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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and characterization of polyamide metallodendrimers and their anti-bacterial and anti-tumor activities

Tansir Ahamad · S. F. Mapolie · Saad M. Alshehri

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Abstract Pt(II) and Pd(II) complexes with aromatic amine terminated first generation (G_1) and second generation (G_2) polyamide dendrimers [PAD-$(NH_2)_n$] ($n = 6$ for G_1 and 12 for G_2) were synthesized. All the synthesized dendrimers and metallodenrimers were characterized by elemental and spectral analysis. These novel dendrimers and their metallodendrimers (metal complexes of dendrimers) were screened for their anti-bacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive) and *Escherichia coli* and *Salmonella typhi* (Gram negative) using agar disk diffusion method. The cytotoxicity assay of the dendrimers and metallodendrimers has been performed against MFC-7 cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Anti-bacterial evaluation indicates that the second generation dendrimer exhibit stronger biological activity against all the pathogenic bacterial strains in comparison to the first generation dendrimers. PdG_2 exhibited lowest value of MIC 70 μg/ml against *E. coli*, while MIC value of PtG_2 was 78 μg/ml. PdG_2 and PtG_2 show lowest anti-bacterial activity against *S. typhi* with 102 and 110 μg/ml (MIC value), respectively. In general, the metallodendrimers showed lower cytotoxicity than cisplatin (standard drug) and Pt(II) containing metallodendrimers were found to be more efficient in the induction of MFC-7 death than Pd(II) containing metallodendrimers.

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Keywords Metallodendrimers · Polyamide · Spectral analysis · Cytotoxicity assay

Introduction

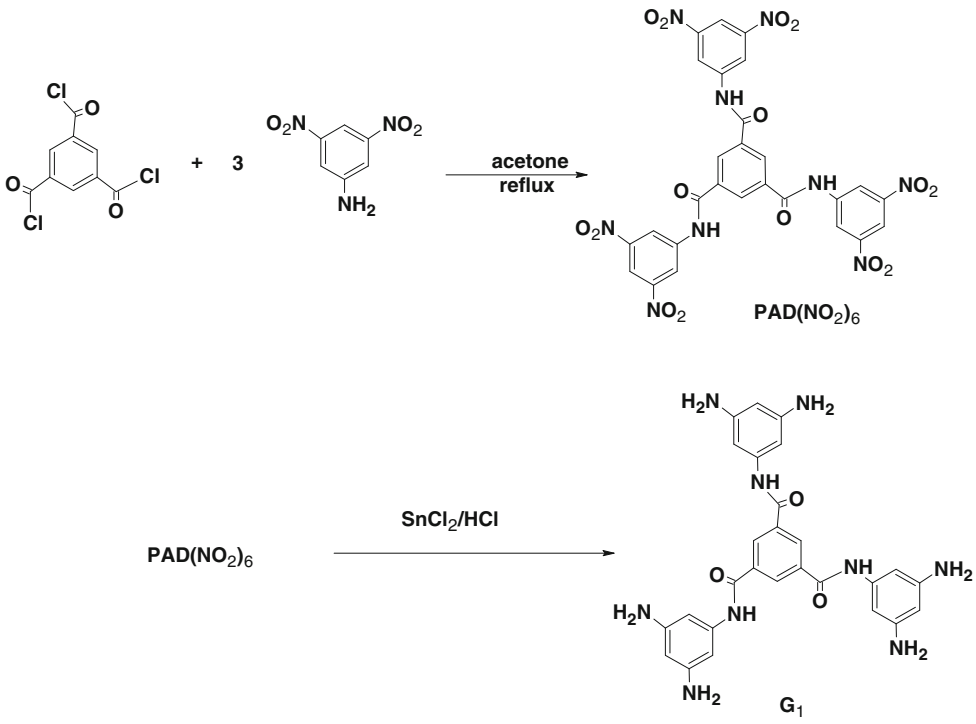
Metallodendrimers are well-defined, highly branched, three dimensional macromolecules with characteristic globular structures for the larger systems (Martinovic *et al.*, 2008). These novel macromolecules functionalized with transition metals have inspired many researchers to develop new materials for pharmaceutical application (Yang and Kao, 2006). The recent impressive strides in synthetic procedures increased the accessibility of functionalized metallodendrimers, resulting in a rapid development of dendrimer chemistry. The potential interests of metallodendrimers as anti-microbial and anti-tumor agents, arise mainly from the control of size and shape along with the placement of functional groups (Emilio *et al.*, 2009; Refat *et al.*, 2009; Machado *et al.*, 2001). Recently, many dendrimers have also been reported and several of them are subjected as preclinical trial as useful additives in drug formulations for increasing the solubility, stability, bio-availability, cellular uptake, targeting ability, and patient compliance of the administrated drugs and for decreasing the drug resistance and irritation (Hou *et al.*, 2009). The periphery-functionalized dendrimers have their ligand systems and able to coordinate with metal ions. Metal coordination to biologically active molecules can be used as a strategy to enhance their activity and overcome resistance. For instance, metal complexes of thiosemicarbazones can be more active than the free ligand, or they can be employed as a vehicle for activation of the ligand as the cytotoxic agent (Patil *et al.*, 2010; Pavan *et al.*, 2010). Pd(II) and Pt(II) complexes with

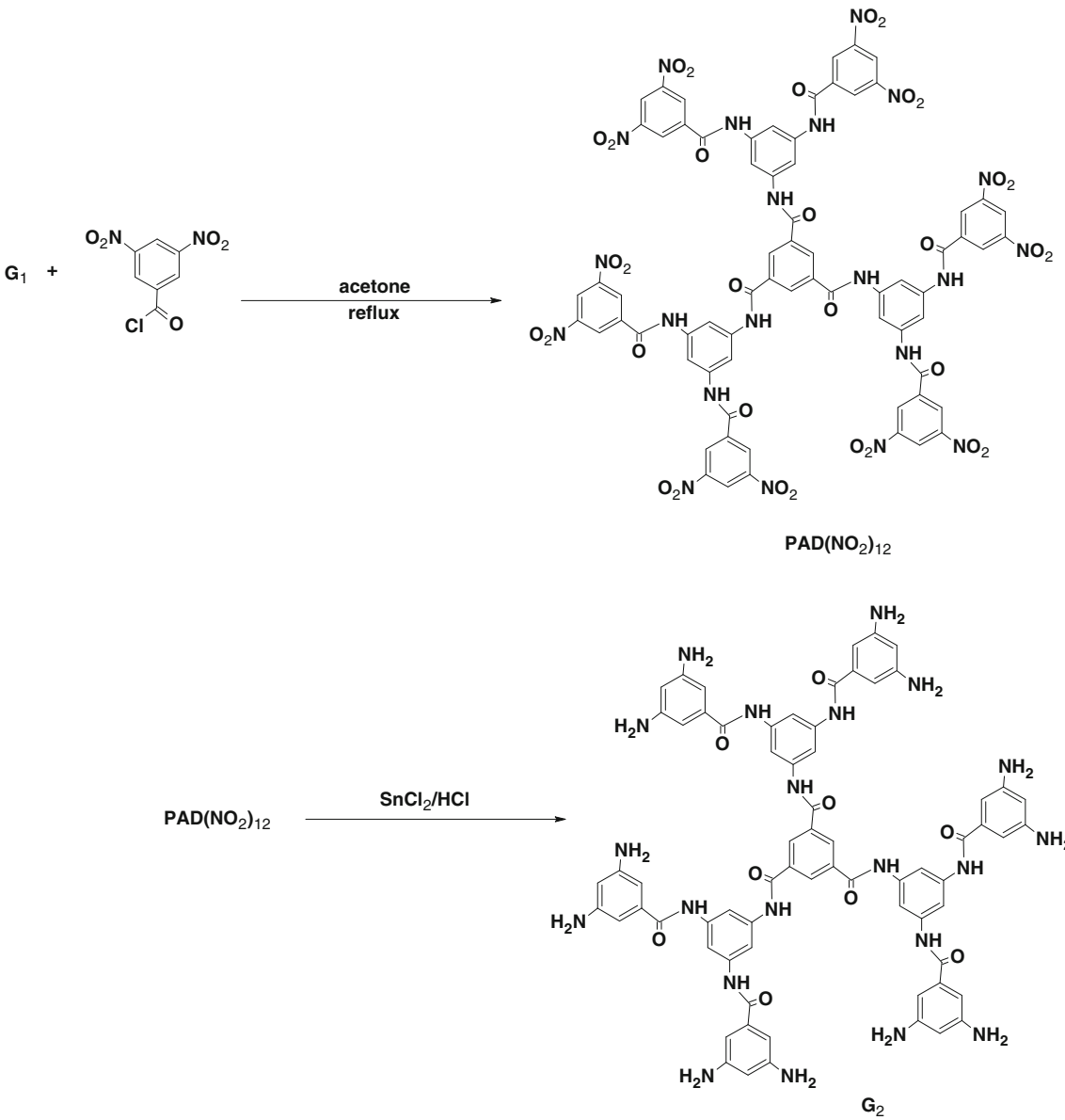
antibiotics of the tetracycline family are more potent against *Escherichia coli* a bacterial strain resistant to tetracycline (Guerra *et al.*, 2005; Zerzankova *et al.*, 2010). Moamen *et al.* (2005) have reported the novel Cu(II) and Zn(II) complexes of first and second generation poly(propylene amine) dendrimers containing 1,8-napthalimide unit on periphery, and represent that the metal coordinating dendrimers is more potent against several pathogenic bacteria then dendritic ligands. The excellent biological activity of the dendrimers inspires us to synthesize periphery-functionalized metallodendrimer and used as anti-tumor and anti-bacterial agents. Herein we selected amino functionalized dendrimers as a ligand support these dendrimers were modified into metallodendrimers with transition metal ions. The present article, we have describes the preparation and characterization of the Pd(II) and Pt(II) metallodendrimers were synthesized using $[PAD\text{-}(NH)_2]_6$ and $[PAD\text{-}(NH)_2]_{12}$ and abbreviated as PdG_1, PtG_1 (complexes of first generation) and PdG_2, PtG_2 (complexes of second generation), respectively. In addition, the antibacterial activity of the synthesized dendrimers and their metallodendrimers has been carried out against against *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive) and *Escherichia coli*, *Salmonella typhi* (Gram negative) using agar disk diffusion method. The cytotoxicity assay of the synthesized compounds has been performed on MFC-7 cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and compared with cisplatin as a standard drug.

Results and discussion

A general synthetic route for first and second generation dendrimeric ligands G_1 and G_2 is shown in Schemes 1 and 2. G_1 and G_2 were prepared using ammonium formate and commercial zinc dust as a reducing agent from their corresponding precursor $[PAD\text{-}(NO_2)_6$ and $PAD\text{-}(NO_2)_{12}]$ according to the literature (Zhou *et al.*, 2006). The yellow color powder of 1,3,5-tris(3,5-dinitrophenyl)benzamide $[PAD\text{-}(NO_2)_6]$ was prepared by the reaction of 1,3,5-benzene tricarboxylchloride with 3,5-dinitroaniline in acetone in 1:3 molar ratio in good yield. $PAD\text{-}(NO_2)_6$ has been purified simply by precipitation in aqueous $NaHCO_3$ in quantitative yield. The formation and isolation of $PAD\text{-}(NO_2)_6$ were confirmed by IR, elemental analysis, ^1H NMR, and ^{13}C NMR spectroscopy. The IR spectrum of $PAD\text{-}(NO_2)_6$ showed strong absorption at 1650 cm^{-1} characteristic to C=O stretching of amide groups and terminal nitro groups at 1588 and 1290 cm^{-1}. The reduction of $PAD\text{-}(NO_2)_6$ by employing $SnCl_2$ and concentrated hydrochloric acid successfully afforded the desired 1,3,5-tris(3,5-diaminophenyl)benzamide (G_1). The FT-IR spectrum of G_1 shows strong peak at 3410 cm^{-1} due to the presence of terminal amino groups (Ueda *et al.*, 1988). The nitro groups band at 1588 cm^{-1} has disappeared completely after reduction. The proton signals at δ 4.36 indicate the presence of NH_2 protons. The 12 nitro-terminated second generation polyamide $PAD\text{-}(NO_2)_{12}$ was synthesized by the reaction of G_1 with 3,5-dinitrobenzolyl

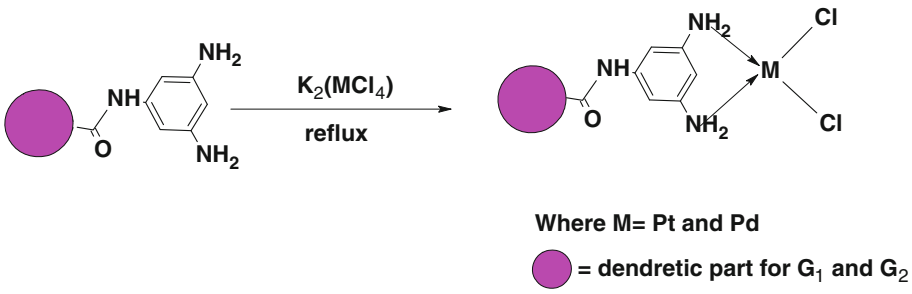
Scheme 1 Synthesis of first generation dendrimeric ligand





Scheme 2 Synthesis of second generation denrimeric ligand

Scheme 3 Synthesis of metalodendrimers



chloride in acetone under nitrogen atmosphere in 75% yield. The structure of PAD-$(NO_2)_{12}$ was confirmed by elemental analysis, IR, ^{1}H NMR, and ^{13}C NMR. The FTIR spectra of PAD-$(NO_2)_{12}$ show a strong band due to the presence of terminal nitro groups at 1588 and 1290 cm^{-1}. The ^{1}H NMR spectra of PAD-$(NO_2)_{12}$ show proton signals for the internal branches amide and outer branch amide at 10.20 and 10.01 ppm, respectively (Okazaki *et al.*, 2003).

The amino terminated second generation dendrimer G_2 was synthesized using the reaction process similar to the reduction of PAD-$(NO_2)_6$ as employed in the synthesis of first generation dendrimer G_1. The overall route for the synthesis of second generation dendrimers G_2 is illustrated in Scheme 2. In the FTIR spectrum of G_2, the nitro peak on 1342 cm^{-1} disappeared, meaning that completely reduction of all the nitro group into corresponding amino groups, it is also supported by the appearance of new resonance signal at 4.82 ppm, when compared with ^1H NMR spectrum of the precursor PAD-$(NO_2)_{12}$.

The metallodendrimers have been synthesized via simple complexation reaction of ethanolic solution of metal salts with G_1 and G_2 in 3:1 and 6:1 molar ratio, respectively. The Pd(II) and Pt(II) complexes were isolated as brown and yellowish brown powders, respectively, in good yields (Scheme 3). The formations of metal complexes were also supported by FTIR and elemental analysis. The results of elemental analysis revealed that the metal ions attached to the terminal aromatic amine groups via coordination-covalent bonds and containing two chloride ions with each metal ion. The FT-IR spectra of the complexes revealed that, the NH stretching at 3405–3410 cm^{-1} was shifted to the lower frequency when compared with their parental dendrimeric ligands (Okazaki *et al.*, 2001). ^{13}C NMR spectra and ^1H NMR spectra were also used to follow the coordination of metal ions with G_1 and G_2. The terminal NH_2 groups peaks was shifted from 4.88 and 4.86 ppm to 4.81 and 4.80 ppm after coordination with metal ions as shown in Figs. 1 and 2. All other peaks of dendrimers protones were observed in corresponding metallodendrimer with slightly shift. The MALDI-TOF MS spectra of G_1 and G_2 showed the expected $[M + H]^+$ peak at 526.23, 1330.51, respectively. While MS measurement of PdG_2 and PtG_2 showed the expected $[M + H]^+$ peak at 2371.14, 2903.04, respectively, as shown in Figs. 3 and 4 and indicated the formation of matellodendrimers.

The results of anti-bacterial activity of dendrimers, metallodendrimers, and standard drug (streptomycin) against four pathaogens [*Bacillus subtilis* and *Staphylococcus aureus* (Gram positive) and *Escherichia coli* and *Salmonella typhi* (Gram negative)] are summarized in Table 1. Both dendrimers exhibit sufficient anti-bacterial activity against all the pathogens. G_1 and G_2 show higher activity 17.5 and 22 mm, respectively, against *S. typhi* and lower activity 12 and 14 mm against *E. coli*, respectively. The anti-bacterial activity of PdG_1 against *B. subtilis* with higher zone of inhibition 16.5 mm, it is higher than that of standard antibiotics 16 mm. The PdG_2 had shows far better anti-bacterial activities against all the pathogens bacteria than streptomycin. The activity of PtG_2 and streptomycin against *B. subtilis* show similar zone of inhibition 16 mm. Therefore, these metallodendrimers have good chemotherapeutic potential for use as antimicrobial agent against these bacteria.

The minimum inhibition concentration (MIC) of synthesized compounds and standard antibiotics streptomycin are given in Table 2. The data revealed that the MIC value of G_1 against *E. coli*, *S. typhi*, *B. subtilis*, and *S. Sureus* was 160, 172, 110, and 112 μg/ml, respectively, while the antibacterial activities of streptomycin against these bacteria was 72, 105, 82, and 80 μg/ml, respectively. The MIC value result also showed that PdG_2 is ore active than all other compounds against all the pathogens used in this study. Although the MIC value of PdG_2 against Gram negative bacteria were less that streptomycin. However, slightly higher in the case of Gram positive bacteria.

The anti-tumor activity of G_1 and G_2 and their metallodendrimers against MFC-7 (Human breast adenocarcinoma) cell lines have carried out using MTT cell proliferation assay and the results are summarized in Fig. 5. Studies were undertaken with different concentrations (10, 20, 30, and 50 μM). In parallel, the influence of widely used anticancer drug (cisplatin) was assayed. While in cell lines cultures, C_{50} was about 30 μM for PtG_1 and nearly 50 μM for the PdG_1. In our experimental system cisplatin was found to have a much higher anti-proliferating activity than that of newly synthesized compound with IC_{50} 2.33 μM in MFC-7 cell cultures. On the other hand, dendrimers had much lower effect on cellular proliferation than the Pt(II) and Pd(II) metallodendrimers, because cell death cannot be distinguished using the MTT assay, the number of dead and alive cells was measured using the trypan blue exclusion test. PdG_2 and PtG_2 showed a much higher cytotoxicity than PdG_1 and PtG_2, respectively. 50 μM PtG_2 caused almost all cells whereas PtG_1, PdG_2, PdG_1, G_1, and G_2 at the same concentration give 85, 60, 55, 40 and 30% of dead cells, respectively.

Conclusion

In this study the first and second generation amino terminated polyamide dendrimers and their metallodendrimers with Pt(II) and Pd(II) have been synthesized. The entire compounds have been characterized using elemental and spectral technique. It was confirmed that both the dendrimers (G_1 and G_2) and metallodendrimers showed promising antimicrobial activities against all the bacteria. The wider spectrum of the antimicrobial activities revealed that PdG_2 exhibit better anti-bacterial activity than PtG_2. Although the Pt(II) metallodendrimers were found to be more cytotoxic than their corresponding Pd(II) metallodendrimers.

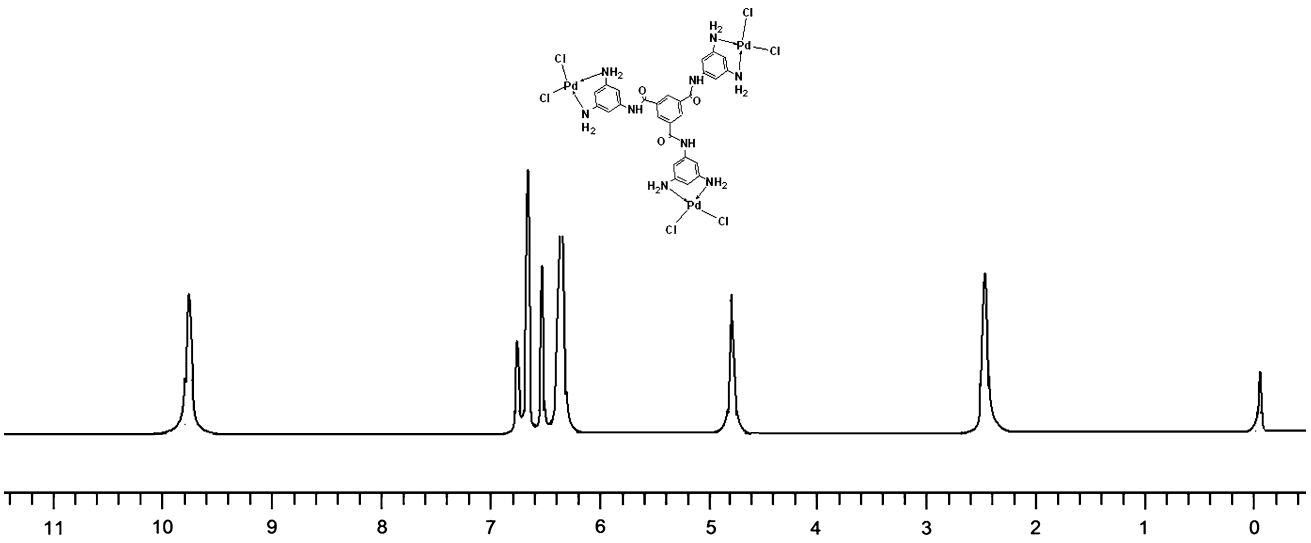
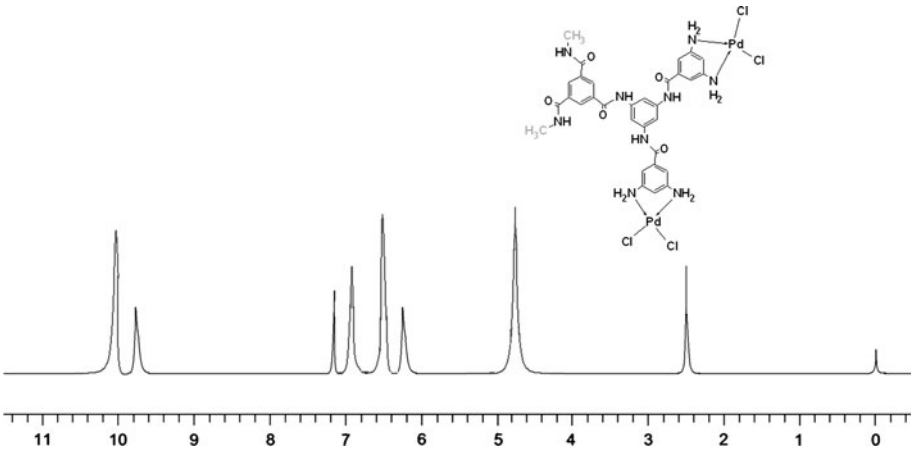


Fig. 1 ^{1}H NMR spectrum of PdG_1

Fig. 2 ^{1}H NMR spectrum of PdG_2



Experimental section

Materials and methods

1,3,5-Benzene tricarboxylchloride, 3,5-dinitro benzoyl chloride, 3,5-dinitroaniline, $SnCl_2$, hydrochloric acid (Sigma-Aldrich). The reagents and solvents were used as received. All manipulations of air and moisture sensitive compounds were performed under a nitrogen atmosphere using standard Schlenk techniques. The elemental analysis of the dendrimers and metallodendrimers were determined with a PerkinElmer elemental analyzer. FTIR spectra were taken on a PerkinElmer IR spectrophotometer model 621 by using KBr pellets in the range of 400–4000 cm^{-1}. ^{1}H NMR spectra were recorded on a JOEL-FX-100 FT-NMR instrument in dimethylsulfoxide (DMSO) solution and tetramethyl silane (TMS) as an internal standard. MALDI-TOF mass spectra were recorded using a KMPACT MALDI mass spectrometer (Shimadzu Kratos) in positive mode.

Synthesis

Synthesis of [1,3,5-tris(3,5-dinitrophenyl) benzamide)] [PAD-$(NO_2)_6$]

In a 250 ml three-neck round-bottom flask, 2.65 g (0.1 mol) 1,3,5-bezene tricarboxylchloride and 2.74 g (0.3 mol) 3,5-dinitroaniline were mixed in 100 ml ethanol. The mixture was refluxed for 6 h under the flow of nitrogen. The resulting yellow mixture was cooled and excess amount of solvent was removed under reduced pressure. The residue was re-precipitated in hexane, resulting precipitate was filtrated and washed off several times. The desired compound [PAD-$(NO_2)_6$] was obtained as a yellow solid powder in 70% yield after purification by column

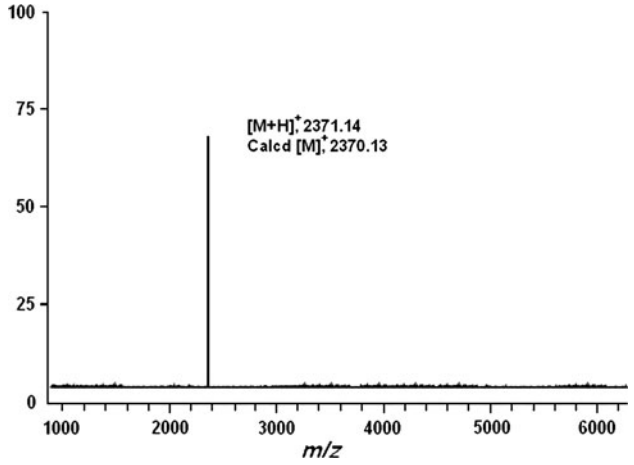


Fig. 3 MALDI-TOF spectra of PdG_2

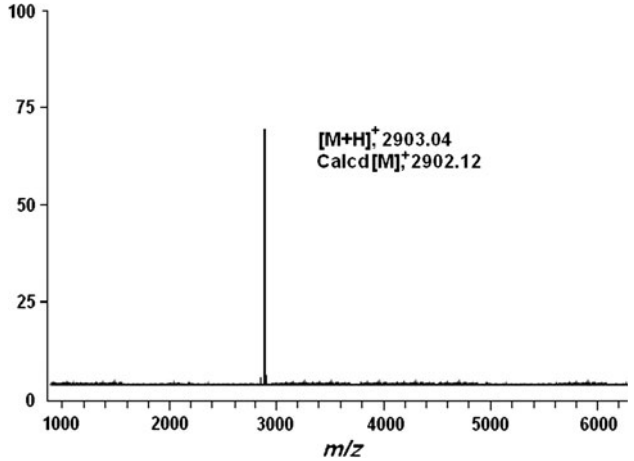


Fig. 4 MALDI-TOF spectra of PtG_2

chromatography (silica gel, 3/1 (v/v) petroleum ether/ethyl acetate). Mp: 190°C. ^{1}H NMR (400 MHz, DMSO, TMS, δ): 10.46 (s, 3H, N*H*CO), 7.12 (s, 3H, Ar*H*), 6.96 (s, 3H, Ar*H*), 6.42 (s, 6H, Ar*H*). FTIR (KBr, cm^{-1}): 1110, 1340, 1560, 1615, 1710, 3265. Anal. Calcd for $C_{27}H_{15}N_9O_{15}$ (705.46): C, 45.97; H, 2.14; N, 17.87. Found: C, 45.95; H, 2.13; N, 17.89.

Synthesis of [1,3,5-tris(3,5-diamminophenyl) benzamide)] [PAD-(NH_2)$_6$] (G_1)

PAD-(NO_2)$_6$ (3.52 g, 50 mmol in 30 ml of acetone) and Sn (7.64 g), conc. HCl (60 ml) were mixed drop-wised in a 100 ml round-bottom flask. The flask was equipped with condenser and the mixture was refluxed for 30 min. The resulting mixture was cooled to room temperature and add an aqueous sodium hydroxide to re-dissolve the initial precipitate. The mixture was transferred into a separating funnel and add 3 g sodium chloride powder with10 ml of chloroform. The organic part of the mixture was separated out and washed off several times with water. The solvent was evaporated at reduced pressure, and the desired compound PAD-(NH_2)$_6$] was obtained as a brown colored powder in 54% yield. Mp: 245°C. ^{1}H NMR (400 MHz, DMSO, TMS, δ): 9.84 (s, 3H, N*H*-CO), 6.81 (s, 3H, Ar*H*), 6.52 (s, 6H, Ar*H*), 6.21 (s, 3H, Ar*H*), and 4.88 (s, 12H, Ar-NH_2). ^{13}C NMR (100 MHz, DMSO, TMS): 102.3, 128.4, 134.9, 136.8, and 174.8 ppm. FTIR (KBr, cm^{-1}): 1511, 1556, 1596, 1622, 1680, 3409. ESI MS (*m/z*): 526.23 ($M + H^+$). Anal. Calcd for $C_{27}H_{27}N_9O_3$ (525.56): C, 61.70; H, 5.18; N, 23.99. Found: C, 61.71; H, 5.17; N, 23.98.

Table 1 Antibacterial activity of dendritic ligands and metallodendrimers

Abbreviation	Zone of inhibition (100 μg/ml)			
	E. coli	*S. typhi*	*B. subtilis*	*S. aureus*
PAD-(NO_2)$_6$	12.0	17.5	13.0	14.0
PAD-(NO_2)$_{12}$	14.0	22.0	14.5	16.2
PdG_1	24.5	27.0	16.5	19.3
PtG_1	22.0	25.5	13.5	15.5
PdG_2	26.0	32.0	18.0	21.0
PtG_2	24.0	28.0	16.0	18.0
Streptomycin	28.5	33.5	16.0	19.5
DMSO	–	–	–	–

Zone of inhibition in millimeters

Streptomycin as a standard drug

DMSO solvent of positive control

Synthesis of nitro group terminated dendrimers [PAD-(NO_2)$_{12}$]

A solution of 5.26 g (10 mmol) [PAD-(NH_2)$_6$] in chloroform (100 ml) was mixed with 13.99 g (60 mmol) 1,3-dinitrobenzoyl chloride and refluxed under nitrogen at 70°C for 5 h. Resulting yellow color mixture was obtained. The process of reaction was monitored by TLC using CH_2Cl_2 and hexane as eluent. After completion of the reaction, the excess amounts of the solvents were removed under reduced pressure and washed off several times using water. A yellow brown powder of [PAD-(NO_2)$_{12}$] was obtained in 60% yield. Mp: 275°C. ^{1}H NMR (400 MHz, DMSO, TMS, δ): 9.81 (s, 3H, N*H*-CO), 9.76 (s, 6H, N*H*-CO), 7.27 (s, 9H, Ar*H*), 6.84 (s, 3H, Ar*H*), 6.62 (s, 6H, Ar*H*), and 6.25 (s, 12H, Ar*H*). ^{13}C NMR (100 MHz, DMSO, TMS): 104.2, 112.5, 114.2, 129.8, 133.3, 135.4, 137.2, 145.2, 171.8, and 174.1 ppm. FTIR (KBr, cm^{-1}): 1115, 1342, 1560, 1558, 1588, 1650, 1720, 3245. Anal. Calcd for $C_{69}H_{39}N_{21}O_{33}$ (1690.17): C, 62.29; H, 4.77; N, 22.11. Found: C, 62.30; H, 4.78; N, 22.10.

Table 2 Minimum inhibitory concentration (MIC) of dendritic ligand and metallodendrimers

Abbreviation	Minimum inhibitory concentration (MIC)			
	E. coli	*S. typhi*	*B. subtilis*	*S. aureus*
PAD-$(NO_2)_6$	160	175	110	112
PAD-$(NO_2)_{12}$	135	140	90	95
PdG_1	90	118	90	88
PtG_1	95	120	90	90
PdG_2	70	102	84	82
PtG_2	78	110	85	87
Streptomycin	72	105	82	80
DMSO	–	–	–	–

Minimum inhibitory concentration in μg/ml

Streptomycin as a standard drug

DMSO solvent of positive control

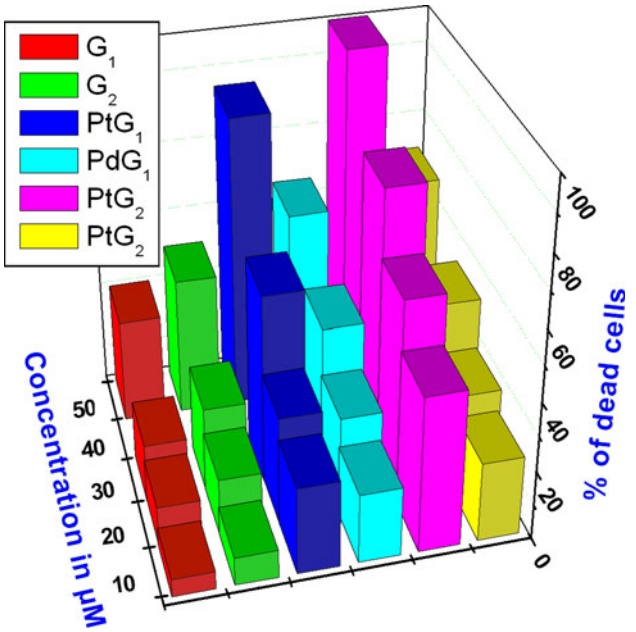


Fig. 5 Cytotoxicity of dendrimers and metallodendrimers

Synthesis of second generation dendritic ligand [PAD-$(NH_2)_{12}$] (G_2)

A similar procedure has been used for the synthesis of [PAD-$(NO_2)_{12}$] as for the synthesis of [PAD-$(NH_2)_6$]. Resulting, a yellow brown powder was in 52% yield. Mp: 348°C. FTIR (KBr, cm^{-1}): 1114, 1340, 1562, 1560, 1586, 1652, 1721, 3265. ^{1}H NMR (400 MHz, DMSO, TMS, δ): 4.86 (s, 48H, N*H*$_2$), 10.10 (s, 3H, N*H*-CO), 9.82 (s, 6H, N*H*-CO), 7.18 (s, 9H, Ar*H*), 6.82 (s, 3H, Ar*H*), 6.48 (s, 6H, Ar*H*), and 6.21 (s, 12H, Ar*H*). ^{13}C NMR (100 MHz, DMSO, TMS): 103.2, 110.8, 115.4, 123.8, 134.2, 136.0, 138.4, 147.0, 170.6, and 173.2 ppm. MALDI-TOF MS (*m*/*z*): 1331.26. Anal. Calcd for $C_{69}H_{39}N_{21}O_9$ (1330.51): C, 49.03; H, 2.33; N, 17.40. Found: C, 49.01; H, 2.24; N, 17.42.

Synthesis of generation 1 dendritic Pt(II) complex (PtG_1)

0.131 g (0.5 mmol) PAD-$(NH_2)_6$] was dissolved in 10 ml ethanol, in a round-bottom flask 0.621 g (0.15 mmol) $K_2(PtCl_4)$ was added and the reaction mixture was stirred magnetically under reflux for 20 h forming a yellowish brown precipitate. The precipitate was filtered off by vacuum filtration and washed extensively with ethanol to afford PtG_1 as a solid in 80% yield. FTIR (KBr, cm^{-1}): 545, 1510, 1555, 1584, 1622, 1685, 3370. MALDI-TOF MS (*m*/*z*): 1324.43 $(M + H)^+$. Anal. Calcd for $C_{27}H_{27}N_9O_3Pt_3Cl_6$ (1323.57): C, 24.50; H, 2.06; N, 9.52; Cl, 16.07; Pt, 44.22. Found: C, 24.53; H, 2.09; N, 9.50; Cl, 16.04; Pt, 44.25.

Synthesis of generation 1 dendritic Pd(II) complex (PdG_1)

Using the same procedure as for the synthesis of PtG_1, when G_1 (0.131 g, 0.5 mmol) in ethanol (10 ml) in a round-bottom flask was added $K_2(PdCl_4)$ (0.485 g, 0.15 mmol) and the reaction mixture was stirred under reflux for 20 h forming a brown precipitate. After filtered, washed off and dried a brown powder was obtained in 52% yield as a brown powder. FTIR (KBr, cm^{-1}): 540, 1515, 1550, 1586, 1622, 1682, 3375. ^{1}H NMR (400 MHz, DMSO, TMS, δ): 9.82 (s, 3H, N*H*-CO), 6.78 (s, 3H, Ar*H*), 6.50 (s, 6H, Ar*H*), 6.20 (s, 3H, Ar*H*), and 4.81 (s, 12H, Ar-N*H*$_2$). ^{13}C NMR (100 MHz, DMSO, TMS): 101.3, 128.2, 134.5, 134.8, and 172.8 ppm. MALDI-TOF MS (*m*/*z*): 1058.28 $(M + H)^+$. Anal. Calcd for $C_{27}H_{27}N_9O_3Pd_3Cl_6$ (1057.53): C, 30.66; H, 2.57; N, 11.92; Cl, 20.11; Pd, 30.19. Found: C, 30.64; H, 2.58; N, 11.91; Cl, 20.13; Pd, 30.14.

Synthesis of generation 2 dendritic Pt(II) complex (PtG_2)

To a solution of the G_2 dendritic ligand (0.665 g, 0.5 mmol) in ethanol (10 ml) was added $K_2(PtCl_4)$ (1.24 g, 0.30 mmol) and the mixture stirred under reflux for 24 h. The solvent was evaporated via rotary evaporation to give a yellowish brown precipitate. The precipitate was then dissolved filtered, washed off, and dried at room temperature. Resulting a yellowish brown powder was found in 76% yield. FTIR (KBr, cm^{-1}): 548, 1110, 1560, 1556, 1648, 1712, 3260. MALDI-TOF MS (*m*/*z*): 2903.04. Anal. Calcd for $C_{69}H_{39}N_{21}O_9Pt_6Cl_{12}$ (2902.13): C, 28.56; H, 1.35; N, 10.14; Cl, 14.66; Pt, 40.33. Found: C, 28.55; H, 1.36; N, 10.12; Cl, 14.68; Pt, 40.30.

Synthesis of generation 2 dendritic Pd(II) complex (PdG_2)

Using the same procedure as for the synthesis of PtG_2 was obtained as a brown powder in 72% yield as. FTIR (KBr, cm^{-1}): 550, 1565, 1550, 1650, 1710, and 3265. ^1H NMR (400 MHz, DMSO, TMS, δ): 4.80 (s, 48H, NH_2), 10.08 (s, 3H, N*H*-CO), 9.78 (s, 6H, N*H*-CO), 7.14 (s, 9H, Ar*H*), 6.81 (s, 3H, Ar*H*), 6.48 (s, 6H, Ar*H*), and 6.20 (s, 12H, Ar*H*). ^{13}C NMR (100 MHz, DMSO, TMS): 103.0, 111.0, 115.3, 123.5, 132.2, 134.0, 138.6, 146.8, 170.4, and 174.0 ppm. MALDI-TOF MS (*m*/*z*): 2371.14. Anal. Calcd for $C_{69}H_{39}N_{21}O_9Pd_6Cl_{12}$ (2370.13): C, 34.97; H, 1.66; N, 12.41; Cl, 17.95; Pd, 26.94. Found: C, 35.00; H, 1.62; N, 12.46; Cl, 17.93; Pd, 26.92.

Anti-bacterial and cytotoxic assay

The anti-bacterial activity of dendrimers and metallodendrimers was determined by disk diffusion method using DMSO as a solvent (Awerbuch *et al.*, 1989). A lawn of bacteria was prepared by pipetting and evenly spreading 10 µl of inoculums, adjusted turbidometrically to 10^5–10^6 CFU/cm^3 (CFU = colony forming units) onto agar set in Petri dishes, using nutrient agar (NA. Whatman No. 1 filter paper discs of 6 mm diameter were impregnated with dimethyl sulfoxide stock solutions of the compounds (100 µg/ml) and dried under sterile conditions. Dried discs were then placed on previously inoculated agar surfaces. The plates were inverted and incubated for 24 h at 37°C. Antimicrobial activity was indicated by the presence of clear inhibition zones around the discs and repeated at least for three times for each anti-microbial agent. The results were compared with Streptomycin as a standard drug for negative control and solvent (DMSO) for positive control. Minimum inhibitory amounts (MIC) of dendrimers and metallodendrimers were determined by the tube dilution method using Mueller–Hinton broth. The bactericidal activity of the minimum inhibitory amount was determined by spreading 0.1 ml of the appropriately diluted broth culture of the MIC positive tube as well as above and below the MIC tubes, on nutrient agar plates without oil or anti-bacterial reference drugs. The Petri plates were incubated at 37°C for 18–24 h. The dilutions that showed no growth were termed as bactericidal and vice versa. In vitro cytotoxicity IC_{50} (concentration of tested agent causing 50% inhibition of cell growth) of these dendrimers and matellodendrimers was studied by using MTT colorimetric assay (Stevens and Olsen, 1993). This assay is based on the cleavage of the yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MTT, Sigma) forming purple formazan crystals by mitochondrial dehydrogenates in viable cell. The MCF-7 cells concentration (3×10^4 ml^{-1}) were adjusted according to cell population doubling time (PDT) 33 h, later they were exposed to compound at different concentration. The IC_{50} values were then estimated after an exposure time of 72 h. One hundred microliters of a 5 mg/ml MTT solution in PBS were added to each well for 4 h. After removal of the medium, DMSO was added to each well to dissolve the formazan crystals, and the absorbance was determined at 540 nM.

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