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Synthesis, Characterisation and BiologicalEvaluation of some novel 2,5-Disubstituted-1,3,4-oxadiazole derivatives of Gallic acid

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Abstract: A series of 2-(3,4,5-trihydroxy phenyl)-5-aryl-1, 3,4- oxadiazole **(3)** (S_1-S_{10}) was synthesized from propyl gallate **(1)** and hydrazine hydrate in presence of ethanol to give 3, 4, 5- trihydroxybenzohydrazide **(2)** followed by reaction with phosphorus oxychloride and various aromatic acids. Structure of the synthesized compounds was established by physicochemical property analysis and spectral data. Synthesized compounds were subjected to anti-microbial, anti-fungal and anti-tubercular activity. Anti-microbial activity was carried out against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus* at a concentration of 100 µg/ml. Streptomycin was used as standard. Anti-fungal activity was performed against *Aspergillus niger*, with test compounds at a concentration of 100µg/ml. Ketaconazole was the standard drug employed. Finally anti tubercular activity was observed at concentrations 100µg/ml on *Mycobacterium tuberculosis* using Rifampicin as standard.
Key words: Gallic acid, 1,3,4 - oxadiazole, Anti-microbial activity, Anti-tubercular activity.

Introduction

Gallic acid is a strong antioxidant and have been reported to posses anti bacterial, anti-fungal[1], anti-inflammatory[2]and anti-cancer activity[3]. Propyl gallate is the propyl ester of gallic acid, which is involved in synthesis as precursor and used as anti-oxidant[4]. It also inhibits histamine release[5]. Also it acts as chelating agent and binds with iron to form stable complex. Gallic acid also acts as astringent. Oxadiazole derivatives are also known to posses anti-microbial[6], anti-parasitic[7], anti-inflammatory[8], anti-cancer[9] and anti-tubercular activity[10, 11]. A novel series of 2,5-diaryl oxadiazole was identified as apoptosis-inducing agents through the cell and chemical genetics-based screening assay for compounds that induce apoptosis using a chemical genetics approach. In view of the above-mentioned findings, the purpose of the present work was to design, synthesize and investigate the *in vitro* anti-bacterial, anti-fungal and anti-tubercular activities of some novel 2,5-disubstituted 1, 3, 4 - oxadiazole derivatives of gallic acid. (S_1-S_{10}).

Experimental

Synthetic method

Synthesis of 3,4,5-Trihydroxybenzohydrazide

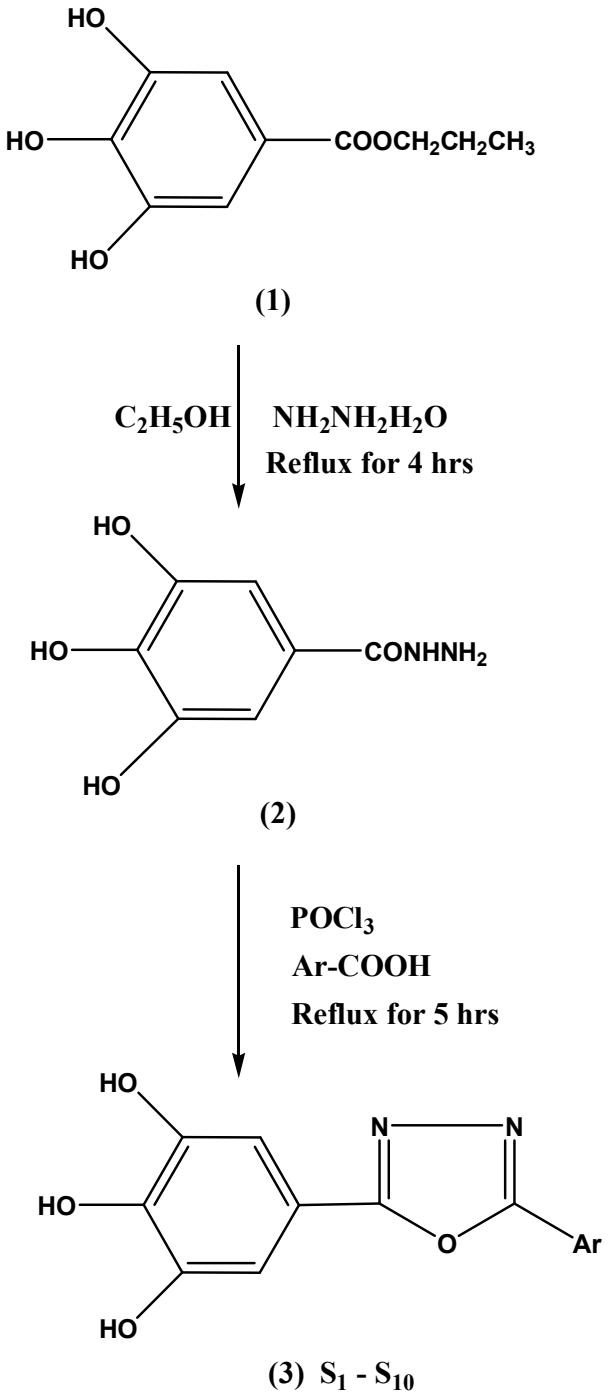
Propyl gallate (0.01 mol) and hydrazine hydrate (0.01 mol) were mixed gently and refluxed for
4 hrs with 30ml of ethanol. The mixture was then cooled and poured into ice cold water with stirring. The mass obtained was filtered, washed with water and recrystallised from ethanol.
(m.p. : 118-120 ºC; yield: 72%).

Synthesis of 2-(3,4,5-trihydroxyphenyl)-5-aryl-1,3,4-Oxadiazole(S_1-S_{10})

A mixture of 3,4,5-trihydroxybenzohydrazide (0.01mol) and appropriate aromatic acid (0.01 mol) were dissolved in phosphorus oxy chloride and refluxed for 5 hrs .The mixture was then cooled and poured into ice cold water under stirring. The mass obtained was filtered and washed with water and recrystallised from ethanol.
Melting point of the synthesized compounds was taken in open-end capillary tubes and was uncorrected. Thin layer chromatography was preformed using plates coated silica

gel of 0.25 mm thickness. Chloroform : methanol (9 : 1) were used as mobile phase. Spots were visualized through the iodine chamber. R_f values of the newly synthesized compounds were calculated. The physicochemical parameters of the synthesized compounds are given in Table 1.

Scheme



The Infra - Red spectroscopy was performed with KBr pellet techniques on Perkin Elmer FT- IR instrument. ^{1}HNMR was recorded on Bruker 400MHz AVANCE. ^{1}HNMR the Chemical shifts were reported as parts per million downfield from tetramethylsilane, Mass spectroscopy was performed on LCMS 2010A using the solvent Dimethyl sulfoxide .The spectral data of the synthesized compounds are summarized.

5- (5-(3-Chlorophenyl)-1,3,4-oxadiazol-2- yl) benzene-1,2,3- triol (S_1)
IR (KBr) (cm $^{-1}$): 3418(phenolic – OH), 1428(C=C), 1074(C-O-C), 1256(N-N=C), 1634 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 5. 05 (s, 3H, OH), 6.45 (s, 2H, Ar H), 7.18-7.80 (m, 4H,Ar H); MS (m/z): 305.09(M^+), 273. 07(B^+).

5- (5- 4- Chlorophenyl) – 1, 3, 4- oxadiazol – yl) benzene – 1, 2, 3- triol (S_2)
IR (KBr) (cm $^{-1}$): 3455(phenolic – OH), 1424(C=C), 1091(C-O-C), 1249 (N-N=C), 1617 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 5.11(s, 3H, OH), 6.32 (s, 2H, Ar H), 7.20-7.60 (m, 4H, Ar H); MS (m/z): 305.09(M^+), 273.01(B^+).

5- (5- (2, 4- Dichlorophenyl) – 1, 3, 4- oxadiazol –2- yl) benzene- 1, 2, 3- triol (S_3)
IR (KBr) (cm $^{-1}$): 3313(phenolic – OH), 1410(C=C), 1110(C-O-C), 1265(N-N=C), 1698 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 5.05 (s,3H, H), 6. 20 (s, 2H, Ar H), 7.18-7.75(m, 4H, Ar H);
MS (m/z): 336. 07(M^+), 283.08(B^+).

5- (5- (2- Hydroxyphenyl) – 1, 3, 4- oxadiazol- 2- yl) benzene- 1, 2, 3- triol (S_4)
IR (KBr) (cm $^{-1}$): 3454(phenolic – OH), 1424(C=C), 1091(C-O-C), 1252(N-N=C), 1619 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 5. 10 (s, 4H, OH), 6. 28 (s, 2H, Ar H),6. 80-7. 65(m, 4H, Ar H); MS (m/z): 287. 13(M^+), 255 .04(B^+).

5- (5- (2,4- Dihydroxyphenyl)- 1, 3, 4- oxadiazol- 2- yl) benzene- 1, 2, 3- triol (S_5)
IR (KBr) (cm $^{-1}$): 3454(phenolic – OH), 1443(C=C), 1095(C-O-C), 1242(N-N=C), 1619 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 5. 12(s, 5H, OH), 6. 30 (s, 2H, Ar H), 6. 20-7. 30(m, 4H, Ar H); MS (m/z): 304.4. (M^+), 287.08. (B^+).

5- (5- (3- Aminophenyl) - 1, 3, 4- oxadiazol- 2- yl) benzene- 1, 2, 3- triol (S_6)
IR (KBr) (cm $^{-1}$): 3452(phenolic – OH), 1467(C=C), 1098(C-O-C), 1249(N-N=C), 1615 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 4. 05(s, 2H, NH_2), 5. 05 (s, 3H, OH), 6. 34(s, 2H,Ar H), 6. 40-7.20(m, 4H, Ar H); MS (m/z): 286.05 (M^+), 241.10. (B^+).

5- (5- (4- Aminophenyl)- 1, 3, 4- oxadiazol- 2- yl) benzene- 1, 2, 3- triol (S_7)
IR (KBr) (cm $^{-1}$): 3452(phenolic – OH), 1467(C=C), 1098(C-O-C), 1249(N-N=C), 1614 (C=N);
^{1}HNMR (DMSO-d_6) δ (ppm): 3. 98 (s, 2H, NH_2), 5. 10 (s, 3H, OH), 6. 25 (s, 2H,Ar H),
6. 50-7.30 (m, 4H, Ar H); MS (m/z): 286. 05 (M^+), 238.04 (B^+).

5-(5- (3- Nitrophenyl)- 1, 3, 4- oxadiazol- 2- yl) benzene- 1, 2, 3- triol (S_8)
IR (KBr) (cm $^{-1}$): 3454(phenolic – OH), 1446(C=C), 1084(C-O-C), 1303(N-N=C), 1618 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 5. 12(s, 3H, OH), 6.32(s, 2H, ArH), 7. 50-8. 50 (m, 4H, ArH);
MS (m/z): 305.12(M^+), 287.05(B^+).

5-(5- (4- Nitrophenyl)- 1, 3, 4- oxadiazol- 2- yl) benzene- 1, 2, 3- triol (S_9)
IR (KBr) (cm $^{-1}$): 3418(phenolic – OH), 1410(C=C), 1091(C-O-C), 1284(N-N=C), 1648 (C=N); ^{1}HNMR (DMSO-d_6) δ (ppm): 5. 05(s, 3H, OH), 6.39(s, 2H, ArH), 7. 50-8. 50(m, 4H, ArH);
MS (m/z): 305.12(M^+), 284.05(B^+)

5- (5-(3- Methoxyphenyl)- 1, 3, 4- oxadiazol- 2- yl) benzene- 1, 2, 3- triol (S_{10})
IR (KBr) (cm $^{-1}$): 3454(phenolic – OH), 1429(C=C), 1087(C-O-C), 1243(N-N=C), 1605 (C=N).; ^{1}HNMR (DMSO-d_6) δ(ppm): 3. 82(s, 3H, CH_3), 5. 10(s,3H, OH), 6. 35(s, 2H,ArH), 6.70-7.40(m, 4H,ArH); MS (m/z): 301. 12(M^+), 269. 10(B^+)

Anti- microbial screening
Anti- bacterial activity
Bacterial strains of *E. coli, K. pneumoniae, S. aereus, P. aeruginosa* were taken. Cup-plate method with nutrient agar medium was used to evaluate *in- vitro* anti- bacterial activity[12] of the synthesized compounds. The standard antibiotic selected for study was Streptomycin. Sub culture was prepared by inoculation of the bacterial cultures in the nutrient agar and incubated at
37^{o}C for 18-24 hrs. Definite volume of this was taken with the help of cotton swab and the organisms were streaked on the entire agar surface and dried. The solution of the test compounds of concentration 100µg/ml were added into the cups by using micropipettes and these plates were subsequently incubated in an inverted position for 24 hours at 37 ^{0}C and observed for anti- bacterial activity. The solvent control (DMSO) was kept separately. After 24 hrs, the diameter of zone of inhibition was measured in mm and is tabulated in Table 2.

Anti- fungal activity
Cup-plate method[13] with nutrient agar media in Petri-dish, was used.. A loopful of fungal strain *Aspergillus niger* was inoculated into nutrient agar media of each plate, and incubated at 25±2ºc for 72 hours. Ketoconazole was used as standard drug.. Minimum inhibitory concentrations of the test compounds at a concentration of 100 µg/ml were determined. A control was prepared using solvent DMSO. Results in the form of percent inhibition are summarized in Table 2.

Anti- tubercular activity
Bacterial strains. *M. tuberculosis $H_{37}Rv$* ATCC 27294 (American Type Culture Collection), *$H_{37}Rv$* inoculate was grown in 100 ml of Middlebrook 7H9 broth (Difco, Detroit, Mich.) supplemented with 0.2% (vol/vol) glycerol (Sigma Chemical Co., Saint Louis, Mo.), 10% (vol/vol) OADC (oleic acid, albumin, dextrose, catalase; Difco), and 0.05% (vol/vol) Tween 80 (Sigma). The complete medium was referred to as 7H9GC-T80. The anti-tubercular activity[14] was performed by Microplate Alamar Blue Assay (MABA). Anti-TB susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Initial drug dilutions were prepared in dimethyl sulfoxide, and subsequent twofold dilutions were performed in 0.1 ml of 7H9GC-T80 media in the microplates. Rifampicin was used as standard drug. Percentage inhiitionof the test compounds were determined after incubated 7 days at 37°C . Results are summarized in Table 3.

Results and discussions
A series of 2-(3,4,5-trihydroxy phenyl)-5 aryl-1, 3,4-oxadiazole (3) (S_1-S_{10}) was synthesized. The synthesized compounds were characterized by IR, ^{1}HNMR and Mass spectral data. All the synthesized compounds show characteristic absorption peaks in IR and NMR spectra. Expected molecular ion peak (M^++1) fragments were observed for the entire compounds in mass spectra.
The biological studies of the compounds were evaluated for Anti-bacterial, Anti-fungal and Anti-tubercular activities. The bacterial screening indicated that among the test compounds S_1, S_2, S_4 and S_{10} showed moderate activity against all the tested bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus*. The remaining compounds were found to be less active, when compared to that of standard drug Streptomycin..
Antifungal screening revealed that the test compounds showed moderate activity against *Aspergillus niger*. The compounds S_4, S_6 and S_{10} showed moderate activity when compared to that of standard drug Ketoconazole. Anti-tubercular activity was performed using Microplate Alamar Blue Assay (MABA) method in Bacterial strains *M. tuberculosis $H_{37}Rv$*. The synthesized compounds S_1, S_2, S_4 and S_6 were found to have similar activity as that of standard. Remaining compounds possess comparable activity as that of standard drug Rifampicin.

Table 1. Physicochemical properties of the synthesized compounds.

Compounds	Ar	% Yield	Melting point (^oC)	Molecular formula	R_f value
S_1	3-Chloro phenyl	78	118-120	$C_{14}H_9N_2O_4Cl$	0.71
S_2	4-Chloro phenyl	82	100-101	$C_{14}H_9N_2O_4Cl$	0.72
S_3	2,4-Dichloro phenyl	79	119-121	$C_{14}H_8N_2O_4Cl_2$	0.65
S_4	2-Hydroxy phenyl	80	110-112	$C_{14}H_{10}N_2O_5$	0.48
S_5	2,4-Dihydroxy phenyl	73	117-119	$C_{14}H_{10}N_2O_6$	0.56
S_6	3-Amino phenyl	78	106-108	$C_{14}H_{11}N_3O_4$	0.71
S_7	4-Amino phenyl	70	98-100	$C_{14}H_{11}N_3O_4$	0.60
S_8	3-Nitro phenyl	75	90-92	$C_{14}H_9N_3O_6$	0.56
S_9	4-Nitro phenyl	83	116-118	$C_{14}H_9N_3O_6$	0.72
S_{10}	3-Methoxy phenyl	88	88-90	$C_{15}H_{12}N_2O_5$	0.66

Table 2. Anti-microbial activity of synthesized compounds

Compounds	Diameter of the Inhibition Zone (mm) 100µg/ml				
	E.coli	***K.pnuemoniae***	***S.aureus***	***P.aeruginosa***	***A. niger***
S_1	15	14	16	14	12
S_2	14	13	15	13	14
S_3	13	15	14	15	12
S_4	16	15	15	14	13
S_5	15	16	16	16	13
S_6	14	14	15	15	14
S_7	12	13	13	12	11
S_8	13	11	13	14	10
S_9	15	16	15	16	12
S_{10}	16	15	17	18	14
Streptomycin	22	25	22	25	-
Ketaconazole	-	-	-	-	24

Streptomycin 10µg/ml was used as standard drug for anti-bacterial activity.
Ketoconazole 10µg/ml was used as standard drug for anti-fungal activity.

Table 3. In-vitro Anti-tubercular activity of synthesized compounds.

Compounds	% Inhibition at 100µg/ml
S_1	100
S_2	99
S_3	93
S_4	98
S_5	95
S_6	100
S_7	95
S_8	92
S_9	89
S_{10}	90
Rifampicin	100

REFERENCES

1. Arunkumar S., Ramalakshmi N., Saraswathi T. and Aruloly L., Synthesis of some derivatives of Gallic acid. Indian J. Heterocyclic chem.2006, 16, 29-32.
2. Indap M.A., Radhika S., Motiwale L. and Rao K. Anticancer activity of phenolic antioxidant against breast cancer cells and a spontaneous mammary tumor, Indian J. Pharm. Sci. 2006, 68, 470-474**.**
3. Catarina A., Gomes T., Cruz G., Jose L.A., Nuno M., Fernanda B. and. Paula M. Anticancer activity of phenolic acids of natural or synthetic origin: A structure-activity study, J.Med.Chem.,2003 (46), 5395-5401.
4. Polwski K. and Slawinska D. Gallic acid a natural antioxidant in aqueous and miceller environment: spectroscopic studies. Current Topics in Biophysics. 2002; 2: 217-227.
5. Sang H. K., Chang D. J. and Kyongho S. Gallic acid inhibits histamine release and pro inflammatory cytokinine production in mast cells. Toxicology Sciences**,** 2006, 1, 123-131.
6. Basavaraja B.M., Vagdevi H.M., Srikrishna L.P., Shubha B.S. and Vaidya V.P. Synthesis and antimicrobial activity of 1, 3, 4-oxadiazole incorporated benzoxazoles. Indian J. Heterocyclic chem. 2008,18, 05-08.
7. Kalluraya B., Jyothi N.R. and Sujith K.V. Microwave assisted one-pot synthesis of some 2, 5-disubstituted 1, 3, 4-oxadiazoles. Indian J. Heterocyclic chem.2008, 17, 359-362.
8. Desai N.C., Bhavsar A.M., Shah M.D. and Saxena A.K. Synthesis and QSAR studies of thiosemicarbazides, 1, 2, 4-triazoles, 1, 3, 4-thiadiazoles derivatives as potential antibacterial agents. Indian J. chem. 2008, 47(B), 579-589.
9. Shivananda W., Airodyvasudeva A. and Suchethakumari N. Synthesis of some new 2-(3-methyl-7-substituted-2-oxoquinoxalinyl)-5-(aryl)-1, 3, 4-oxadiazoles as potential non – steroidal anti-inflammatory and analgesic agents. Indian J. chem.2008; 47(B): 439-448.
10. Pinaki S., Deepakkumar D., Veerendra C.Y., Murgesh K., Rajalingam D.and Maity T.K. Evaluation of anticancer activity of some 1, 3, 4-oxadiazole derivatives. Indian J chem. 2008, 47(B), 460-462.
11. Vagadevi H.M., Joshi S.D., Vaidya V.P. and Gadaginamath G.S. Synthesis of new 4-pyrrol-yl benzoic acid hydrazide analogs and some derived oxadiazole,triazole and pyrolle ring system:A novel class of potential antibacterial and antitubercular agents. Eur J Med. Chem. 2008, 43,1989-1996
12. Jayamma Y., Sarangapani M., Reddy V.M. Synthesis and anti microbial activity of 2-[(3, 4-

dihydro-3-oxa-2H-1,4-benzoxazin-2-yl) methyl]-5-(alkyl/arythio)-1, 3, 4-oxadiazoles. Indian J Heterocyclic chem.1996, 6, 111-114.

13. Mari S.K., Dasappa J.P., Manjathuru M., Shivaramaholla B. and Suchethakumari N. Antimicrobial studies of 2,4-dichloro-5-fluorophenyl containing oxadiazole. Eur J Med. Chem. 2008, 43, 25-31
14. Collins L.A. and Franzblau S.G. Micro plate Alamar blue assay versus BACTEC 460 system for high –throughput screening of compounds against *Mycobacterium avium* Antimicrob Agents Chemother. 1997, 41, 1004-1009.

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