8.25 ± 0.21	10.58 ± 0.153	310.26
$3a_2$	3.66± 0.30	4.5 ± 0.18	4.75 ± 0.28	5.25 ± 0.28	5.91 ± 0.15	6.75 ± 0.21	184.42
$3a_4$	3.75 ± 0.21	4.5 ± 0.22	4.8 ± 0.21	5.08 ± 0.23	5.25 ± 0.28	6.33 ± 0.37	168.80
$3a_6$	3.75 ± 0.21	4.83 ± 0.10	5.33 ± 0.10	5.75 ± 0.21	6.16 ± 0.27	6.91 ± 0.23	184.26
$3a_7$	3.75 ± 0.21	4.75 ± 0.11	5.41 ± 0.08	6.61 ± 0.10	6.83 ± 0.10	7.33 ± 0.10	195.47
$3a_8$	3.83 ± 0.16	5.41 ± 0.15	6.08 ± 0.23	6.75 ± 0.28	7.25 ± 0.30	7.83 ± 0.24	204.44
$3a_9$	3.41 ± 0.21	4.83 ± 0.24	5.5 ± 0.18	6.33 ± 0.10	6.91 ± 0.20	7.58 ± 0.15	222.29

Experimental

Melting points were determined on a Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel-GF254 plates using ethyl acetate: n-hexane (1:1) solvent system as irrigant and UV light as visualizing agent. IR spectra in KBr (cm^{-1}) was recorded on a Shimadzu FTIR-8400 series spectrophotometer and ^{1}HNMR spectra (DMSO-d_6) on Bruker spectrospin 200 MHz spectrometer using TMS as internal standard (chemical shifts are expressed in δ ppm). All the compounds showed satisfactory analytical results for C, H and N.

Synthesis of thiocarbohydrazide

Absolute ethanol (25 ml) and hydrazine hydrate (16 ml) were taken in a beaker and stirred. To this ammonium hydroxide solution (10 ml) was added with stirring. Carbon disulphide (4 ml) was added drop wise at this stage for 10 min with stirring and the mixture was allowed to stand for 30 min. Later sodium chloro acetate (4.5 gm) followed by hydrazine hydrate (6 ml) was added to the above mixture and stirring was continued for 3-4 hrs. The reaction mixture was kept in refrigerator for whole night. The crystalline product obtained was filtered, washed with ethanol, dried and recrystallized from hot water (white crystalline solid, mp 168 ºC, yield 80 %). IR (KBr) cm^{-1}: 3422 (NH), 3275 (NH_2), 1649 NH bending, 1287 (C=S).

Synthesis of 4-formyl phenoxy acetic acid:

The 4-hydroxy benzaldehyde (3 gm, 0.26 mol) was dissolved in 33 % aqueous NaOH solution to which was added slowly chloroacetic acid solution (2.5 gm, 0.26 mol) with constant stirring, sufficient water was added to dissolve the solid obtained and the reaction mixture was refluxed for 7 hrs. On cooling it was acidified with HCl and the precipitate obtained was dissolved in 10 % $NaHCO_3$ solution and again reprecipitated with HCl. The solid was filtered, washed 2-3 times with water, dried and recrystallized from aqueous ethanol (Whitish crystalline solid, mp 197 ºC, yield 75 %).

Synthesis of Schiff bases

Method-1

Equimolar quantities of 4-formyl phenoxy acetic acid and substituted primary amine/hydrazide were taken in sufficient quantity of absolute ethanol with catalytic amount of glacial acetic acid/p-toluene sulphonic acid and the reaction mixture was refluxed for 10-12 hrs. Excess of ethanol was distilled off. The obtained solid product was washed with sod.bisulphite, dil.HCl followed by water and recrystallized from aqueous ethanol.

Method-2

Eqimolar quantities of 4-formyl phenoxy acetic acid (0.01 mol) and primary amine (0.01 mol) were taken in sufficient quantity of glacial acetic acid and refluxed for 6-8 hrs. The reaction mixture was concentrated and poured on crushed ice when require Schiff base precipitates out. It was filtered washed with water and recrystallised from aqueous ethanol.

In the both methods the progress and purity of the compounds were monitored by TLC studies using silica gel plates. The spots on the plates were visualized in UV chamber and Rf values were noted. The other compounds of this series were prepared following the same procedure and characterized.

$2a_1$: IR : 3530 (OH), 3061 (aromatic C-H stretching), 2928 and 2858 C-H stretching of OCH_2 asymmetric and symmetric, 1745 (C=0), 1599 (C=N) and (C=C); 1420-1312 (C-H bending of OCH_2 asymmetric and symmetric), 1172 (C-O-C), 835 (1, 4-disubstituted phenyl ring), 760 (monosubstituted phenyl ring), **^{1}HNMR** 4.8 (2H, s, OCH_2),

7.00-7.80 (9H, m, Ar-H), 8.80 (1H, s, CH=N), 11.70 (1H, s, OH of COOH), Mass m/z 255 (M^+).[Found C, 70.53; H, 4.98; N, 5.45 $C_{15}H_{13}O_3N$ requires C, 70.58; H,5.09 ; N, 5.49 %]

2a2: IR: 3452 (OH), 3060 (aromatic C-H stretching), 2970 and 2854 (C-H stretching of OCH_2, asymmetric and symmetric), 1712 (C=O), 1601 (C=N), 1576, 1512 (C=C), 1410 and 1375 (C-H bending of OCH_2 asymmetric and symmetric), 1175 (C-O-C), 839 (1,4-disubstituted phenyl ring), 592 (C-Cl), **^{1}HNMR** 4.8 (2H, s, OCH_2), 7.00-7.80 (8H, m, Ar-H), 8.90 (1H, s, CH=N), 11.74 (1H, s, OH of COOH).[Found C, 62.22; H, 4.08; N, 4.80; $C_{15}H_{12}O_3NCl$ requires C, 62.28; H, 4.15; N, 4.84 %]

2a4: IR: 3450 (OH), 3062 (aromatic C-H stretching), 2962-2848 (C-H stretching of OCH_2, CH_3 groups), 1718 (C=O), 1604 (C=N), 1599, 1524, 1476 (C=C ring stretching), 1422-1380 (C-H bending OCH_2& CH_3 groups) 1190 (C-O-C), 842 (1,4-disubstituted benzene), **^{1}HNMR** 2.30 (3H, s, CH_3), 4.80 (2H, s, -OCH_2), 7.00-7.80 (8H, m, Ar-H), 8.90 (1H, s, HC=N), 11.70 (1H, s, -OH), Mass m/z 269 (M^+).[Found C, 71.29; H, 5.53; N, 5.16; $C_{16}H_{15}O_3N$ requires C, 71.37; H, 5.57; N, 5.20 %].

2a8: IR: 3462 (OH), 3348 (NH), 3065 (aromatic C-H stretching), 2930-2838 (C-H stretching of CH_3, CH_2 and OCH_2 groups), 1728 (C=O), 1668 (C=O of CONH), 1608 (C=N), 1602,1580, 1465 (C=C ring stretching), 1428 (C-N), 1418-1368 (C-H bending of CH_3, CH_2 and OCH_2 groups), 1192 (O-H bending), 1185 (C-O-C), 838 (1,4-disubstituted phenyl rings), **^{1}HNMR** 0.90 (6H, d, 2xCH_3 of $CH(CH_3)_2$, 1.40 (3H, d, CH_3 of CH-CH_3), 1.80 (1H, m, CH of $CH(CH_3)_2$), 2.40 (2H, d, CH_2 of CH_2-$CH(CH_3)_2$), 3.80 (2H, s, CH_2 of OCH_2), 3.90 (1H, q, CH of CH-CH_3), 7.10-8.00 (8H, m, Ar-H), 8.70 (1H, s, HC=N), 10.60 (1H, s, NH of –CONH), 11.80 (1H, s, OH of COOH).[Found C, 69.02; H, 6.72; N, 7.29; $C_{22}H_{26}O_4N_2$ requires C, 69.10; H, 6.80; N, 7.32 %].

Synthesis of triazole

Equimolar quantities of Schiff base and thiocarbohydrazide were taken in rb flask fitted with thermometer. The reaction mixture was heated in an oil bath on hot plate till the contents started melting. The mixture was maintained at the same temperature for 1-2 hrs with occasional shaking the flask. The reaction mixture was cooled, washed, with 10 % sodium bicarbonate solution, followed by water, dried and recrystallized from DMF. The progress and purity of the compounds was monitored by TLC studies using silicagel **GF254** plates. The solvent system used was ethyl acetate : Petroleum ether.

The compounds of this series were prepared following the same procedure and characterised in

Table I: Physical data of compounds ($2a_1$-a_{10}) and ($3a_1$-a_{10}).

S. No.	Code	Z	mp °C	% Yield	Rf value
1	$2a_1$	Phenyl	207	60	0.50
2	$2a_2$	4-Chlorophenyl	165	78	0.52
3	$2a_3$	2-Chlorophenyl	154	48	0.50
4	$2a_4$	4-methyl phenyl	178	81	0.50
5	$2a_5$	2-methyl phenyl	69	58	0.45
6	$2a_6$	2-fluoro phenyl	206	60	0.56
7	$2a_7$	4-hydroxy phenyl	271	66	0.66
8	$2a_8$	2[4'-Isobutyl phenyl] propionyl carboxamido	155	40	0.67
9	$2a_9$	2[5'-methoxynaphthyl] propionyl carboxamido	184	40	0.68
10	$2a_{10}$	2[1'-amino, 2',6'-dichloro phenyl] benzyl carboxamido	176	35	0.60
11	$3a_1$	Phenyl	220-224	64	0.50
12	$3a_2$	4-Chloro phenyl	203	65	0.55
13	$3a_3$	2-Chloro phenyl	192	45	0.56
14	$3a_4$	4-methyl phenyl	223	65	0.62
15	$3a_5$	2-methyl phenyl	165	45	0.56
16	$3a_6$	2-fluoro phenyl	246	40	0.62
17	$3a_7$	4-hydroxy phenyl	250	55	0.58
18	$3a_8$	2(4'-isobutyl phenyl) propionyl carboxamido	214	38	0.65
19	$3a_9$	2(5'-methoxy naphthyl) propionyl carboxamido	250	35	0.65
20	$3a_{10}$	2(1'-amino, 2',6'-dichloro phenyl) benzyl carboxamido	252	40	0.68

3a1: IR: 3439 (NH_2), 3072 (aromatic C-H stretching), 2926, 2857 (C-H stretching of $-OCH_2$ asymmetric and symmetric), 2620 (SH), disappearance of C=O of COOH at 1745, 1651 (N-H bending), 1599 (C=N), 1560, 1512, 1460 (C=C ring stretching), 1160 (C-O-C), 840 (1,4-disubstituted phenyl ring), 730 (monosubstituted phenyl ring), 690 (C-S), **^{1}HNMR** 5.10 (2H, s, OCH_2), 5.89 (2H, s, NH_2), 7.85-8.28 (9H, m, 8H of Ar-H and 1H of CH=N), 13.18 (1H, s, SH), Mass m/z 325 (M^+), 210, 180, 115, 107, 104, 77, 65.[Found C, 70.54; H, 5.00; N, 5.47; $C_{15}H_{13}O_3N$ requires C, 70.58; H, 5.09; N, 5.49 %].

3a2: IR: 3417 (NH_2), 3068 (aromatic C-H stretching), 2931 (C-H stretching of OCH_2), 1640 (N-H bending), 1606 (C=N), 1598, 1514, 1455 (C=C ring stretching), 1242 (C=S), 824 (1,4-disubstituted phenyl ring), 698 (C-S), 569 (C-Cl); **^{1}HNMR** 5.10 (2H, s, -OCH_2), 6.00 (2H, s, NH_2), 7.30-8.70 (9H, m, 8H of Ar-H and 1H of HC=N), 13.2 (1H, s, SH), Mass m/z 359, 244, 139, 115, 107, 77, 65.[Found C, 53.41; H, 3.79; N, 19.44; $C_{16}H_{14}ON_5SCl$ requires C, 53.48; H, 3.89; N, 19.49 %].

3a4:IR: 3421 (NH_2), 3070 (aromatic C-H stretching), 2924 (C-H stretching of –OCH_2), 1643 (NH bending), 1606 (C=N), 1585, 1510, 1479, (C=C ring stretching), 1246 (C=S), 1174 (C-O-C), 833 (1,4-disubstituted phenyl ring), 698 (C-S), **^{1}HNMR** 2.52 (3H, s, CH_3), 5.00 (2H, s, -OCH_2), 5.68 (2H, s, NH_2), 6.70-7.90 (9H, m, 8H of Ar-H and 1H of HC=N), 13.23 (1H, s, -SH), Mass m/z 339 (M^+), 224, 128, 115, 107, 77, 65. [Found C, 60.11; H, 4.95; N, 20.59; $C_{17}H_{17}N_5OS$ requires C, 60.17; H, 5.00; N, 20.64 %]

3a8: IR: 3449 (NH_2), 2986, 2955, 2868 (C-H stretching of OCH_2, CH_2& CH_3 group), 1640 (N-H bending), 1602 (C=N), 1580, 1520, 1452 (C=C ring stretching), 1261 (C=S), 1170 (C-O-C), 840 (1,4-disubstituted phenyl ring), 1696 (C-S), **^{1}HNMR** 0.90 (6H, d, 2xCH_3 of $CH(CH_3)_2$), 1.4 (3H, d, CH_3 of CH-CH_3), 1.8 (1H, m, CH of $CH(CH_3)_2$), 2.4 (2H, d, CH_2 of CH_2-$CH(CH_3)_2$), 3.9 (1H, q, CH of CH-CH_3), 4.8 (2H, s, CH_2 of OCH_2), 5.6 (2H, s, NH_2), 6.70-8.30 (9H, m, 8H of Ar-H and 1H of HC=N), 10.2 (1H, s, NH of CONH), 13.20 (1H, s, SH). [Found C, 60.09; H, 6.09; N, 19.08; $C_{22}H_{27}O_2N_6S$ requires C, 60.13; H, 6.15; N, 19.13 %].

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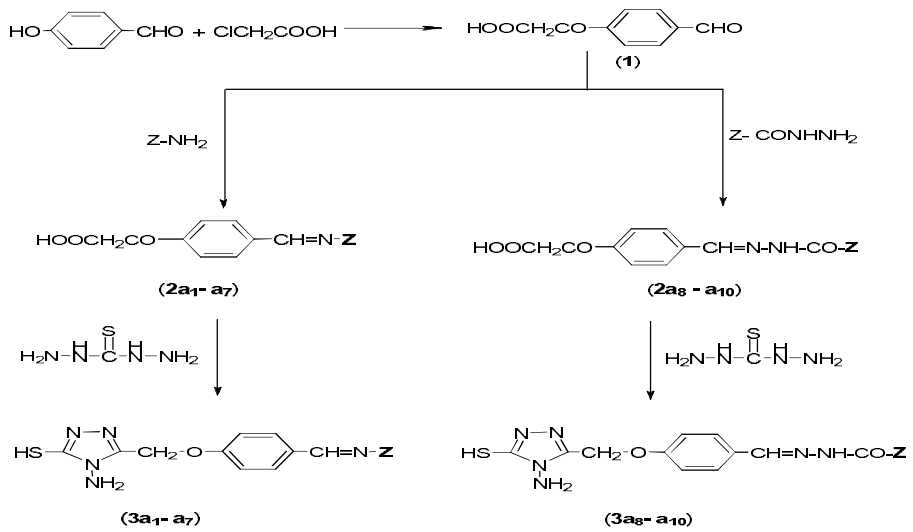

Scheme-1

Table II: Antibacterial and antifungal activity of triazole derivatives.

Compound	Antibacterial activity zone of inhibition in mm and concentration in µg/ml.								Antifungal activity zone of inhibition in mm and concentration in µg/ml.			
	S. aureus		B. subtilis		E. coli		P. aeruginosa		A. oryzae		C. albicans	
	100	200	100	200	100	200	100	200	100	200	100	200
Control	0	0	0	0	0	0	0	0	0	0	0	0
Streptomycin	12	20	8	18	20	32	18	26	-	-	-	-
Ampicillin	12	22	10	18	14	20	12	22	-	-	-	-
$3a_2$	0	8	0	9	0	10	0	10	3	7	2	7
$3a_4$	0	6	0	9	0	8	5	8	2	9	3	8
$3a_6$	8	12	10	13	5	12	6	10	5	9	4	11
$3a_7$	7	11	8	13	10	15	5	12	7	12	5	10
$3a_8$	6	12	7	14	8	13	7	13	8	14	6	12
$3a_9$	7	14	8	15	7	16	6	12	10	16	8	14
Griseofulvin	-	-	-	-	-	-	-	-	12	20	10	20
Clotimazole	-	-	-	-	-	-	-	-	14	20	12	22

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