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Synthesis and Antimicrobial activity Evaluation of Poly ethylene Imine (PEI) dendrimer modified with 1,3,4 oxadiazole derivatives.

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ABSTRACT

Dendrimers have wide range of applications in industry, Pharmaceutical and medicine. Oxadiazole compounds also have many applications in Pharmaceutical and medicine. Modification of dendrimers which have many branches like $-NH_2$ functional groups with heterocyclic derivatives make them capable to show variety properties. In this article, PEI-dend-4[N[(Ts)(2-(methyl)-5-aryl-1,3,4 oxadiazole)]] **8a-f** have been synthesized from PEI (Poly ethylene imine) dendrimer which modified with 1,3,4 oxadiazole derivatives and investigated their antimicrobial activities.

Keywords: Poly ethylene imine(PEI) Dendrimer. 1,3,4 Oxadiazole. Antimicrobial.

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INTRODUCTION

Dendrimers are an interesting class of molecules, because they are relatively easily may be modified at their surface to obtain specific biological properties. Therefore by appropriate variation of the surface motifs, dendrimers with specific biological properties can be constructed and their biological properties can be modulated in endless ways. The biological properties of dendrimers are to a large extent determined by the nature of the surface groups, opening up for the possibility to specifically tailor the dendrimers for the desire biological effects. Dendrimers can also be synthesized to possess intrinsic biological properties that can be useful for treatment and other medical interventions. Dendrimers offer a range of advantages with respect to mediating biological effects in the living organism.

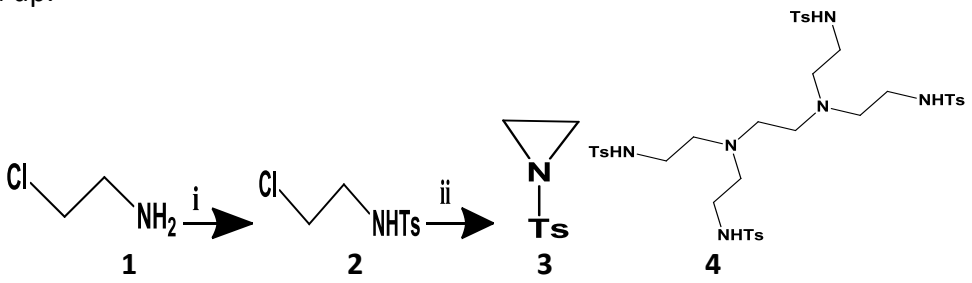
Oxadiazoles and their analogues can be considered as simple membered heterocycles possessing one oxygen and two nitrogen atoms. 1,3,4 oxadiazoles is the only isomers not containing a nitrogen-oxygen bond and are thermally stable neutral aromatic molecules[1] and its estimated resonance energy is 167.4 KJ/mol. 1,3,4 oxadiazole are associated with potent. Pharmacological activity due to the presence of toxophoric linkage[2] considerable evidence have been accumulated to demonstrate the efficacy of 1,3,4 oxadiazoles including anti protelytic, analgesic[3]anti malarial, anti cetylcholine esterase[4], anti inflammatory[5][6] diuretic[7] [8] , anti pyretic[9] and anti emetic activities.

MATERIALS AND METHODS

All the solvents and reagents were used as AR grade and used as such without further purification. The NMR spectra were recorded on Agilent 400 MHz spectrometer using DMSO-d_6, $CDCl_3$ solvents. Silica gel column chromatography was performed using Merck silica gel (100-200 mesh) and Merck made TLC were used for reaction monitoring.

General procedure for the synthesis of DEN-4[N[(Ts)(1,3,4 oxadiazole)]].

Dendrimer dissolved in dry DMF and added at 0°C to 60% NaH under N_2 atmosphere.1,3,4 oxadiazole dissolved in dry DMF and added to dendrimer mixture drop wise in 15 min. The reaction mixture heated to 80°C overnight then extracted with EtOAc and worked up.



Sheme1: i) Tosyl Chloride/MDC,TEA/RT, 2h ii)Tolene/NaOH 20%/0°C, 2h iii)Toluene/Acetonitrile/ethylenediamine/Reflux.

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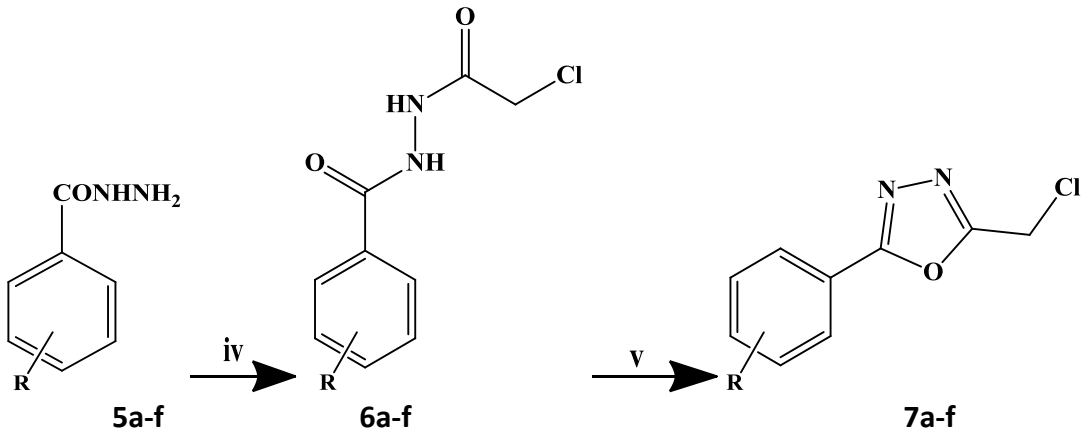
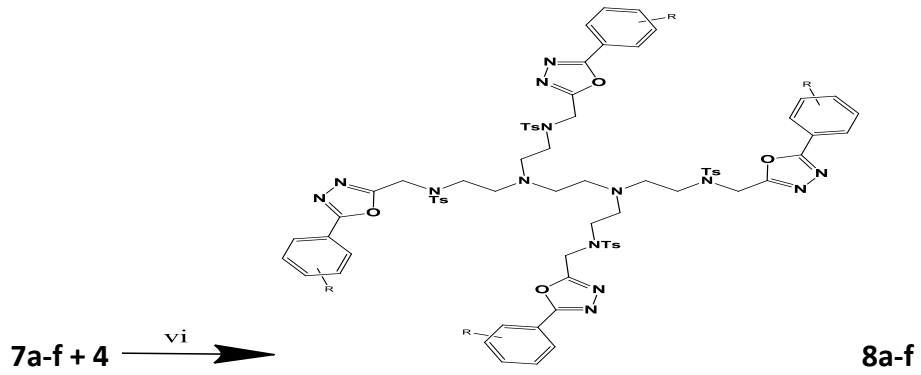


R:a:**4Me,** R:b:**4Cl,** R:c:**4F,** R:d:**4OMe,** R:e:**4Br**, R:f:**H**

Scheme 2: iv)Chloro acetyl chloride/THF/reflux v) $POCl_3$/reflux



R:a:**4Me,** R:b:**4Cl,** R:c:**4F,** R:d:**4OMe,** R:e:**4Br**, R:f:**H**

Scheme 3: vi) DMF/NaH 60%/0^{o}c/reflux.

Typical procedure for the synthesis of PEI-dend-4[N[(Ts)(2-(methyl)-5-aryl-1,3,4 oxadiazole)]] 8a-f.

Dendrimer (4),(0.2gr-0.0023 mol) dissolved in 4 ml of dry DMF was added to 60% NaH (14.4gr-0.6mol) in dry DMF (10ml) under nitrogen atmosphere. The reaction mixture heated to 80^{o}C for 4 h and cooled to 0^{o}C.

2-(Chloromethyl)-5-aryl-1,3,4 oxadiazole (7_{a-f}),(0.2gr-0.0118mol) dissolved in 4 ml dry DMF and added to dendrimer solution drop wise in 15 min. The reaction mixture heated to 80^{o}C overnight. The completion of the reaction was monitored by TLC and after completion, quenched by iced water then extracted with 2x10ml EtOAc and washed with 3x20ml water and brine solution then passed over Na_2SO_4, concentrated under reduced presser to give crude product. The crude product was purified by column chromatography to afford 74% yield product.

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DEN-4[N[(Ts)(2-(methyl)-5-(4-methylphenyl)-1,3,4 oxadiazole)]] 8a .
^{1}H NMR ($CDCl_3$): 2.07(s, 1H), 2.17-2.21(t, 2H), 2.32-2.37(dd, 6H), 2.62(t, 2H), 5.10(s, 2H), 7.31-7.41(dd, 4H), 7.62-7.64(d, 2H), 7.86-7.88(d, 2H).
DEN-4[N[(Ts)(2-(methyl)-5-(4-Chlorophenyl)-1,3,4 oxadiazole)]] 8b .
^{1}H NMR ($CDCl_3$): 2.01(s, 1H), 2.13-2.17(t, 2H), 2.31(s, 3H), 2.59(t, 2H), 5.08(s, 2H), 7.39-7.49(dd, 4H), 7.52-7.54(d, 2H), 7.66-7.68(d, 2H).
DEN-4[N[(Ts)(2-(methyl)-5-(4-Fluorophenyl)-1,3,4 oxadiazole)]] 8c .
^{1}H NMR ($CDCl_3$): 2.03(s, 1H), 2.15-2.17(t, 2H), 2.35 (s, 3H), 2.60(t, 2H), 5.12(s, 2H), 7.38-7.48(dd, 4H), 7.66-7.68(d, 2H), 8.62-8.64(d, 2H).
DEN-4[N[(Ts)(2-(methyl)-5-(4-methoxyphenyl)-1,3,4 oxadiazole)]] 8d .
^{1}H NMR ($CDCl_3$): 2.10(s, 1H), 2.2-2.26(t, 2H), 2.48(s, 3H), 2.69(t, 2H), 3.81(s, 3H), 5.30(s, 2H), 7.41-7.51(dd, 4H), 7.72-7.74(d, 2H), 8.01-8.03(d, 2H).
DEN-4[N[(Ts)(2-(methyl)-5-(4-Bromophenyl)-1,3,4 oxadiazole)]] 8e .
^{1}H NMR ($CDCl_3$): 2.03(s, 1H), 2.11-2.13(t, 2H), 2.24(s, 3H), 2.58(t, 2H), 5.15(s, 2H), 7.29-7.39(dd, 4H), 7.32-7.34(d, 2H), 7.42-7.44(d, 2H).
DEN-4[N[(Ts)(2-(methyl)-5-(phenyl)-1,3,4 oxadiazole)]] 8f .
^{1}H NMR ($CDCl_3$): 2.00(s, 1H), 2.18-2.22(t, 2H), 2.68(t, 1H), 5.11(s, 2H), 7.49-7.59(m, 5H), 7.59-7.61(d, 2H), 8.11-8.13(d, 2H).

Biology

Primary Screening by Agar well diffusion method

The antibacterial activity of the newly synthesized compounds were evaluated by agar well diffusion method [10]. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5×10^8 CFU/ml. 20 ml of Muller Hinton agar media was poured into each Petri plate and plates were swabbed with 100 ml inoculate of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl volume with concentration of 1.0 mg/ml of each compound reconstituted in the dimethyl formamide (DMF). All the plates were incubated at 37 ˚C for 24 h. Antibacterial activity of all the synthesized compounds was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). The dimethyl formamide (DMF) solvent and gentamicin 10µg/well (standard antibiotic) were used negative and positive control respectively. The experiments were performed in triplicates.

Determination of Minimum inhibitory concentration:

The broth micro dilution method was used to determine the minimum inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001) [11].All tests were performed in a Mueller-Hinton broth for the bacterial strains. Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 2×10^6 CFU/ ml. Compounds were dissolved in dimethyl formamide (DMF) and then diluted to the highest concentration. Two-fold serial concentrations of the compounds

were prepared (over the range 1000–0.19 µg/ml) in a 96-well micro titer plate. In the tests, triphenyl tetrazolium chloride (TTC) (Aldrich Chemical Company Inc., USA) was also added to the culture medium as a growth indicator. The final concentration of TTC after inoculation was 0.05%. The microbial growth was determined by the absorbance at 600 nm using a universal micro plate reader after incubation at 37° C for 24 h. The MIC is defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth.

Table1: Antibacterial activity data of PEI-dend-4[N[(Ts)(2-(methyl)-5-aryl-1,3,4 oxadiazole)]] 8a-f.

Compound No	Zone of inhibition in mm (MIC in µg/ml)						
	Test Bacteria						
	Gram positive bacteria			Gram negative bacteria			
	Bacillus subtilis	Staphylococcus aureus	Staphylococcus epidermidis	E.coli	Xanthomonas campestris	Salmonella typhi	Pseudomonas aeruginosa
1 Best	23(12.5)	27(6.25)	20(12.5)	18(25)	16(25)	20(12.5)	17(25.5)
2 Mod	12(25)	15(25)	-	16(25.0)		-	-
3 Low	14(25)	18(25)	-	-	-	-	-
4 Best	25(12.5)	23(12.5)	21(25)	20(25)	20(12.5)	18(25)	15(25)
5 Low	12(50)	14(25)	-	12(50)	-	-	-
6 Mod	18(25)	20(25)	20(12.5)	16(12.5)	-	-	-
Gentamicin	34(2)	32(2)	29(4)	30(4)	26(8)	28(4)	20(4)
Nystatin	ND	ND	ND	ND	ND	ND	ND

"-" : No activity , "ND" : No determined

Table 2: Antifungal activity data of PEI-dend-4[N[(Ts)(2-(methyl)-5-aryl-1,3,4 oxadiazole)]] 8a-f.

Compound No	Zone of inhibition in mm (MIC in µg/ml)	
	Test fungi	
	Filamentous	Yeast
	Aspergillus niger	Candida albicans
1 Best	16(6.25)	18(6.25)
2 Mod	-	14(12.5)
3 Low	-	12(25)
4 Best	17(6.25)	18(6.25)
5 Low	14(25)	-
6 Mod	-	14(25)
Gentamicin	ND	ND
Nystatin	18(6.25)	20(6.25)

"-" : No activity , "ND" : No determined

RESULTS AND DISCUSSION

Chemistry

The desired compounds **8a-f** were synthesized as outlined in the scheme3. Compounds **8a-f** were synthesized by introducing heterocyclic compounds **7a-f** (scheme2) to dendrimer **4** (scheme1).

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The dendrimer 4 was synthesized has shown in the scheme 1.

i) A solution of 2-Chloroethylamine hydrochloride was dissolved in dichloro methane (MDC) and added drop wise to a solution of p-methyl tosylchloride (Ts-Cl) dissolved in MDC and TEA at 0^{o} C then stirred for 2 hour in room temperature. The solid Chloroethylamine tosylate (ClEtNHTs) was collected by suction filtration and washed with distilled water and dried in vacuum.
ii) ClEtNHTs was dissolved in toluene and a solution of NaOH added drop wise in a ice/salt bath. The reaction mixture stirred for 2 h then white solid tosyl aziridine collected and washed with distilled water and dried with vacuum.
iii) Tosyl aziridine dissolved in toluene and acetonitrile and a solution of ethylenediamine which dissolved in acetonitrile drop wise added to tosyl aziridine solution, then refluxed at 60^{o} C overnight. The reaction mixture cooled to room temperature and the white solid collected and washed with acetonitrile and dried by vacuum.

The heterocyclic compounds 7a-f were synthesized has shown in the scheme 2.

By refluxing 85% hydrazine hydrate and aryl esters in methanol aryl acid hydrazides **5a-f** prepared.
iv) Aryl acid hydrazides **5a-f** treated with Chloroacetylchloride in dry THF at 0^{o}C followed by refluxing for 1 h, for preparing N(Chloroacetyl)-aryl acid hydrazides **6a-f** .
v) By refluxing with phosphorous oxychloride afforded 2-(chloromethyl)-5-aryl-1,3,4 oxadiazoles **7a-f** .

Modification of dendrimer has shown in the scheme3.

vi) Dendrimer was dissolved in dry DMF and added at 0^{o} C to 60% NaH under N_2 atmosphere.1,3,4 oxadiazole dissolved in dry DMF and added to dendrimer mixture drop wise in 15 min. The reaction mixture heated to 80^{o} C overnight then extracted with EtOAc and worked up.

The formation of compounds **8a-f** was confirmed by ^{1}H NMR. The proton of –NH-Ts groups in dendrimer 4, (δ : 7.32), was disappeared in **8a-f** and aromatic protons for compounds **7a-f** with different substitute was appeared in (δ : 7.86-7.88 2H) for *Meta* protons and (δ : 7.35-7.37 4H) for *Ortho* protons merged with Tosyl group protons.

Biology

The antimicrobial activity of compounds **8a-f** was evaluated *in vitro* against some human pathogenic Gram positive bacteria such Bacillus subtilis (MTCC 121), Staphylococcus epidermis (MTCC 435), Staphylococcus aureus (MTCC 7433) and Gram negative bacteria E.Coli (MTCC 7440), Xanthomonas campestris (MTCC 7408), salmonella typhi (MTCC 733), Pseudomonas aeruginosa. Also compounds **8a-f** were evaluated against fiamatory fungi such as aspergillus niger (MTCC 378) and yeast Candida albicans (MTCC 183).The corresponding zone of inhibition and minimum inhibitory concentrations were summarized in **tables 1** and **2**.

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Results indicate that the compounds No: 1, **8d** and No: 4, **8a** have exhibited broad spectrum antimicrobial activity against both bacteria and fungi. Whereas that the rest of the compounds in the series have exhibited moderate antimicrobial activity when compared to positive controls. Antimicrobial spectrum indicates that the gram positive bacteria and fungi were more susceptible to the synthesized compounds than gram negative bacteria. The thorough investigation of our synthesized compounds **8a-f** pertaining to quantitative structure activity relationship highlighted that presence of more electron donating groups at *Para* position in phenyl ring bearing oxadiazole has influenced the antimicrobial activity, while it counterpart electron withdrawing groups has shown ins opposite effect. The compound No: 2, **8b** have shown moderate activity when compared to positive controls. The un substituted compound No:5, **8f** has shown better activity when comparing with compound No:3, **8c** and compound No:6, **8e** which has Fluorine and Bromo substituent.

CONCLUSION

In conclusion we have reported a facile route for the synthesis of **PEI-dend-4[N[(Ts)(2-(methyl)-5-aryl-1,3,4 oxadiazole)]] 8a-f,** from PEI (poly ethylene imine) dendrimer **4** and 2-(chloromethyl)-5-aryl 1,3,4 oxadiazole) **7a-f**. The new molecular framework has shown broad spectrum antimicrobial activity which is substantiated by the presence of electron donating groups. Among the synthesized compounds **8a-f**, compounds **8a** and **8d** has exhibited potent antimicrobial activity whereas the rest of the analogues have shown moderate activity when compared to the standard positive controls.

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