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Synthesis and Antimicrobial Activity Evaluation of Poly ethylen imine (PEI) Dendrimer modified with 1,2,4-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)(5-(methyl)-3-aryl-1,2,4 oxadiazole)]] **8a-g** have been synthesized from PEI (Poly ethylene imine) dendrimer which modified with 1,2,4 oxadiazole derivatives and investigated their antimicrobial activities.

Keywords: Poly ethylene imine (PEI). Dendrimer. 1,2,4-Oxadiazole, Antimicrobial.

Contents

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1. Introduction

Dendrimers are molecular architectures of well-defined size and number of terminal groups, starting from a multi-functional core unit, the structure branches often irregular layers. Multiply branched structures are frequently encountered in nature, science, technology and everyday life. Nature utility dendritic structures have wide range of applications in the design of animals and plants. The reason in the beneficial behavior of these dendritic structures is their ability to expose a highly multivalent surface to ensure maximum interaction with surroundings. In order to incorporate dendrimers as biological agents, introducing them to biological systems, certain properties have to be present or fine-tuned through preclinical chemical modification. Profile of a dendrimer is to a large extent governed by the size of the dendrimer and the surface group present on the particular dendrimer. The inner dendritic structure are generally of less importance as interaction between the dendrimer and the surroundings generally take place via the groups exposed on the surface, which may enable the dendrimer to penetrate.

Oxadiazoles are five membered heterocyclic compounds containing one oxygen and two nitrogen atoms. Tiemann and Kruger[1] prepared the diphenyl derivatives by heating O-benzoyl benzamidoxime above its melting point. The physical properties of 1,2,4 oxadiazoles are unexceptional. The boiling point of the parent compound is 87^o C within the normal range of other two carbon compounds. 1,2,4 oxadiazoles have received considerable attention in the

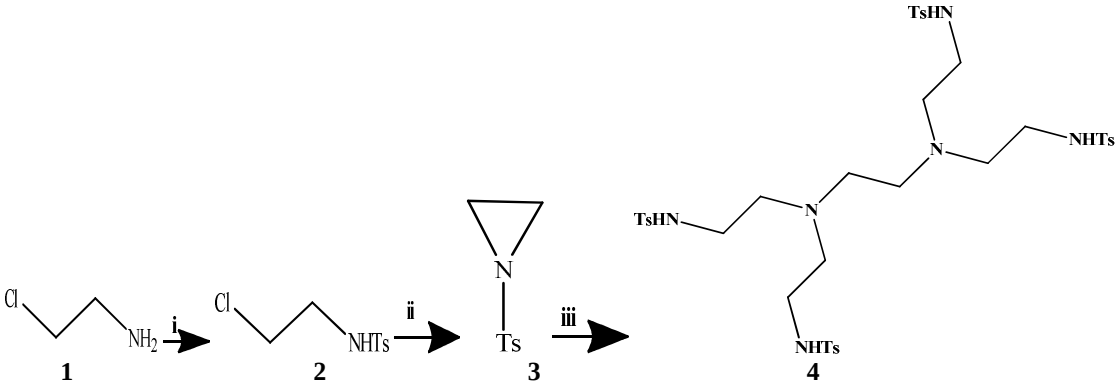
pharmaceutical industry as heterocyclic amide and bioisosteres [2][3] and is found in several drugs and drug leads[4] and the metabotropic glutamate subtype5(mELU5) receptor an tiagainist[5][6]**.** Furthermore oxadiazoles have been employed in the design of numerous biologically active templates, examples include muscarinic agonists[7] tyrosine kinase inhibitors[8]**,** anti-inflammatory agent[9]**,** histamine H_3 antagonist[10]**,** antitumor agent[11]**,** and monoamine oxidase inhibitors[12]**.**

2. Materials and methods

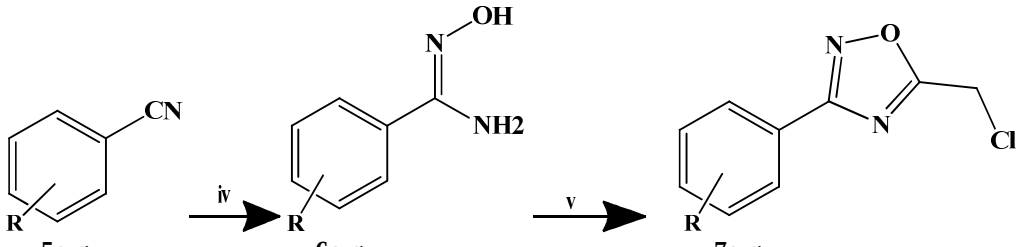
All the solvents and reagents were used as AR grade and used as such without further purification. The NMR spectra were recorded on Agilent 400 MHz spectrometer using DMSO-d_6, $CDCl_3$ solvents. Silica gel column chromatography was performed using Merck silica gel (100-200 mesh) and Merck made TLC were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

General procedure for the synthesis of PEI-dend-4[N[(Ts)(1,2,4 oxadiazole)]] 8a-g.

Dendrimer dissolved in dry DMF and added at 0^{o}C to 60% NaH under N_2 atmosphere.1,2,4 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.

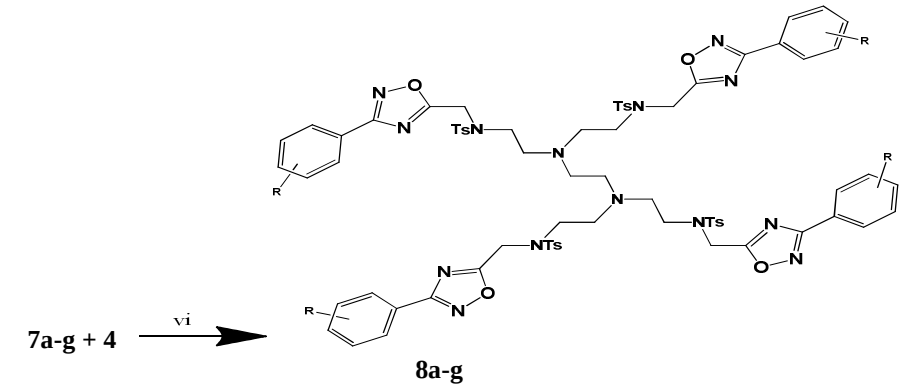


Sheme1: i) Tosyl Chloride/MDC, TEA/RT, 2h ii) Toluene/20%NaOH /0^o C, 2h
iii) Toluene/Acetonitrile/ethylenediamine/Reflux.



R**:a:4Cl,** R**:b:H,** R**:c:4CH$_3$,** R**:d:4OMe,** R**:e:2OMe,** R**:f:4Fl,** R**:g:2Me**

Sheme2: iv) Hydroxylamine hydrochloride/K_2CO_3, EtOH v) Chloroacetyl chloride/toluene/reflux.



R**:a:4Cl,** R**:b:H,** R**:c:4CH$_3$,** R**:d:4OMe,** R**:e:2OMe,** R**:f:4Fl,** R**:g:2Me**

Sheme3: vi) DMF/60%NaH /80^{o}C/reflux.

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

Dendrimer **4**,(0.2gr-0.0023 mol) were 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 were heated to 80^{o}C for 4 h and cooled to 0^{o} C. 5-(Chloromethyl)-3-aryl-1,2,4 oxadiazole **7a-g** (0.2g 0.0118mol) were 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 ice water then extracted with 2x10ml EtOAc and washed with 3x20ml water and brine solution then passed over Na_2SO_4, concentrated under reduced pressure to give crude product. The crude product was purified by column chromatography to afford 71% yield product.

DEN-4[N[(Ts)(5-(methyl)-3-(4-ChloroPhenyl)-1,2,4 oxadiazole)]] 8a

^{1}H NMR ($CDCl_3$): 2.11(s, 1H), 2.23-2.26(t, 2H), 2.37(s, 3H), 2.67(t, 2H), 5.20(s, 2H), 7.35-7.37(d, 4H), 7.66-7.68(d, 2H), 8.03-8.05(d, 2H).

DEN-4[N[(Ts)(5-(methyl)-3-(phenyl)-1,2,4 oxadiazole)]] 8b .

^{1}H NMR ($CDCl_3$): 2.13(s, 1H), 2.20-2.23(t, 2H), 2.35(s, 3H), 2.70(t, 2H), 5.21(s, 2H), 7.21-7.36(m, 5H), 7.59-7.61(d, 2H), 7.93-7.95(d, 2H).

DEN-4[N[(Ts)(5-(methyl)-3-(4-methylphenyl)-1,2,4 oxadiazole)]] 8c .

^{1}H NMR ($CDCl_3$): 2.12s, 1H), 2.25-2.28(t, 2H), 2.38-2.44(m, 6H), 2.69(t, 2H), 5.25(s, 2H), 7.39-7.41(d, 4H), 7.81-7.83(d, 2H), 8.33-8.35(d, 2H).

DEN-4[N[(Ts)(5-(methyl)-3-(4-methoxyphenyl)-1,2,4 oxadiazole)]] 8d .

^{1}H NMR ($CDCl_3$): 2.13(s, 1H), 2.21-2.24(t, 2H), 2.31(s, 3H), 2.63(t, 2H), 3.83(s, 3H), 5.29(s, 2H), 7.25-7.27(d, 4H), 7.41-7.43(d, 2H), 7.93-7.95(d, 2H).

DEN-4[N[(Ts)(5-(methyl)-3-(2-methoxyphenyl)-1,2,4 oxadiazole)]] 8e .

^{1}H NMR ($CDCl_3$): 2.08(s, 1H), 2.17-2.20(t, 2H), 2.31(s, 3H), 2.60(t, 2H), 3.80(s, 3H), 5.15(s, 2H), 7.31-7.37(m, 3H), 7.61-7.70(m, 3H), 7.70-7.72(d, 2H).

DEN-4[N[(Ts)(5-(methyl)-3-(4-Fluorophenyl)-1,2,4 oxadiazole)]] 8f .

^{1}H NMR ($CDCl_3$): 2.13(s, 1H), 2.29-2.32(t, 2H), 2.36(s, 3H), 2.61(t, 2H), 5.18(s, 2H), 7.25-7.27(d, 4H), 7.59-7.61(d, 2H), 7.63-7.65(d, 2H).

DEN-4[N[(Ts)(5-(methyl)-3-(2-methylphenyl)-1,2,4 oxadiazole)]] 8g .

^{1}H NMR ($CDCl_3$): 2.10(s, 1H), 2.21-2.23(t, 2H), 2.35(s, 3H), 2.59(s, 3H), 2.65(t, 2H), 5.10(s, 2H), 7.35-7.37(d, 4H), 7.66-7.68(d, 2H), 8.03-8.05(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.[13] 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 dimethylformamide (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 dimethylformamide (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 microdilution method was used to determine the minimum inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001).[14] 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 dimethylformamide (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, triphenyltetrazolium 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 of PEI-dend-4[N[(Ts)(5-(methyl)-3-aryl-1,2,4 oxadiazole)]] 8a-g

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.colli	Xanthomonas campestris	Salmonella typhi	Pseudomonas aeruginosa
1 Low	-	14(25)	-	10(50)	-	-	-
2 Best	20(12.5)	20(12.4)	-	15(25)	-	-	-
3 Mod	14(25)	18(25)	-	-	-	12(5)	-
4 Best	20(12.5)	18(25)	18(25)	20(12.5)	20(12.5)	14(25)	15(25)
5 Low	14(50)	12(50)	-	-	-	-	-
6 Best	24(12.5)	20(25)	20(12.5)	15(25)	16(25)	16(25)	12(50)
7 Mod	13(50)	15(25)	18(25)	12(5)	-	-	12(12.5)
Gentamicin	34(2)	32(2)	285(4)	30(4)	25(8)	25(4)	18(8)
Nystatin	ND	ND	ND	ND	ND	ND	ND

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

Table2: Antifungal activity of PEI-dend-4[N[(Ts)(5-(methyl)-3-aryl-1,2,4 oxadiazole)]] 8a-g

Compound No	Zone of inhibition in mm (MIC in µg/ml)	
	Test fungi	
	Filamentous	Yeast
	Aspergillus niger	Candida albicans
1 Low	-	-
2 Best	12(50)	14(25)
3 Mod	-	14(25)
4 Best	13(50)	18(12.5)
5 Low	-	12(50)
6 Best	17(25)	15(25)
7 Mod	-	-
Gentamicin	ND	ND
Nystatin	16(3.125)	18(3.125)

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

3. Results and Discussion

Chemistry

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

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

i) The solution of 2-Chloroethylamine hydrochloride which was dissolved in dichloromethane (MDC) added drop wise to a solution of p-methyl tosylchloride (Ts-Cl) which dissolved in MDC and TEA at 0^o C, then stirred for 2 hour in room temperature. The solid Chloroethyl amine 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 was 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-g were synthesized has shown in the scheme 2.

iv) The solution of benzonitrile in ethanol was added to a solution of hydroxylamine hydrochloride and potassium carbonate each dissolved in water and then refluxed for 8 h. After work up solid compounds Benzamidoxime has got **6a-g**.

v) Benzamidoxime **6a-g** dissolved in toluene and added drop wise Chloroacethyl chloride at 0^o C, stirred for 4 h at room temperature then refluxed overnight. After work up 5-(Chloro methyl)-3-(phenyl) -1,2,4 oxadiazole **7a-g** produced.

Modification of dendrimer has shown in the scheme 3.

vi) Dendrimer **4** was dissolved in dry DMF and added at 0^o C to 60% NaH under N_2 atmosphere.1,2,4 oxadiazole **7a-g** was 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-g** was confirmed by ^{1}H NMR. The proton of –NH-Ts groups in dendrimer **4**, (δ : 7.32), was disappeared in **8a-g** and aromatic protons for compounds **7a-g** with different substitute was appeared in (δ : 8.03-8.05 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-g** 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-g** were evaluated against filamentous 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.** Results indicate that the compound No: 6, **8d** and No: 4, **8c** 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-g** , 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 its counterpart electron withdrawing groups have shown in opposite effect. The compounds No: 7 **8e**, No: 3, **8g** and No: 2, **8a** have shown moderate activity when compared to positive controls. The un substituted compound No: 5, **8b** has shown better activity when compared to compound No: 1, **8f** which has Fluorine substituent.

4. Conclusion

In conclusion we have reported a facile route for the synthesis of **PEI-dend-4[N[(Ts)(5-(methyl)-3-aryl-1,2,4 oxadiazole)]] 8a-g ,** from PEI (poly ethylene imine) dendrimer **4** and 5-(chloromethyl)-3-aryl 1,2,4 oxadiazole) **7a-g**. 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-g**, compounds **8c** 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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