Synthesis and biological evaluation of some novel triazolo, thiadiazole derivatives of gallic acid

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Abstract

In the present study, a new series of 5-{6-(substituted phenyl)-5,6-dihydro-(1,2,4) triazolo(3,4-b)(1,3,4)thiadiazol-3-yl}benzene-1,2,3-triol **7a-j** have been synthesized. The reaction of Propyl gallate **1** and hydrazine hydrate **2** yielded galloylhydrazide **3,**which on treatment with potassium hydroxide and carbondisulfide furnished 5-(-5-mercapto-1,3,4-oxadiazol-2-yl) benzene-1,2,3-triol **4** .This on hydrazinolysis gave 5-(4-amino-5mercapto - 4H-1,2,4 – triazol-3-yl) benzene - 1,2,3-triol **5**. Finally cyclization of **5** with various aromatic aldehyde **6a-j** and piperidine converted into the title compounds **7a-j**. These compounds have been characterized by IR, ^{1}HNMR, Mass spectral analysis. These newly synthesized compounds were screened for their antimicrobial activity and anti-inflammatory. The anti-inflammatory activity has been done by carrageenan induced acute paw oedma in rats. The *in vitro* antimicrobial activity has been carried out by cup plate method and minimum inhibitory concentration value was determined for the titled compound by agar streak dilution method.

Keywords: Gallic acid, triazolothidiazole, antimicrobial, anti-inflammatory activity.

Introduction

Triazolothiadiazole and its derivatives are biologically important active compounds. They may be viewed as a cyclic analogue of two very important compounds thiosemicarbazide and biguanide which often display diverse biological activity [1]. The substituted triazolothiadiazole in the 3 and 6 positions by aryl, alkyl or heterocyclic moiety posses pharmacological activities such as antibacterial [2], antifungal [3], anticancer [4], anti-inflammatory [5], antihelmintic [6], antiparasitic [7] and antioxidant [8] activities. Some new 1,2,3-triazolo derivatives were reported for inhibiting tumor proliferation, invasion and metastasin and are useful in treating chronic renal failure related disorder, particularly anxiety, depression, other psychotic neurology disorders as well as in the treatment of immunology and cardiovascular disorders. Gallic acid is a strong natural antioxidant, able to scavenge hypochlorous acid [9,10]. It also inhibit histamine release and pro-inflammatory cytokine production in mast cells [11]. Propyl gallate is the propyl ester of gallic acid which is involved in synthesis as precursor and used as antioxidant [12]. A vast

literature reveals that 1,2,4-triazole and 1,3,4 triazolo thiadizole derivatives play vital role as synthetic drugs.

In the present study, substituted thiadiazoles **7a-j** were prepared by reacting propylgallate with hydrazine hydrate yielded galloyl hydrazide in the presence of ethanol refluxed for 6hrs. This was treated with carbon disulfide in the presence of potassium hydroxide in ethanol refluxed for 6hrs yielded the product 4. The products 4 were mixed with hydrazine hydrate refluxed for 6hrs in ethanol yielded the product **5.** Which on treatment with various aromatic aldehydes **6a-j** and piperidine in ethanol, refluxed for 3 hrs, the product **7a-j** were produced**. (Scheme – I)**

The *in vitro* antibacterial *(Staphylococcus aures, Klebisiella pneamoniae, Pseudomonas aeruginosa, Escherichia coli)* and antifungal *(Asperigillus niger)* activity of compounds were evaluated by cup plate method, the minimum inhibitory concentration (MIC) of the compounds were also determined by agar streak dilution method [13]. The *in vivo* anti-inflammatory activity, of synthesized compounds **7a-j** were evaluated by carrageenan induced paw oedma in rats and the results are compared with standard drug Diclofenac sodium.

Result and Discussion

All the compounds exhibited significant anti-inflammatory activity. Among the ten compounds, the synthesized compounds substituted with hydroxyl benzaldehyde **7a,7b and 7c**, nitrobenzaldehyde **7d,7e and 7f** and dimethyl amino benzaldehyde **7i** were found to be more active as that of standard drug Diclofenac sodium and compound substituted with trimethoxy benzaldehyde **7j** was moderately active. Moreover, all the compounds exhibited moderately potent antibactierial and antifungal activity.

The compounds were active against the entire tested microorganism compared to ciprofloxacin as standard and the MIC value of 150µg/ml against *Escherichia coli, Staphylococcus aures, Pseudomonas aeruginosa, Klebisiella pneamoniae and Asperigillus niger*. Among all the compounds **7i** and **7j** showed potent antimicrobial activity.

Materials and Methods

The melting points were determined in open capillary tubes and are uncorrected. The IR spectra of the compounds were recorded on Perkin Elmer FTIR spectrometer in KBr pellets. ^{1}HNMR spectra were recorded on Bruker 400 MHZ AVANCE ^{1}HNMR spectrometer. The chemical shifts are reported in parts per million downfield from tetramethylsilane. Mass spectra were recorded on LC-MS Schimadzu 2010A using dimethyl sulfoxide as solvent.

All the compounds gave satisfactory chemical analysis the homogencity of the compounds was checked by TLC on aluminum foil packed precoated silica gel plates using n-hexane and ethyl acetate (8:2) as mobile phase and visualized by iodine vapors. The results are in table I.

Table I - Physicochemical parameters

S. No	Compound	Mol. formula	% Yield	Melting point (°C)	R_f value
1.	7a	$C_{15}H_{12}N_4O_4S$	80	240-243	0.71
2.	7b	$C_{15}H_{12}N_4O_4S$	82	246-249	0.72
3.	7c	$C_{15}H_{12}N_4O_4S$	80	243-245	0.65
4.	7d	$C_{15}H_{11}N_5O_5S$	80	168-171	0.48
5.	7e	$C_{15}H_{11}N_5O_5S$	73	188-190	0.56
6.	7f	$C_{15}H_{11}N_5O_5S$	78	210-213	0.71
7.	7g	$C_{15}H_{11}N_4O_3SCl$	70	185-188	0.60
8.	7h	$C_{15}H_{11}N_4O_3SCl$	75	184-186	0.56
9.	7i	$C_{17}H_{17}N_5O_3S$	83	228-230	0.72
10.	7j	$C_{18}H_{18}N_4O_6S$	88	190-193	0.66

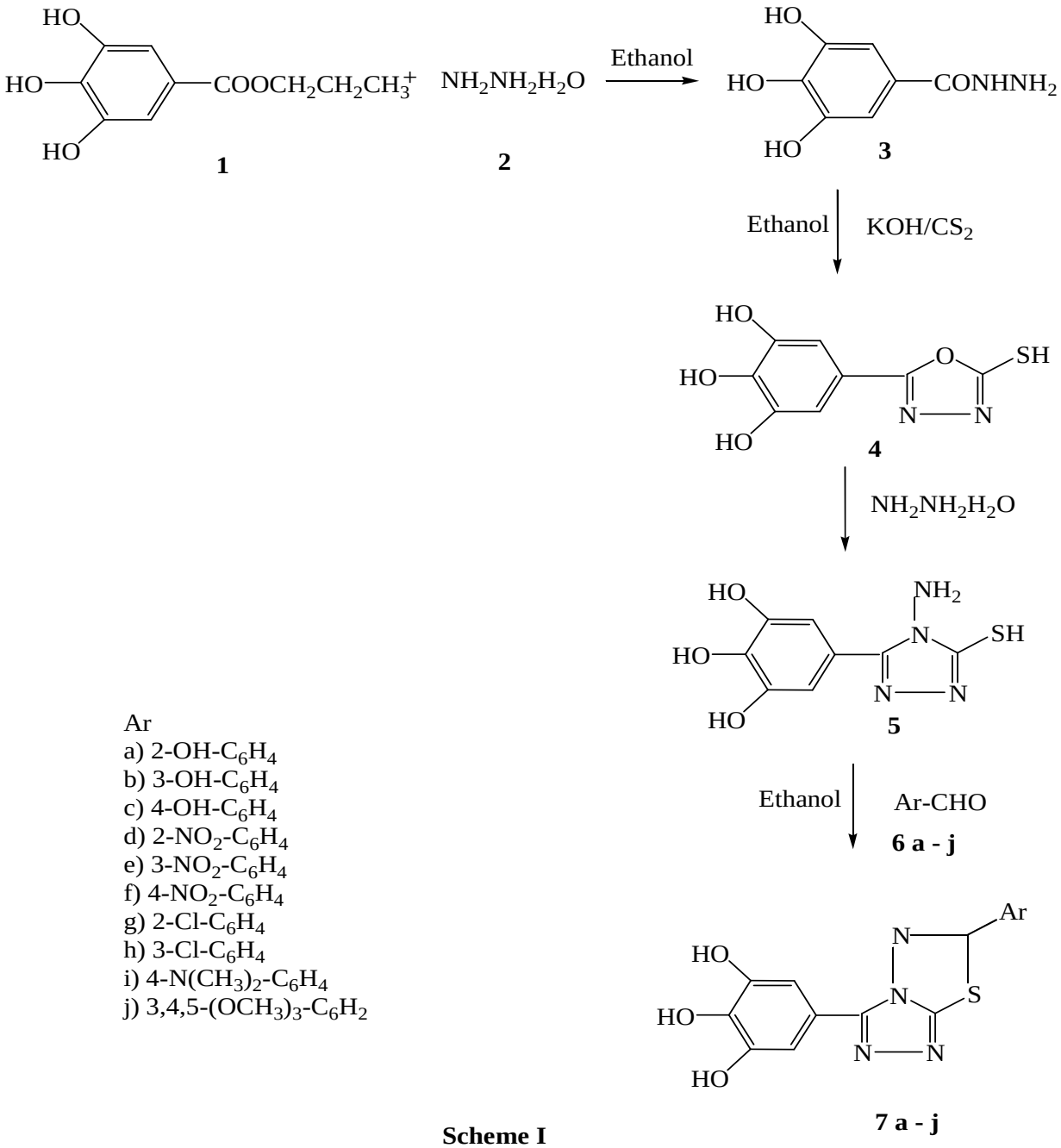
General procedure for synthesized compounds 7a-j

Galloyl hydrazide **3** was synthesized from propyl gallate **1** and hydrazine hydrate **2** from the reported procedure [9].

Galloyl hydrazide (0.01moles) **3** was treated with carbon disulfide (0.01moles) in the presence of potassium hydroxide (0.25g) in 10ml of ethanol, medium at room temp the potassium salt of dithiocarbazate on refluxed for 6hrs yielded the product, was filtered, dried and recrystallized with methanol and compound **4** was collected.

The substituted oxadiazoles (0.01moles) **4** from previous step and hydrazine hydrate (0.01moles) were mixed gently and refluxed for 6hrs with 15ml of ethanol. The mixture was then cooled and poured into ice cold water with stirring. Then neutralized with dil. Hcl and the mass obtained was filtered and recrystallized with ethanol to yield compound **5**.

A mixture of 5-(4-amino-5-mercapto-4H-1,2,4,-triazol (0.01moles) **5**, aromatic aldehydes (0.01moles) **6a-j** and few drops of piperidine in 15ml of ethanol were under refluxed for 3hrs. The reaction mixture was cooled and treated with crushed ice. The residue was dried and recrystallized with ethanol and formations of the desired products 7a-j were confirmed by TLC. **(Scheme – I)**



Ar
a) 2-OH-C_6H_4
b) 3-OH-C_6H_4
c) 4-OH-C_6H_4
d) 2-NO_2-C_6H_4
e) 3-NO_2-C_6H_4
f) 4-NO_2-C_6H_4
g) 2-Cl-C_6H_4
h) 3-Cl-C_6H_4
i) 4-$N(CH_3)_2$-C_6H_4
j) 3,4,5-$(OCH_3)_3$-C_6H_2

Scheme I

Spectral Data

5-(6-(2-Hydroxyphenyl)-5,6-dihydro(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3yl) benzene-1,2,3-triol ***7a:*** Yield: 80%. m.p. 240-242^{0}C. ^{1}HNMR (DMSO-d_6): δ 1.23 (S,1H,CH), 5.05 (S,4H,OH), 6.55 (S,1H,NH), 7.78-7.69 (m,6H,ArH); IR (KBr) cm^{-1}: 3435 (OH phenolic), 1462 (C=C), 1602 (C=N), 1250 (C-N), 618 (C-S); MS: m/z 344 $[M]^+$

5-(6-(3-Hydroxyphenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3yl) benzene-1,2,3-triol ***7b:*** Yield: 82%. m.p 246-248^{0}C. ^{1}HNMR (DMSO-d_6): δ 1.55 (s,1H,CH), 4.85 (s,1H,NH),4.85 (s,4H,OH), 7.46-7.53 (m,6H,Ar.H); IR (KBr) cm^{-1}: 3420(OH phenolic**)**,1576 (C=C), 1617 (C=N), 1280 (C-N), 688 (C-S); MS: m/z 344 $[M]^+$

5-(6-(4-Hydroxyphenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b) (1,3,4) thiadiazol-3yl) benzene-1,2,3-triol ***7c:*** Yield: 82%. m.p 243-246^{0}C, ^{1}HNMR (DMSO-d_6): δ 2.50 (s,1H,CH), 4.127 (s,4H,OH), 6.83 (s,1H,NH), 7.68-7.80 (m,6H,Ar.H); IR (KBr) cm^{-1}: 3421 (OH phenolic),1597 (C=C), 1617 (C=N), 1312 (C-N), 688 (C-S); MS: m/z 344 $[M]^+$

5-(6-2(4-Nitrophenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b) (1,3,4) thiadiazol-3yl) benzene-1,2,3-triol ***7d:*** Yield: 80%. m.p 168-170^{0}C, ^{1}HNMR (DMSO-d_6): δ 1.23 (s,1H,CH), 4.11 (s,3H,OH), 5.05 (s,1H,NH), 7.17-7.19 (m,6H,ArH); IR (KBr) cm^{-1}: 3412 (OH phenolic), 1444 (C=C), 1606 (C=N), 1346 (C-N), 691 (C-S); MS: m/z 373 $[M]^+$

5-(6-(4-Nitrophenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b) (1,3,4) thiadiazol-3yl) benzene-1,2,3-triol ***7e:*** Yield: 73%. m.p 188-190^{0}C, ^{1}HNMR (DMSO-d_6): δ 1.65 (s,1H,CH), 4.12 (s,3H,OH), 6.82 (s,1H,NH), 7.63-7.81 (m,6H,ArH); IR (KBr) cm^{-1}: 3436 (OH phenolic), 1354 (C=C),1627 (C=N),1266 (C-N), 699 (C-S); MS: m/z 373 $[M]^+$

5-(6-(3-Hydroxyphenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b)(1,3,4)thiadiazol-3yl) benzene-1,2,3-triol ***7f:*** Yield: 78%, m.p 210-213^{0}C, ^{1}HNMR (DMSO-d_6): δ 1.59 (s,1H,CH), 4.69 (s,3H,OH), 4.85 (s,1H,NH), 7.50-7.56 (m,6H,ArH); IR (KBr) cm^{-1}: 3446 (OH phenolic),1343(C=C),1521 (C=N),1106 (C-N), 700 (C-S); MS: m/z 373 $[M]^+$

5-(6-(4-Chloropenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b) (1,3,4) thiadiazol-3yl) benzene-1,2,3-triol ***7g:*** Yield: 70%. m.p 185-188^{0}C, ^{1}HNMR (DMSO-d_6): δ 1.54 (s,1H,CH), 4.12 (s,3H,OH), 6.76 (s,1H,NH), 7.57-7.77 (m,6H,ArH); IR (KBr) cm^{-1}: 3422 (OH phenolic), 1444 (C=C), 1667 (C=N), 1286 (C-N), 698 (C-S); MS: m/z 362 $[M]^+$

5-(6-(2-Chloropenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b) (1,3,4) thiadiazol-3yl) benzene-1,2,3-triol ***7h:*** Yield: 70%. m.p 184-186^{0}C, ^{1}HNMR (DMSO-d_6): δ 1.59 (s,1H,CH), 5.05 (s,3H,OH), 6.76 (s,1H,NH), 7.46-7.60 (m,6H,ArH); IR (KBr) cm^{-1}: 3413 (OH phenolic), 1465 (C=C), 1614 (C=N), 1270 (C-N), 724 (C-S); MS: m/z 362 $[M]^+$

5-(6-(4-Dimethylpenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b) (1,3,4) thiadiazol-3yl) benzene-1,2,3-triol ***7i:*** Yield: 83%. m.p 228-230^{0}C, ^{1}HNMR (DMSO-d_6): δ 1.03 (s,1H,CH), 2.48 (s,6H,N-$(CH_3)_2$), 5.64 (s,3H,OH),6.24 (s,1H,NH),7.42-7.49 (m,6H,ArH); IR (KBr) cm^{-1}: 3401 (OH phenolic), 1551 (C=C),1605 (C=N), 1179 (C-N), 744 (C-S); MS: m/z 371 $[M]^+$

5-(6-(3,4,5-Trimethoxypenyl)-5,6-dihydro-(1,2,4)triazolo(3,4-b) (1,3,4) thiadiazol-3yl) benzene-1,2,3-triol ***7j:*** Yield: 88%. m.p 190-193^{0}C, ^{1}HNMR (DMSO-d_6): δ 1.54 (s,1H,CH), 4.21

(s,9H,OCH_3), 4.50 (s,3H,OH), 6.55 (s,1H,NH), 7.42-7.51 (m,4H,ArH); IR (KBr) cm^{-1}: 3394 (OH phenolic), 1580 (C=C), 1622 (C=N), 1184 (C-N) 736 (C-S); MS: m/z 418 $[M]^+$

In vitro Antimicrobial activity

All the synthesized compound were tested for their antimicrobial activity against two gram +ve (*Staphylococcus aures and Klebisiella pneamoniae),* two gram –ve (*Escherichia coli and Pseudomonas aeruginosa*) bacterial and one fungal strains (*Asperigillus niger*) at a concentration of 150 µg/ml using cup plate method and MIC was determined by agar streak dilution method. Ciprofloxacin at a concentration of 10µg/ml was used as standard for antibacterial activity and Ketaconazole at a concentration of 20 µg/ml was used as standard for antifungal activity. The observed Zone of inhibition and the MIC values for all the synthesized compounds are presented in Table-II.

Table II - Antimicrobial activity of synthesized compounds **7a-j** *In vitro* activity Zone of inhibition (MIC)

Compound	*Escherichia coli*	*Klebisiella pneamoniae*	*Staphylococcus aures*	*Pseudomonas aeruginosa*	*Asperigillus niger*
7a	15(25)	14(22)	16(30)	14(24)	13(15)
7b	14(22)	13(31)	15(22)	13(25)	14(15)
7c	13(24)	15(30)	14(22)	15(22)	13(22)
7d	16(30)	15(25)	15(31)	14(26)	15(24)
7e	15(24)	16(24)	16(24)	16(24)	13(15)
7f	14(24)	14(26)	15(30)	15(30)	12(24)
7g	12(31)	13(30)	13(24)	12(22)	11(25)
7h	13(25)	11(31)	13(31)	14(30)	13(15)
7i	15(26)	16(30)	15(33)	16(32)	15(22)
7j	16(33)	15(32)	17(35)	18(30)	16(34)
Ciprofloxacin 10µg/ml	22(1.5)	25(0.9)	22(1.2)	25(1.4)	-
Ketaconazole 20µg/ml	-	-	-	-	24(2.2)

Zone of inhibition in mm, MIC in µg mL^{-1}

Anti-inflammatory Activity

The anti-inflammatory activity was determined by carrageen an-induced acute paw edema in rats [14]. Rats of either sex were selected by random sampling technique and used for the study. Diclofenac sodium 20mg/kg of was administered as standard drug for comparison. The test compounds **7a-j** was administered orally by intragastric tube at a dose of 20mg/kg body weight. After half an hour of administration of test compounds 0.1ml of carrageenan was injected in to

the lateral malleolus of the sub planter region of the left hind paw. The inflammation of the paw was measured for all the animals by using plethysmograph before the administration of the carrageenan and after the administration of the carrageenan at 60 and 180 min. The percentage protection of the compound was calculated and tabulated in Table –III.

Table III - Anti-inflammatory activity of the synthesized compound 7a-j Carrageenan induced Paw Oedema

Group	Oedema volume		% inhibition	
	1hr	3rd hr	1hr	3rd hr
Group I 1%Tween-80	0.47±0.01	0.50±0.02	-	-
Group II Compound-7a (20mg/kg)	0.26±0.02	0.23±0.01	43.33±2.15	38.32±2.86
Control III Compound-7b (20mg/kg)	0.21±0.01	0.25±0.03	54.86±1.42	49.69±3.80
Group IV Compound-7c (20mg/kg)	0.23±0.02	0.27±0.02	54.24±2.77	44.74±2.73
Group V Compound-7d (20mg/kg)	0.27±0.02	0.31±0.02	41.26±2.09	36.49±2.68
Group VI Compound-7e (20mg/kg)	0.22±0.02	0.26±0.02	52.73±3.42	47.91±1.40
Group VII Compound-7f (20mg/kg)	0.22±0.02	0.27±0.03	51.92±1.88	45.27±4.27
Group VIII Compound-7g (20mg/kg)	0.26±0.01	0.30±0.02	43.86±1.67	40.22±1.97
Group IX Compound-7h (20mg/kg)	0.25±0.02	0.29±0.02	46.99±2.26	41.22±3.01
Group X Compound-7i (20mg/kg)	0.23±0.02	0.27±0.02	50.31±1.81	45.82±2.68
Group XI Compound-7j (20mg/kg)	0.24±0.01	0.27±0.02	49.14±2.83	45.27±1.04
Group XII Standard Diclofenac (20mg/kg)	0.21±0.01	0.23±0.01	55.5±1.97	53.20±0.38

All values are mean ± SEM values using 6 animals in each group.

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