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Synthesis, characterization and biological evaluation of novel 1,2,4-triazole derivatives as potent antibacterial and anti-inflammatory agents

Rakesh Kumar*[1], M. Shahar Yar[2], Birendra Srivastava[1] and A. K. Rai[3]

[1]*School of Pharmaceutical Science, Jaipur National University, Jaipur, Rajasthan, India*
[2]*Deptt. of Pharmaceutical Science, Jamia Hamdard, Hamdard University, New Delhi, India*
[3]*Pranveer Singh Institute of Technology, Kanpur, India*

ABSTRACT

To synthesize various new 1,2,4-Ttriazole derivatives and evaluating them for their Anti-inflammatory and antimicrobial Activity. Therefore, the discovery of new safer Anti-inflammatory and antimicrobial drugs represents a challenging goal for this research area. The synthesized compounds 1,2,4-Triazole have showed good positive effects as Anti-inflammatory and antimicrobial agents. Anti-inflammatory and antimicrobial activities of test compounds at compared to standard drugs.

Key words: Anti-inflammatory Activity, antimicrobial Activity, 1,2,4-Triazole.

INTRODUCTION

Now a day's research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The heterocyclic are enjoying their importance as being the center of activity. The triazole nucleus is one of the most important and well known heterocycles which is a common and integral feature of a variety of natural products and medicinal agents. Triazole and Thaidiazole constitute an important class for new drug development in order to discover an effective compound against multi drug resistant microbial infection. A large number of heterocyclic compounds containing a symmetrical 1,2,4-triazole ring are associated with diverse pharmacological activities such as antiviral [1] antimicrobial[2,3] , anti-Inflammatory [4-6] and anti tumor[7]. The Triazole derivative possess a wide a range of pharmacological such as analgesic [8], local anesthetics [9], anti convulsant[10], anti neoplastic[11], anti malarial[12] , anti viral[13] , anti proliferative[14], and anti cancer activities[15]. The broad and potent activity of triazole and their derivatives has established them as pharmacologically significant. The basic heterocyclic rings present in the various medicinal agents are 1,2,4-triazole. A large volume of research has been carried out on triazole and their derivatives, which has proved the pharmacological importance of this heterocyclic nucleus.

MATERIALS AND METHODS

All the reagents and solvents used in the synthesis work were of laboratory grade and procured from SD Fine Chemicals and Sigma Aldrich India.A new class of 1,2,4-Triazole have been synthesized from Biphenyl 4-carboxylic acid on treatment with various chemicals & synthesized 3-(biphenyl-4-yl)-4-phenyl-1H-1,2,4-triazole-5(4H)-thione derivatives. The synthesized compounds were characterized by FTIR, 1H-NMR and mass spectrometry. The Anti-inflammatory activity of test compounds was determined by Carrgenan induced mice paw edema inhibition method. The antimicrobial Activity studies were carried out for the synthesized compounds which were also evaluated against the representative panel of *Staphylococcus aureus* and *Bacillus subtilis* gram positive *Escherichia coli* and *Pseudomonas aeroginosa* gram negative bacteria.

1. Synthesis of 1,2,4-triazole was similar to describe in the literature [16].

General procedure for the synthesis of 3-(biphenyl-4-yl)-4-phenyl-1H-1,2,4-triazole-5(4H)-thione derivatives (R1-R12).

A mixture of corresponding 2-(biphenyl carbonyl)-N-phenyl hydrazine carbothioamide (10mmole) in ethanol (50ml) was dissolved in 4N aq. Sodium hydroxide solution (2ml) resulting in the formation of clear solution. The reaction mixture was refluxed for 4-6 hr on water bath, concentrated cooled and filtered. The ph was filtrate was adjust between 5 and 6 with acetic acid and kept aside for 1-2 hr. The solid separated filtered, washed with water, dried and recrystalysed from ethanol (R1-R12).

R1*-3-(biphenyl-4-yl)-4-phenyl-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2947 (C-H), 1254 (C=S), 1615 (C=N). ^{1}HNMR (300 MHz, $CDCl_3$, δ ppmA): 7.06-7.58 (m, 14, aromatic), 12.61 (S,1H,NH). MS: m/z 329 (M^+). Anal. Cacld. For $C_{20}H_{15}N_3S$: C,72.92; H,4.59; N,12.59 Found C,72.90; H,4.57; N,12.58

R2*- 4-benzyl-3-(biphenyl-4-yl)-1H-1,2,4-triazole-5 (4H)-thione.*

IR (γ max cm-^{1}): 2932 (C-H), 1252 (C=S), 1610 (C=N).^{1} HNMR (300 MHz, $CDCl_3$, δ ppm): 6.88-7.65 (m, 14, aromatic), 12.47 (S,1H,NH). MS: m/z 343 (M^+). Anal. Cacld. For $C_{21}H_{17}N_3S$: C,73.44; H,4.99; N,12.23, S,9.31 Found C,73.46; H,4.97; N,12.21; S,9.30.

R3*- 3-(biphenyl-4-yl)-4-(4-chloro phenyl)-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2914 (C-H), 1249 (C=S), 1606 (C=N).^{1} HNMR (300 MHz, $CDCl_3$, δ ppm): 6.78-7.89 (m, 13, aromatic), 12.59 (S,1H,NH). MS: m/z 364 (M^+), 365 (M^+ +1), Anal. Cacld. For $C_{20}H_{14}ClN_3S$: C,66.02; H,3.88; N,11.55, Found C,66.04; H,3.86; N,11.50.

R4*-3-(biphenyl-4-yl)-4-(4-methyl phenyl)-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2922 (C-H), 1258 (C=S),1568 (C=N). ^{1} HNMR (300 MHz, $CDCl_3$, δ ppm): 6.81-7.52 (m, 13, aromatic), 12.64 (S,1H,NH), 2.17 (S,3H,CH_3). MS: m/z 343 (M^+),Anal. Cacld. For $C_{21}H_{17}N_3S$: C,73.44; H,;4.99; N,12.23 Found, C,73.40; H,;4.97; N,12.20.

R5*- 3-(biphenyl-4-yl)-4-(2-methoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2935 (C-H), 1255 (C=S),1608 (C=N), 1240 (C-O-C). ; ^{1}HNMR (300 MHz, $CDCl_3$, δ ppm): 6.83-7.54 (m, 13, aromatic), 12.46 (S,1H,NH). 2.19 (S,3H,OCH_3) MS: m/z 359 (M^+), Anal. Cacld. For $C_{21}H_{17}N_3$ OS : C,70.17; H,;4.77; N,11.69; Found C,70.16; H,;4.75; N,11.67.

R6*- 3-(biphenyl-4-yl)-4-(4-methoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2936 (C-H), 1253 (C=S), 1601 (C=N). ^{1} HNMR (300 MHz, $CDCl_3$, δ ppm): 7.08-7.51 (m, 13, aromatic), 12.54 (S,1H,NH). 2.18 (S,3H,OCH_3). MS: m/z 359 (M^+). Anal. Cacld. For $C_{21}H_{17}N_3OS$: C,70.17; H,4.77; N,11.69 Found C,70.15; H,4.75; N,11.71.

R7*- 3-(biphenyl-4-yl)-4-(4-fluoro phenyl)-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2919 (C-H), 1251 (C=S), 1616 (C=N). ^{1} HNMR (300 MHz, $CDCl_3$, δ ppm): 7.11-7.52 (m, 13, aromatic), 12.67 (S,1H,NH). MS: m/z 347 (M^+), 348 (M^+1), Anal. Cacld. For $C_{20}H_{14}FN_3S$: C,69.14; H,4.06; N,5.46 Found C,69.19; H,4.16; N,5.43.

R8*-3-(biphenyl-4-yl)-4-(2,4-dichlorophenyl)-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2946 (C-H), 1253 (C=S), 1613 (C=N).^{1} HNMR (300 MHz, $CDCl_3$,δ ppm): 7.04-7.58 (m, 12, aromatic) 12.63 (S,1H,NH). . MS: m/z 398 (M^+), 400 (M^+ +2). Anal. Cacld. For $C_{20}H_{13}Cl_2N_3S$: C, 60.31; H, 3.29; N,10.55, Found C,60.33; H,3.28; N,10.53.

R9*- 3-(biphenyl-4-yl)-4-(3-methoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione*

IR (γ max cm-^{1}): 2928 (C-H), 1252 (C=S), 1611 (C=N). ^{1} HNMR (300 MHz, $CDCl_3$, δ ppm): 7.16-7.78 (m, 13, aromatic) 12.59 (S,1H,NH). MS: m/z 359 (M^+). Anal. Cacld. For $C_{21}H_{17}N_3OS$: C,70.17; H,4.77; N,11.69 Found C,70.15; H,4.78; N,11.68.

R10*- 3-(biphenyl-4-yl)-5-thioxo-1H-1,2,4-triazole-4(5H)-yl)(phenyl)methaone*

IR (γ max cm-^{1}): 2935 (C-H), 1256 (C=S), 1509 (C=N). ^{1} HNMR (300 MHz, $CDCl_3$, δ ppm): 7.26-7.68 (m, 14, aromatic) 12.50 (S,1H,NH). MS: m/z 357 (M^+). Anal. Cacld. For $C_{21}H_{15}N_3OS$: C,70.57; H,4.23; N,11.76 Found C,70.59; H,4.25; N,11.78.

R11- *3-(biphenyl-4-yl)-4-(4-nitrophenyl)-1H-1,2,4-triazole-5(4H)-thione*
IR (γ max cm-^{1}): 2940 (C-H), 1247 (C=S), 1610 (C=N). ^{1}HNMR (300 MHz, $CDCl_3$, δ ppm): 7.09-7.68 (m, 13, aromatic) 12.57 (S,1H,NH). MS: m/z 374 (M^+), Anal. Cacld. For $C_{20}H_{14}N_4O_2S$: C,64.16; H,3.77; N,14.96 Found C,64.18; H,3.78; N,14.94.

R12- *3-(biphenyl-4-yl)-4-(2-chloro-5-(trifluoro methyl) phenyl)-1H-1,2,4-triazole-5(4H)- thione.*
IR (γ max cm-^{1}): 2931 (C-H), 1252 (C=S), 1610 (C=N). ^{1}HNMR (300 MHz, $CDCl_3$, δ ppm): 6.86-7.48 (m, 12, aromatic) 12.62 (S,1H,NH). MS: m/z 331 (M^+), 333 (M^+ +2). Anal. Cacld. For C21H13ClF3N3S: C,58.40; H,3.03; N,9.73 Found C,58.42; H,3.06; N,9.71.

2. Characterization
The Melting points were determined in open capillary tube and are uncorrected. Infra red spectra were recorded on Perkin Elmer spectrum spectrophotometer. ^{1}HNMR spectra were run on BRUCKER spectrometer (300MHz) using TMS as internal standard. Elemental analyses were doing using Carbo Erba 1106 CHN analyses. The progress of the reaction was monitored by thin layer chromatography on TLC silica gel plates. The purity of synthesized compounds was as curtained by TLC on silica gel G in various solvent systems using Iodine vapors as detecting agents.

3. Study of anti-inflammatory and antimicrobial activities
Anti-inflammatory activity was evaluated using Carrgennan Induced oedema in rat paw as per method given by winter et al [17]. The animals were allocated into twelve groups. Control group received only 0.5% sodium carboxyl methyl cellulose (CMC) solution. Carrgennan solution (0.1% in sterile 0.9% Nacl solution) in a volume of 0.1 ml was injected subcutaneously into the sub-planter region of the right hind paw of each rat. The volume of paw was measured with a plethysmometer at 0 hour immediately after injected Carrgennan; the same procedure was repeated at 1 hour, 3hour and 4 hour. The percentage inhibition of paw edema was calculated to the following equation.

% anti-inflammatory activity = (1-Vt / VC) × 100, where Vt represent mean paw volume in test group of rats and V_C correspond to mean paw volume in control group of rats.

Antimicrobial Activity:
Antimicrobial activities of the synthesized compounds against two Gram-positive bacteria (*Staphylococcus aureus* & *Bacillus subtilis*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeroginosa*) were expressed as zone of inhibition values in mm [18,19]. The zone of inhibition values were determined by cup plate agar diffusion method. Zone of inhibition values of the synthesized compounds and the standard drugs, Ofloxacin were compared at concentration of 100 µg/ml. The stock solutions of the compounds were prepared in dimethyl sulphoxide (DMSO) as solvent which was also used as control.

Table- 1.: Anti-inflammatory Activity (% Inhibition) of Test Compounds

Compound Code	% Inhibition			
	0 hr	1hr	3hr	4hr
R1	5.38	8.20	11.58	14.83
R2	27.69	26.11	33.12	43.95
R3	26.89	29.10	27.14	26.10
R4	31.53	29.10	33.53	58.24
R5	21.20	21.25	22.32	24.50
R6	20.16	21.10	23.21	22.68
R7	23.84	20.14	31.09	44.5
R8	22.54	24.40	23.80	25.95
R9	20.44	21.50	22.43	24.46
R10	11.30	11.94	13.86	14.60
R11	29.13	29.69	30.42	30.97
R12	35.38	27.61	36.58	42.85
Control	-	-	-	-
Indomethacin	26.92	26.11	54.87	68.13

Numbers of mice in each group were four.

The compounds were screened for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeroginosa* in Nutrient Agar medium. The petri plates were washed thoroughly and sterilized in hot air oven at 160°C for one hour. Sterile nutrient agar medium (15 ml) was poured into sterile Petri dishes of 9 cm diameter and allowed to solidify. The Petri plates were incubated at 37°C for 24 hours to check for sterility. The medium was seeded with the suspension of microorganism (1 ml) by spread plate method using sterilized triangular loop. Bores were made on the medium using sterilized stainless steel cylinder of 8 mm diameter

to make cups or cavities. The solutions of test compounds (100 µg/ml in DMSO) were added serially in the cups or cavities with the help of micropipette (0.1 ml). A control having only DMSO in the cup was maintained in each plate. The Petri plates were kept in refrigerator at 4°C for 1 hour, allowing diffusion to take place. These plates were incubated at 37°C for 24 hours for antibacterial activity respectively. The radius of zone of inhibition (in mm) was observed around the cup or cavity after respective incubation was assured and measured in triplicate sets by using a scale.

Table-2: Antibacterial Activity of Test compounds

Compound Code	Zone of Inhibition (mm) ± SEM and MIC (µg/ml)			
	SA	BS	EC	PA
R1	10.96	11.6	12.05	11.99
R2	13.78	11.32	14.76	12.65
R3	28.75	28.32	28.97	29.39
R4	15.65	16.35	-	16.80
R5	16.24	15.45	16.31	14.75
R6	-	16.85	17.10	18.39
R7	30.04	31.29	31.78	31.88
R8	20.65	21.48	-	23.42
R9	14.10	15.28	15.98	16.50
R10	12.56	14.65	-	12.54
R11	21.76	22.56	24.58	23.65
R12	20.45	23.20	22.56	21.65
Control	-	-	-	-
Ofloxacin	33.6	34.92	35.12	35.88

Values in bracket are MIC values. (-) did not show activity)
SA- Staphylococcus aureus BS- bacillus subtilis EC- Escherichia coli PA- Pseudomonas aeroginosa

RESULTS AND DISCUSSION

The synthesized compounds Carrgennan-induced mice paw oedema test for acute inflammation, the synthesized compounds 3-(biphenyl-4-yl)-4-(2-chloro-5-(trifluoro methyl) phenyl)-1H-1,2,4-triazole-5(4H)-thione (R12), 3-(biphenyl-4-yl)-4-(4-methyl phenyl)-1H-1,2,4-triazole-5(4H)-thione (R4) 3-(biphenyl-4-yl)-4-(4-nitrophenyl)-1H-1,2,4-triazole 5(4H)-thione (R11), 3-(biphenyl-4-yl)-4-(4-chloro phenyl)-1H-1,2,4-triazole-5(4H)-thione (R3) derivatives showed excellent anti-inflammatory activity**,** and another compounds showed good and mild anti-inflammatory activity. Therefore according to structure activity relationship, the functional groups like chloro, Nitro, amino, methoxy group's electron withdrawing group responsible for the excellent activity against rat foot inflammation.

The compounds were screened for antibacterial activity against *Staphylococcu*s *aureu*s, *Bacillus subtilis*, *Escherich*ia *coli* and *Pseudomona*s *aeroginosa*. The investigation of antibacterial screening data are revealed all the tested compounds and showed good antibacterial activity. The test compounds3-(biphenyl-4-yl)-4-(4-fluoro phenyl)-1H-1,2,4-triazole-5(4H)-thione (R7), 3-(biphenyl-4-yl)-4-(4-chloro phenyl)-1H-1,2,4-triazole-5(4H)-thione (R3), 3-(biphenyl-4-yl)-4-(4-nitrophenyl)-1H-1,2,4-triazole 5(4H)-thione (R11), 3-(biphenyl-4-yl)-4-(2-chloro-5-(trifluoro methyl) phenyl)-1H-1,2,4-triazole-5(4H)-thione (R12), showed excellent activity against all four bacterial strains. All these compounds contain electron withdrawing or electron releasing groups in their structure. This suggests that electron withdrawing groups and bulkier groups are responsible for the activity. At last, it was interesting to observe that remaining test compounds of the above mentioned series did not show appreciable antibacterial activity when compared with standard drug Ofloxacin.

Table 3: Value of R, Physical data for synthesized Compounds

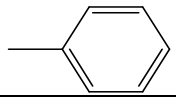
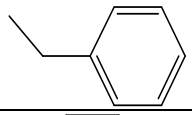
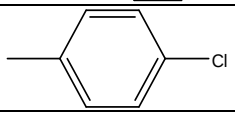
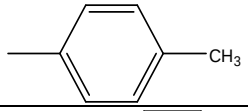
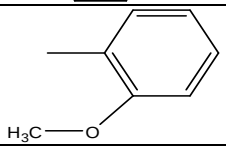
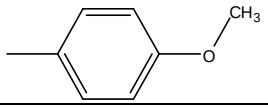
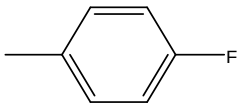
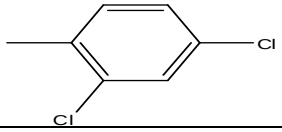
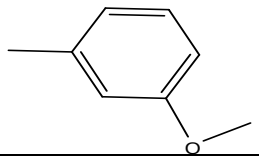
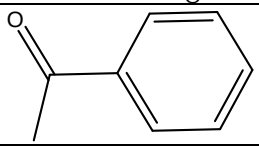
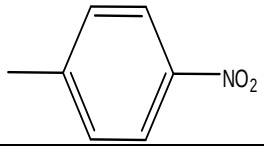
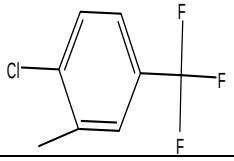
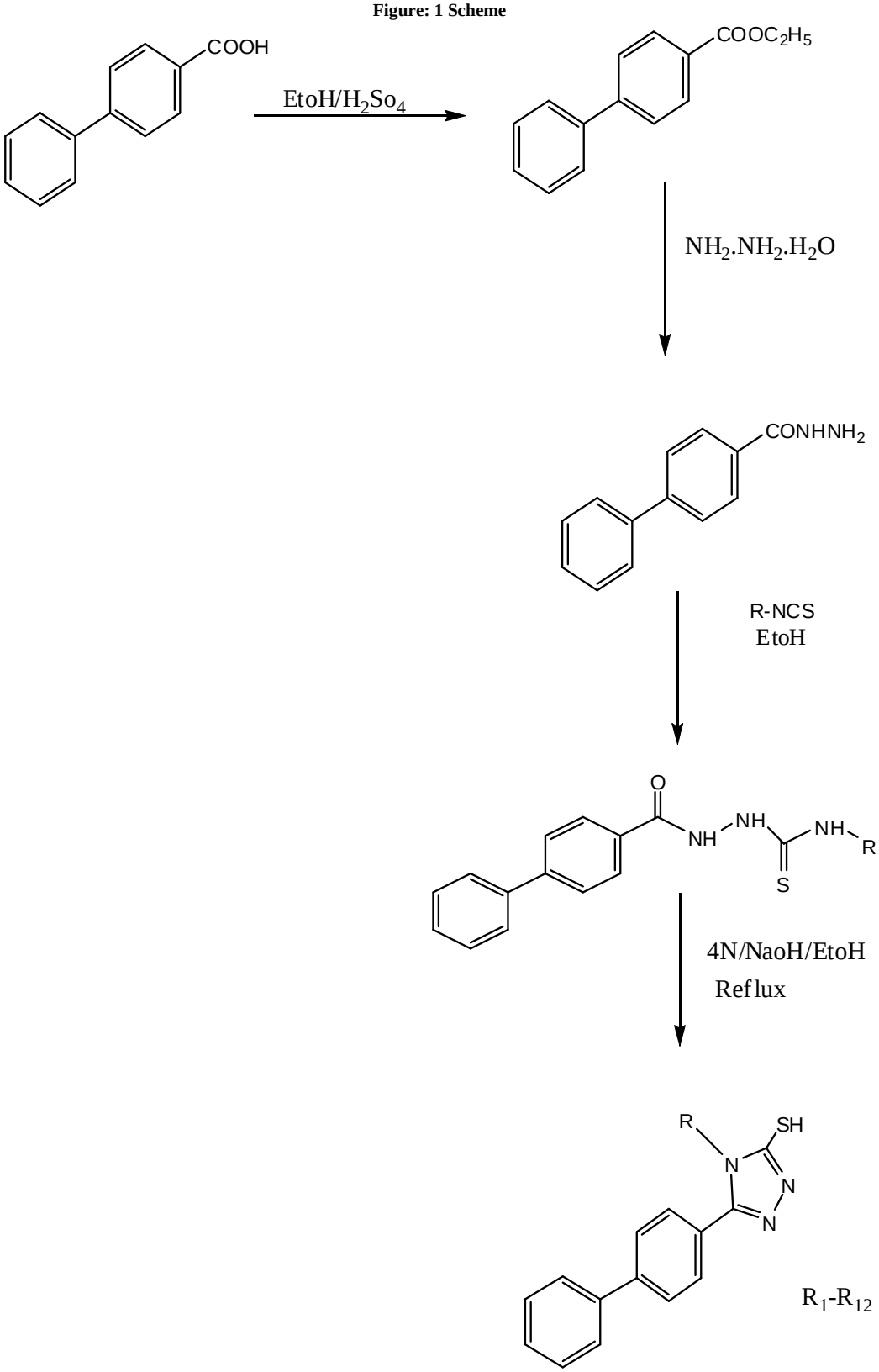
S.N	Compound	R	Chemical Formula	Mole.Wt.	Yield	M.P. (^{0}c)
1	R_1		$C_{20}H_{15}N_3S$	329	71 %	154-156
2	R_2		$C_{21}H_{17}N_3S$	343	65%	163-165
3	R_3		$C_{20}H_{14Cl}N_3S$	364	70%	188-190
4	R_4		$C_{21}H_{17}N_3S$	343	78%	172-174
5	R^5		$C_{21}H_{17}N_3OS$	359	80%	148-150
6	R^6		$C_{21}H_{17}N_3OS$	359	65%	127-129
7	R_7		$C_{20}H_{14}FN_3S$	347	70%	102-194
8	R_8		$C_{20}H_{13}Cl_2N_3S$	398	75%	134-136
9	R_9		$C_{21}H_{17}N_3OS$	359	60%	107-109
10	R_{10}		$C_{21}H_{15}N_3OS$	357	71%	121-123
11	R_{11}		$C_{20}H_{14}N_4O_2S$	374	62%	143-145
12	R_{12}		$C_{21}H_{13}ClF_3N_3S$	331	58%	165-167

Figure: 1 Scheme



CONCLUSION

A new class of heterocycles 1,2,4-Triazole were developed adopting simple, elegant and well versed methodologies. 1,2,4-Triazole are useful templates for further development through modification or derivitization to design more potent biologically active compounds .Various 1,2,4-Triazole from biphenyl 4- carboxylic acid were prepared with

the objective with of developing antimicrobial molecules and we have also evaluated preliminary antimicrobial activity. The 1,2,4-Triazole can be considered as potent Anti-inflammatory agents.Various compounds show potent inhibitory action against test organism. Synthesized compounds are useful templates for further development through modification or derivitization to design more potent biologically active compounds.

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