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Synthesis, characterization, optical, electrochemical properties and antifungal, anticancer activities of ferrocenyl conjugated novel dendrimers

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Abstract

New class triazoloferrocenyl conjugates **1**, **2** and **3** were obtained by copper (I) catalyzed click chemistry of alkyne azide cycloaddition (CuAAC) of bis(β–hydroxyl)arylether with suitable ferrocenyl azides. The synthesized bis(β–hydroxyl)arylether linked ferrocenyl conjugates **1**, **2** and **3** exhibit antifungal activity against the fungal pathogens viz Candida albicans, Candida glabrata and Candida crusi comparable to that of amphotericin B and also shows excellent anticancer activity against the MCF-7 cell lines.

Keywords: Ferrocenyl dendrimers, β–hydroxypropargylether, click chemistry, antifungal and anticancer activity amphotericin B.

Introduction

Copper (I) catalyzed azide–alkyne cycloaddition (CuAAC) known as click chemistry[1] developed before few decades has been quickly applied for the synthesis of new dendritic architectures.[2] The triazolyl moiety is a perfect choice known for its interaction with many anions, transition metals cations and neutral molecules and also has wide range of applications in the field of pharmacological and biological chemistry. The introduction of triazolyl group into the dendrimer system would rather enable the encapsulation of guest molecules. Triazolyl moiety is a privileged class of five member heterocyclic systems, which has attracted both medicinal and synthetic chemists due to its synthetic and biological applications. The triazolyl group is highly stable under basic, acidic, reductive and oxidative

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conditions and also have high dipole moment, rigidity, stability and capability to form hydrogen bonding with biological targets. Ferrocenyl motif has played a unique role in the modern medicine because of its excellent features including non-toxicity and stability under physiological conditions.[3] The ferrocenyl unit, has now been recognized as a useful motif for the development of new class and effective drugs viz. anti-androgen nilutamide and ferrocenyl analogs of commercial anti-estrogen tamoxifen which display higher cytotoxicity against breast and prostate cancer cells respectively in comparison to the reference drugs.[4] Certainly, the ferrocene appended chloroquine (CQ) known as Ferroquine (FQ), has enhanced the biological activity of the drug molecule by 22 times more than CQ against CQ-R strain of P. Falciparum.[5] Ferrocene derivatives are highly active in vitro and in vivo, against several fungal and bacterial infections,[6] including Human Immunodeficiency Virus (HIV),[7] Malaria[8] and Cancer.[9] Ferrocenyl conjugates play an important role in electron-transfer reactions which are involved in many biological systems and also in synthetic chemistry as multi-redox nanosystems, viz., as biosensors,[10] vectors,[11] biological redox processes,[12] catalysis,[13] semiconductors,[14] redox recognition,[15] etc., and biological properties like antitumour agent,[16] antimalarial,[17] anti-cancer effect in human lung cancer cells,[18] Many ferrocenyl compounds display interesting cytotoxic, anti-tumor, antimalarial, antifungal and DNA cleaving activities.[19]

In particular, ferrocenyl compounds have been synthesized for a wide range of applications in the medicinal and pharmaceutical disciplines.[20] The drug resistance in the microbial pathogens has drastically increased and surpassed the development and delivery of new effective antimicrobial drugs. As a result there is a threat to the global health and the currently available therapeutics will no longer be effective in treating infections due to increasing drug resistance in microbial pathogens. To fight against such increase in drug resistance, discovery of new antimicrobial agents is essential.

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The occurrence of breast cancer in Asian women has been increasing in the last few decades. The breast tumors are resistant to the conventional treatment which shows side effects. Radiation therapy and surgical treatments are not sufficient and not satisfactory for most of the developing countries. According to World Health Organization (WHO) the chemotherapy is employed for more than 90% of women affected with breast cancer. Owing to the need of new drugs for the chemotherapeutic treatment of breast cancer and to find the compounds with effective antifungal activity, we report herein the synthesis of ferrocenyl conjugates **1**-**3** with β –hydroxyarylether focal point using the copper (I) catalyzed click chemistry.[21] The promising antifungal and anticancer activities of dendrimers **1**, **2** and **3** provide useful information and serve as a new potential area of research.

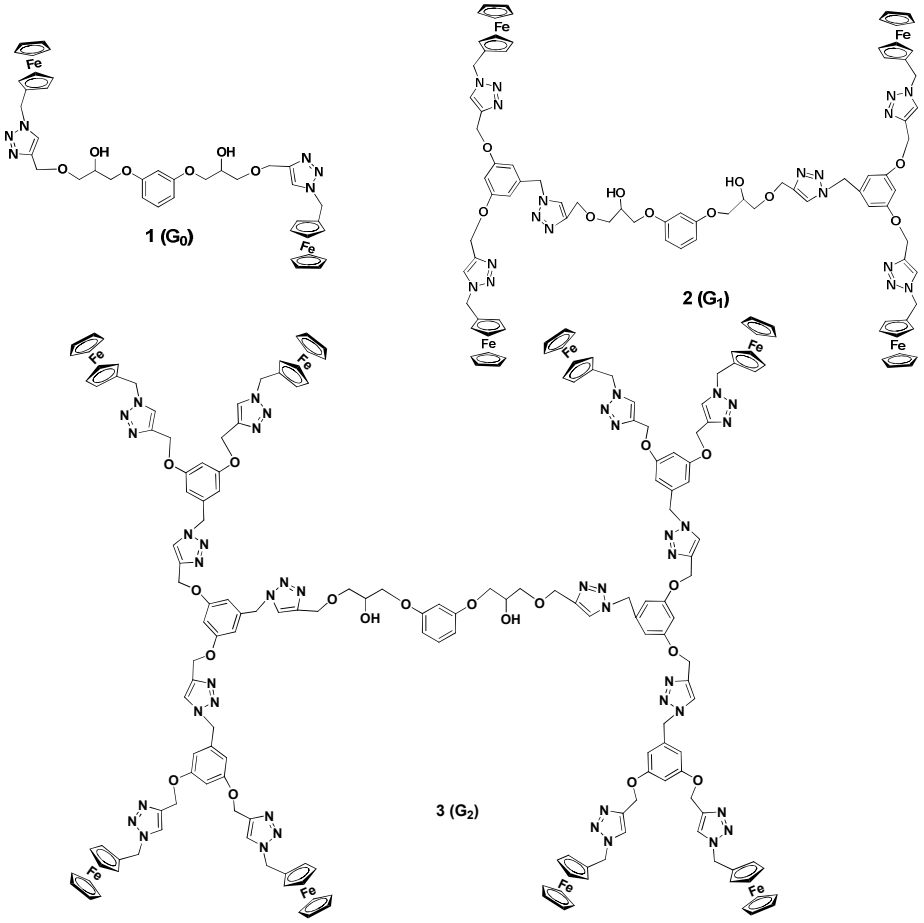

Figure 1: Structure of the ferrocenyl conjugates **1**, **2** and **3.**

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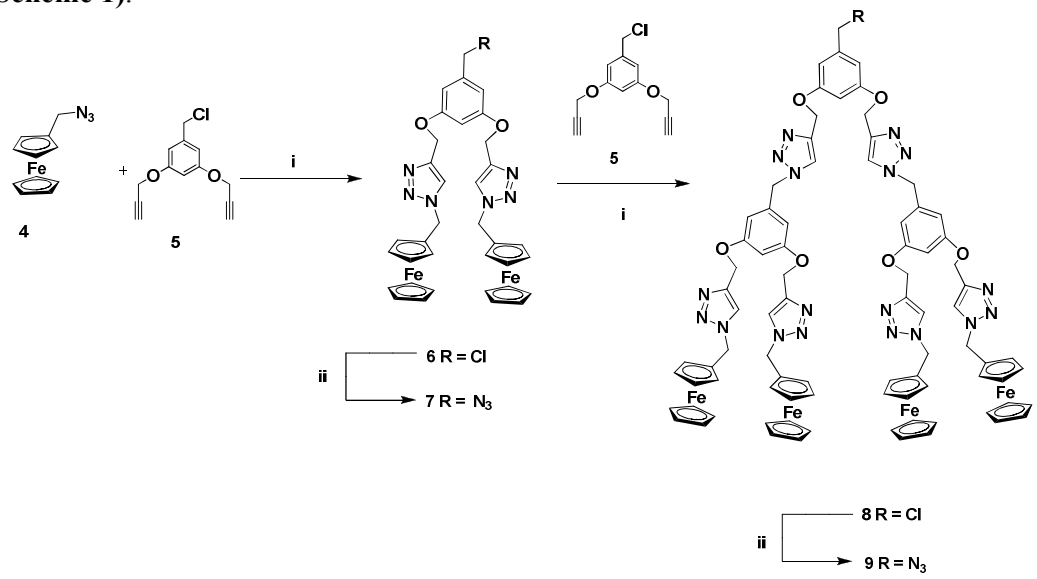
Results and discussion

Reaction of 3,5-bis(propargyloxy)benzyl chloride **5** with 2.1 equiv. of ferrocenylazide **4** under Cu(I)-catalyzed click reaction conditions gave the first generation dendritic chloride **6** in 92% yield, which on further treatment with NaN_3 in a mixture of acetone-water (4:1) at 60 ^oC gave the dendritic azide**7** in 96% yield. The second generation dendritic azide **9** was obtained by a similar sequence by repeating of the above reaction sequence namely Cu(I)-catalyzed click reaction followed by reaction with sodium azide. Reaction of 1.0 equiv. of 3,5-bis (propargyloxy) benzyl chloride **5** with 2.1 equiv. of the azidodendron **7** under click chemistry conditions gave the dendritic chloride **8** in 83% yield, which was further converted into the dendritic azide **9** in 85% yield using NaN_3 in a mixture of acetone-water (4:1) at 60 ^oC **(Scheme 1)**.



Scheme 1 Reagents and conditions: (i) $CuSO_4$ (5 mol%), sodium ascorbate (10 mol%), H_2O-THF (1:1), rt, 12 h, **6** (92%), **8** (83%) (ii) 1.5 equiv. NaN_3, CH_3COCH_3-H_2O (1:1), 60 ºC, 1-3 h, **7** (96%), **9** (85%).

The ^1H NMR spectrum of the dendritic azide **7** displayed three different singlets at δ 4.16, 4.21 and 4.27 for the ferrocenyl protons, two different singlets at δ5.06 and 5.28 for the

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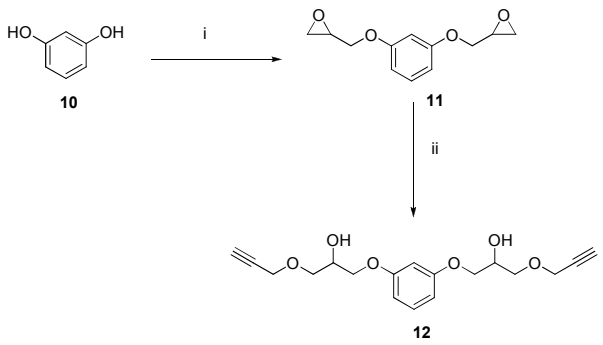
O-methylene and *N*-methylene protons, a singlet at δ 7.51 for the triazolyl proton. The ^{13}C NMR spectrum of the dendritic azide **7** displayed the ferrocenyl carbons at δ 68.6, 68.8, 68.9 and 69.1, and two different triazolyl carbons at 143.3 and 144.1 respectively. The appearance of molecular ion peak at *m/z* 723 also confirmed the structure of the dendritic azide **7**. Further the constitution of dendritic azide **7** was confirmed from elemental analysis.

The ^{1}H-NMR spectrum of the ferrocenyl dendritic azide **9** displayed two different singlets at δ5.12 and 5.29 for the *O*-methylene and *N*-methylene protons, a singlet at δ 7.51 for the triazole proton in addition to the signals for aromatic and ferrocenyl protons. The ^{13}C NMR spectrum of the dendritic azide **9** displayed the triazole carbon at δ 143.6. The appearance of molecular ion peak at *m/z* 723 also confirmed the structure of the dendritic azide **9**. Elemental analysis also supported the constitution of the dendritic azide **9**.

The synthesis of the key intermediate, asymmetric bisoxirane **11** begins from the readily available resorcinol. Reaction of 2.1 equiv. of epichlorohydrin with 1.0 equiv of resorcinol in the presence of NaH in DMF at room temperature gave the bisoxirane **11** in 86% yield. The bisoxirane **11** was subjected to ring opening with 2.1 equiv. of propargyl alcohol in dry DMF to afford the β-hydroxypropargyloxy ether **12** in 74% yield (**Scheme 2)**.



Scheme 2 Reagents and conditions: (i) 2.1 equiv. Epichlorohydrin, NaH, DMF, 0–25 °C, N_2, 12 h, **11** (86%). (ii) 2.1 equiv. Propargyl alcohol, Dry DMF, RT, 3h, **12** (74%)

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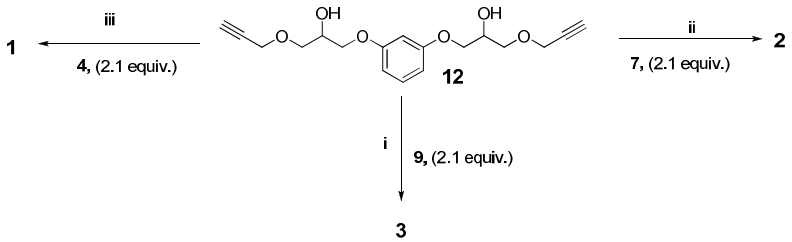
The ^{1}H NMR spectrum of the bisoxirane **11** displayed two doublets of doublet at δ 4.20, 3.90 for *OCH*$_2$ protons, the oxirane protons appeared as doublets of doublet at **δ** 2.90, 2.73 in addition to the signals for the other aliphatic and aromatic protons. The ^{13}C NMR spectrum of the bisoxirane **11** displayed the oxirane carbons at δ 44.6, 50.1 and the *OCH*$_2$ carbon at δ 68.8 in addition to the signals for the other aromatic carbons. The appearance of molecular ion peak at *m/z* 222 in mass spectrum and also elemental analysis confirmed the structure of the bisoxirane **11**. The ^{1}H NMR spectrum of the β-hydroxypropargyloxy ether **12** displayed triplet at δ 2.47 for acetylenic protons, the *OCH*$_2$ protons appeared as a doublet at δ 4.02 in addition to the signals for the other aliphatic and aromatic protons. The ^{13}C NMR spectrum of β-hydroxypropargyloxy ether **12** displayed the acetylenic carbons at δ 75.0 and 79.3, the propargyl -*CH*$_2$ carbon appeared at δ 58.7 in addition to the signals for the other aliphatic and aromatic carbons. The appearance of molecular ion peak at *m/z* 334 in mass spectrum and also the elemental analysis confirmed the structure of the β-hydroxypropargyloxy ether **12**.

Reaction of the 1.0 equiv. of the β-hydroxypropargyloxy ether **12** with 2.1 equiv. of the dentric azide **4**, **7** and **9** under click chemistry conditions in presence of copper sulphate (5 mole%) and sodium ascorbate (10 mol %) in a mixture of THF - H_2O (1:1) at room temperature afforded the ferrocenyl conjugates **1**, **2** and **3** in 92%, 89%, 82% yields, respectively (**Scheme 3**).



Scheme 3. **Ragents and conditions:** (i) $CuSO_4$ (5 mol %), sodium ascorbate (10 mol %), H_2O/THF (1:1), rt, 12 h, **1** (92%), **2** (89%) and **3** (82%).

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Optical and property of ferrocenyldendrimers 1, 2 and 3

The UV-Vis absorption spectrum of the ferrocenyl conjugates **1**, **2** and **3** was obtained in DCM (1 X 10^{-5} M) and the absorption maxima is observed at 433, 435 and 434 nm, for the dendrimer **1**, **2** and **3** respectively (**Figure 2A**). In the absorption spectra, the absorption intensity of the UV-visible band increases from ferrocenyl conjugate **1** to **3** at the same concentration though λ_{max} remains almost constant, which is due to the presence of more number of ferrocenyl units, usually called valence effect in dendrimer chemistry.[22] Figure 2B shows the linear increase in the absorption intensity of the ferrocenyl dendrimers with increase in the number of ferrocenyl units.

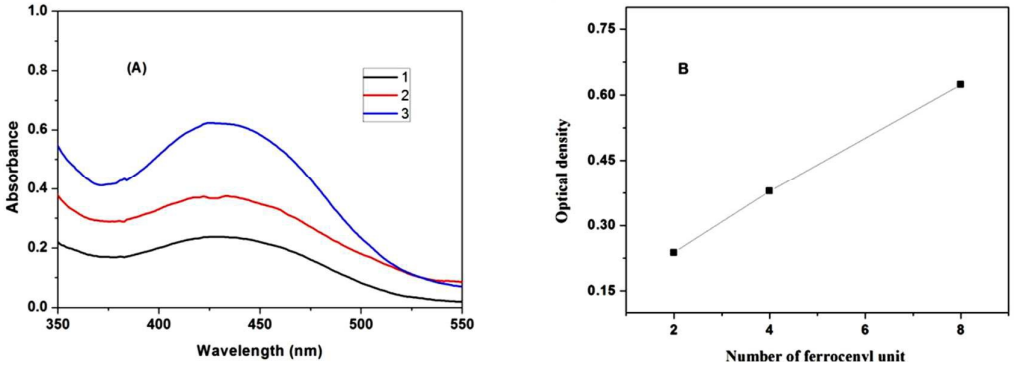

Figure 2: (A) UV-vis absorption spectra of ferrocenyl conjugate **1, 2** and **3** in DCM (1X10^{-5} M) at room temperature: (B) Linear relation of absorption with number of ferrocenyl units.

Electrochemical studies

The redox properties of the ferrocenyl dendrimers **1**, **2** and **3** was studied using cyclic voltammetry (CV) technique. The cyclic voltammogram for dendrimer **1** to **3** was obtained in the potential range of 0.0 V to 1.0 V at room temperature in CH_2Cl_2 containing 0.1 M TBAP as the supporting electrolyte at the scan rate 50 mV/s (Figure 3). The reversible oxidation potential for the ferrocenyl dendrimer **1-3** is obtained at 419, 497 and 599 mV and the

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reversible reduction peak is observed at 597, 613 and 649 mV respectively. The increase in the dendrimer generation from **1** to **3** increases the redox peak potential from less positive to more positive peak pontential due to the presence of more number of triazolyl units and ferrocenyl groups in **3** than in dendremers **1** and **2**, though the current intensity remains constant.

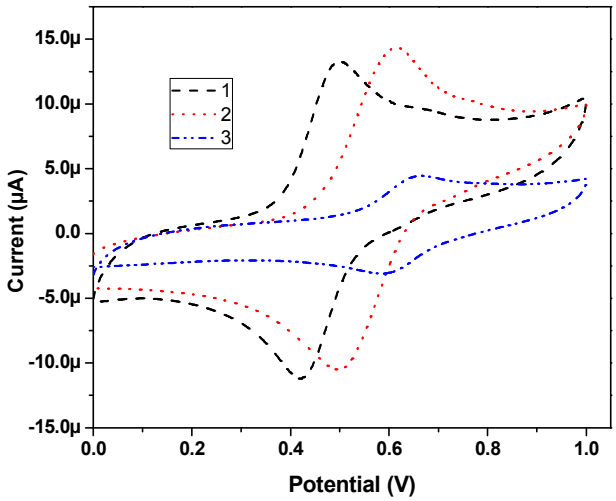

Figure 3. Cyclic votammogram of ferrocenyldendrimers **1**, **2** and **3** in DCM at room temperature. (scan rate at 50 mV s^{-1}) and 0.1 m tetrabutylammonium perchlorate as a supporting electrolyte in dry DCM.

Table 1. Optical and electrochemical parameters of ferrocenyl dendrimers **1**, **2** and **3** in DCM (1 X 10^{-5} M). Ferrocenyl dendrimers **1**, **2** and **3** are almost identical and exhibit an intense absorption band at 433-435 nm (**Figure 2**).

Dendrimers	λ_{abs} max (nm)	Optical density	Molar absorptivity coefficient (ε) L mol^{-1} cm^{-1})	E_{pa}	E_{pc}
1	433	0.24	24000	0.42	0.60
2	435	0.38	38000	0.50	0.61
3	434	0.62	62000	0.60	0.65

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Antifungal activity of ferrocenyl dendrimers 1, 2 and 3

Minimum Inhibitory Concentration (MIC):

The MIC for the antifungal activity of the dendrimers is determined by resazurin dye reduction assay method.[23] The change of the colour of the dye from blue or purple to pink is considered as the indication of MIC values. The conversion of resazurin to pink colored resorufin is due to the presence of enzyme oxido-reductase in unicellular fungus. The reduction of resazurin indicates presence of viable cells. But when the fungal cells dies, then it could not convert the resazurin to resorufin and thus the colour of the dye remains blue. The ferrocenyl dendrimers **1**, **2** and **3** when added to the assay wells kills the fungal cells after appropriate incubation, viz., the fungal suspension was added in well plate then incubated in 37° C at 18-24 h then the dye was added to the proper well and then incubated in 37° C at 2-3 h and the colour change from blue to colour was observed pink. This was observed by the blue colour in the respective wells. The pink colour formation in the wells even after treating with ferrocene dendrimers or commercial drug indicates the presence of viable cells. Thus the least dilution in which the colour remains blue was taken as the MIC value of the particular compound. The ferrocenyl dendrimers **1**, **2** and **3** showed antifungal activity against the fungal viz Candida albicans, Candida glabrata and Candida crusi. The MIC values lie between from 3.12 to 50 μg/mL for all the synthesized compounds. The ferrocenyl dendrimer **1** was found to show be better activity than **2** and **3** in antifungal assay against Candida albicans, Candida glabrata and the results are shown in Table **2**. Figure **4** shows the antifungal activity of the dendrimer **1**, **2** and **3** with amphotericin B as the standard.

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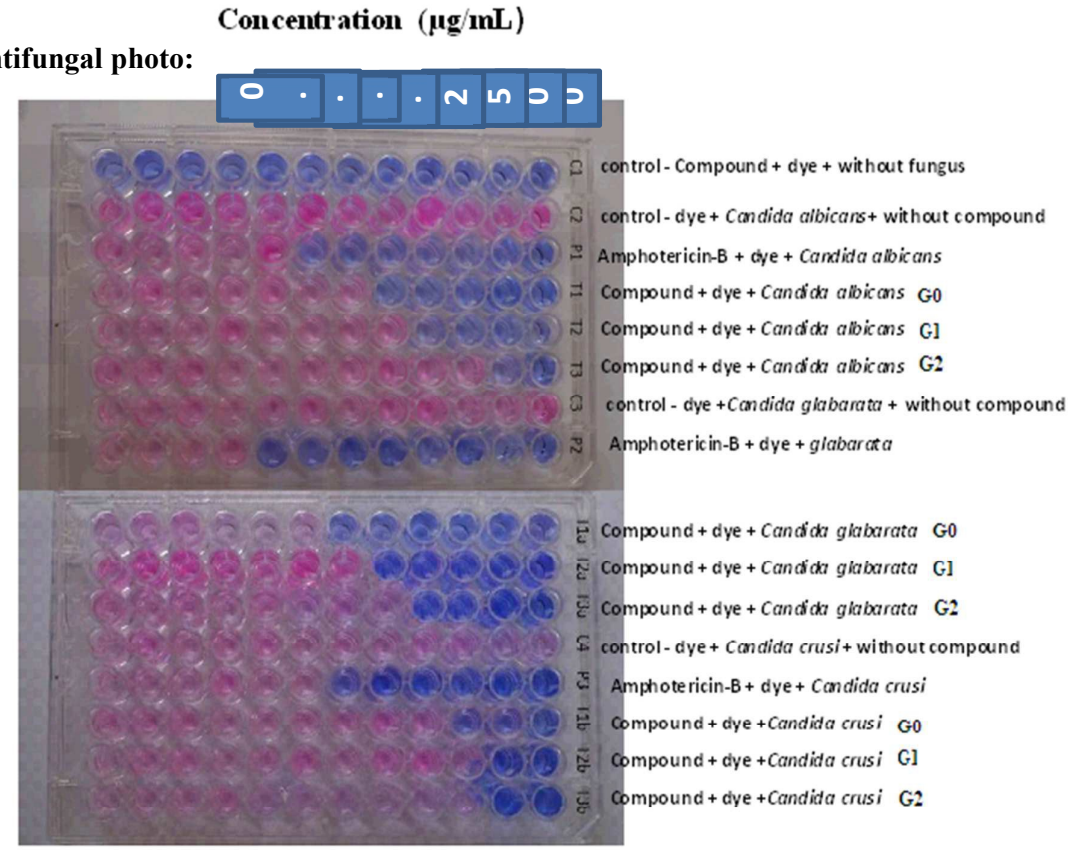

Figure 4: The antifungal activity of the ferrocenyl dendrimer **1**, **2** and **3.**

For unicellular and multicellular organism, iron happens to be an essential element.[24] It is interesting to note that some of the fungal pathogens use iron from host molecule for their metabolism, growth and invasion during infection.[25] Microorganism such as *Candida albicans*, *Candida glabarata* and *Candida crusi* can even utilise haemoglobin as an iron resource.[26] In our current study, surprisingly addition of ferrocenyl dendrimer help the fungal pathogens to utilise the iron present in the host molecule for their generation. Dendrimer **1** has lower iron content and hence its can effectively inhibit the growth of the three fungal pathogens viz *Candida albicans*, *Candida glabarata* and *Candida crusi.* However as we move on to the first and second generation dendrimer **2** and **3,** the iron content increases and hence the inhibition of the growth of the fungal organism decreases rapidly. In fact the second generation ferrocenyl dendrimer **3** do not inhibit the growth of the fungal organism and hence the MIC value are much larger than the standard viz Amphotericin-B. In

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conclusion the presence of ferrocenyl dendrimer do not inhibit that much the growth of fungal organism viz *Candida albicans*, *Candida glabarata* and *Candida crusi* mainly due to the presence of iron in it. Further from the study it is clear that though the presence of triazolyl group could try to increase the inhibition of the growth of fungal pathogens the iron present in the dendrimers helps the growth of the tested fungal pathogens and hence the MIC values are much larger than the standard.

Table 2: Minimum Inhibitory Concentration of the ferrocenyl dendrimer **1**, **2** and **3** by resazurin reduction assay against fungal pathogens

	MIC μg/mL		
Compounds/generation	Fungal pathogens		
	Candida albicans	*Candida glabrata*	*Candida crusi*
1(G_0)	6.25	3.12	12.5
2(G_1)	12.5	6.25	50
3(G_2)	50	12.5	50
Standard **- Amphotericin-B**	1.56	0.78	3.12

Anticancer activity of ferrocenyl dendrimers 1, 2 and 3

The ferrocene dendrimers were then tested for anticancer activity by MTT assay against the MCF-7 cell lines and all the three ferrocene dendrimers exhibited good anticancer activity against the MCF-7 cell lines. The viability of MCF-7 cells decreases gradually as the concentration of the compounds increases. A dose response graph was plotted and the IC_{50} value was calculated. The IC_{50} value of the compounds are calculated by the three parameter logistic fixed bottom (3PLFB) model[27] and IC_{50} values of the compounds 1, 2 and 3 are found to be 20.55 μg/mL, 10.91 μg/mL, 3.12 μg/mL and for SD viz, doxorubicin the IC_{50} values is 2.61 μg/mL. The error was less by 3PLFB model and hence fixing bottom at 100% is

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employed as the activity base was unable to fit a fixed top model and the IC_{50} value increases by more than two-fold. Among the three ferrocenyl dendrimers tested, the ferrocenyl dendrimer **3** was found to be more effective against the MCF-7 cells with effective IC_{50} value (Table **3** and Figure **5**). Further molecular docking studies of these ferrocenyl dendrimers are underway to find their mode of actions against the MCF-7 cells.

Table 3: Anticancer activity of ferrocenyl dendrimers

S.No	Concentration (µg/mL)				Absorbance (OD in nm)				Cell viability (%)			
	Den 1	Den 2	Den 3	SD	Den 1	Den 2	Den 3	SD	Den1 (Go)	Den2 (G1)	Den 3 (G2)	SD
1	100	100	100	50	0.09	0.07	0.04	0.02	15.51	12.06	6.89	3.44
2	50	50	50	25	0.12	0.12	0.10	0.06	20.69	20.69	17.24	10.34
3	25	25	25	12.50	0.28	0.24	0.19	0.10	48.27	41.37	32.75	17.24
4	12.50	12.50	12.50	6.25	0.31	0.28	0.25	0.16	53.44	48.27	34.48	27.58
5	6.25	6.25	6.25	3.12	0.37	0.32	0.29	0.27	63.79	55.17	43.10	46.55
6	3.12	3.12	3.12	1.56	0.43	0.40	0.33	0.36	74.13	68.96	50.00	62.06
7	Control	Control	Control	control	0.58	0.58	0.58	0.58	100	100	100	100

Den 1= dendrimer 1, Den 2 = dendrimer 2, Den 3 = dendrimer 3, SD- standard drug- doxorubicin

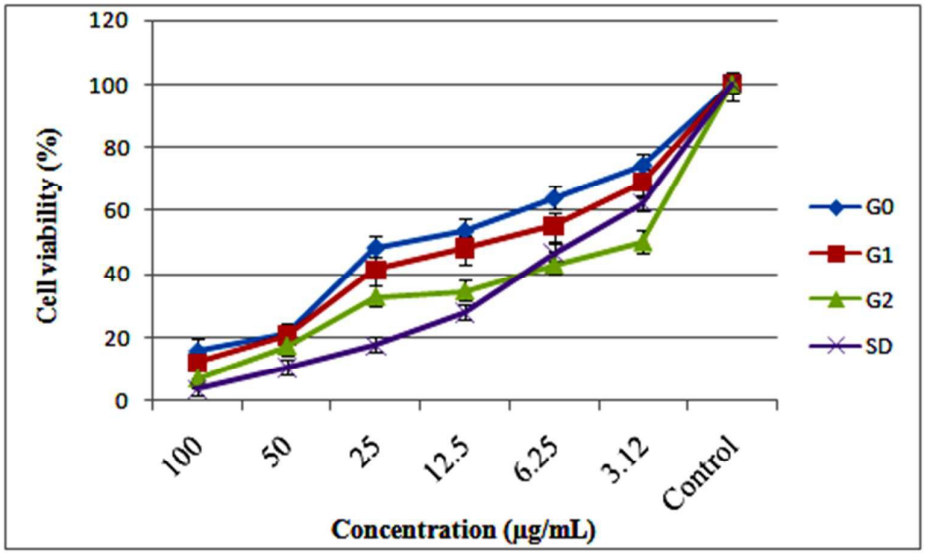

Figure 5: Anticancer activity of ferrocenyl dendrimers **1** (G0), **2** (G1) and **3** (G2) against MCF-7 cells

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Conclusion

In conclusion, ferrocenyl conjugated dendrimers **1**, **2** and **3** with triazole as the bridging unit has been obtained through click chemistry in excellent yield. The zeroth generation ferrocenyl dendrimer **1** showed better activity against fungal pathogens candida albicans, candida glabrata and candida crusi when compared to the first and second generation dendrimer **2** and **3**, whereas second generation dendrimer **3** showed excellent anticancer activity against the MCF-7 cells, when compared with the zeroth and first generation dendrimer **1** and **2**. Further studies on such these biological active ferrocenyl dendrimers are essential to explore their complete biological potentials.

Experimental

Material and methods

All reagents were commercially available and used as such unless otherwise stated. Analytical TLC was performed on commercial Merk plates coated with Silica Gel GF254. Analytical samples were obtained from silica gel chromatography, using silica gel of 100-200 mesh and elution with the solvent system as mentioned under each experiment section. The melting points were determined by using a Metler Toledo melting point apparatus by open capillary tube method and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz BRUKER AVANCE (75 MHz for ^{13}C NMR,) spectrometer. UV-Vis absorption spectra were measured with a Perkin–Elmer Lambda 35 UV-vis spectrometer, Cyclic voltammetry measurements were performed in a conventional three electrode system on a CHI model 1100A series electrochemical analyzer (CH Instrument, Austin, TX). The measurements were based on a three electrode system, with a glassy carbon (GC) electrode (of geometric area 0.07 cm^2) being used as the working electrode, a Pt wire in the form of a spiral (with a high

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geometrical surface area B20 cm^2) being used as the auxiliary electrode and Ag/AgCl as the reference electrode with a scan rate of 50 mV s^{-1}. The supporting electrolyte of 0.1 M TBAP.

General procedure for the Cu (I) catalyzed click reaction (procedure A)

A mixture of acetylenic derivative (1.0 mmol, 1equiv.) and azido derivative (5.1 mmol, 5.1equiv.) was dissolved in THF-H_2O (1:1; 8 mL) and added sodium ascorbate (0.4 mmol, 0.4 equiv.) followed by the addition of $CuSO_4$ (0.2 mmol, 0.2 equiv.). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated and the crude product was dissolved with EtOAc (2 X 100 mL), washed with NH_4Cl solution (50 mL) and brine solution (50 mL), dried over Na_2SO_4 and concentrated to give a residue, which was purified by column chromatography (SiO_2), using the eluent as mentioned under each compound.

General procedure for convertion of dendritic chloride to azide (procedure B)

Dendritic chloride (1.0 mmol, 1.0 equiv.) was dissolved in a mixture of acetone-water (4:1; 8 mL) and added NaN_3 (1.5 mmol, 1.5 equiv./ 2.5 mmol, 2.5 equiv.), and the mixture was heated to 60 ^{0}C for 3 h. The reaction mixture was cooled to room temperature, acetone was evaporated, diluted with water (100 mL), and extracted with EtOAc (2 X 100 mL). The organic layer was washed with saturated NaCl (100 mL), dried over Na_2SO_4 and evaporated to give the corresponding azido compounds.

Azidomethylferrocene 4:

Azidomethylferrocene **4** was prepared as reported in the literature [28] from hydroxyl methyl ferrocene (2g, 9.3 mmol) and NaN_3**(**1.2g, 18.5 mmol**).**

3,5-bis(propargyloxy)benzyl chloride 5:

3,5-Bis(propargyloxy)benzyl chloride **5** was prepared as reported in the literature [29]

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from 3,5-bis(propargyloxy)benzyl alchohol (1.2g, 5.6 mmol) and thionyl chloride (0.8 mL, 11.1 mmol).

Ferrocenyldendritic chloride 6:

The dendritic chloride **6** (0.45 g) was obtained as pale yellow solid from 3,5-bis (propargyloxy) benzyl chloride **5** (0.16 g, 0.68 mmol) and azidomethylferrocene **4** (0.35 g, 1.4 mmol) under click chemistry conditions following the procedure A. Yield: 92 % MP: 124-126 ^{o}C ^{1}H NMR: 300 MHz, $CDCl_3$): δ_H 4.18 (s, 10H), 4.22 (s, 6H), 4.28 (s, 4H), 5.12 (s, 4H), 5.29 (s, 4H), 6.53 (s, 1H), 6.56 (s, 2H), 7.51 (s, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$): δ_C 50.2, 62.2, (68.7, 68.9. 69.1,Cp) 80.6, 101.8, 107.5, 122.2, 137.8, 143.6, 159.7. MS (ES): *m/z* = 716 [M^+]. Elemental Anal. Calcd for $C_{35}H_{33}Fe_2N_6O_2Cl$: C, 58.64, H, 4.64, N, 11.72%. Found: C, 58.54, H, 4.61, N, 11.68%.

Ferrocenyldendritic azide 7:

Following the general procedure B, the dendritic azide **7** (0.95 g) was obtained as light yellow solid from the dendritic chloride **6** (1 g, 1.4 mmol) and NaN_3 (0.18 g, 2.8 mmol). Yield: 96% MP: 134-136 ^{o}C ^{1}H NMR: (300 MHz, $CDCl_3$): δ_H 4.16 (s, 10H), 4.22 (s, 6H), 4.28 (s, 4H), 5.12 (s, 4H), 5.29 (s, 4H), 6.53 (s, 1H), 6.56 (s, 2H), 7.51 (s, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$): δ_C 50.2, 62.2, (68.7, 68.9. 69.1,Cp) 80.6, 101.8, 107.5, 122.2, 137.8, 143.6, 159.7. MS (ES): *m/z* = 724 [M^+]. Elemental Anal. Calcd for $C_{35}H_{33}Fe_2N_9O_2$: C, 58.11, H, 4.60, N, 17.43%. Found: C, 58.00, H, 4.47, N, 17.36%.

Second generation ferrocenyldendritic chloride 8:

The ferrocenyl dendritic chloride **8** (0.77 g) was obtained as yellow solid from 3,5-bis (propargyloxy) benzyl chloride **5** (0.13 g, 0.55 mmol) and the dendritic azide **7** (0.86 g, 1.16 mmol) under click chemistry conditions following the procedure A. Yield: 83% MP: 130-132 ^{o}C ^{1}H NMR: (300 MHz, $CDCl_3$): δ_H 4.10 (s, 2H), 4.16, 4.21, 4.27 (s, 36H; Cp), 5.06 (s, 8H),

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5.14 (s, 4H), 5.28 (s, 8H), 5.39 (s, 4H), 6.46 (s, 4H), 6.53 (s, 3H), 6.56 (s, 2H), 7.51 (s, 4H), 7.59 (s, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$): δ_C 50.1, 54.1, 54.6, 62.0, (68.6, 68.8, 68.9, 69.1, Cp), 80.7, 101.7, 102.0, 107.5, 122.4, 123.1, 133.5, 136.8, 137.8, 143.3, 144.1, 159.6, 159.8. MS (ES): *m/z* = 1680 [M^+]. Elemental Anal. Calcd for $C_{83}H_{77}Fe_4N_{18}O_6Cl$: C, 59.29, H, 4.62, N, 14.99%. Found: C, 59.23, H, 4.58, N, 14.91%.

Second generation ferrocenyldendritic azide 9:

The dendritic azide **9** (0.43 g) was obtained as pale yellow solid from the dendritic chloride **8** (0.5 g, 0.3 mmol) and NaN_3 (0.04 g, 0.6 mmol) following the procedure B. Yield: 85% MP: 134-136 ^{o}C ^{1}H NMR: (300 MHz, $CDCl_3$): δ_H 4.12 (s, 2H), 4.16, 4.21, 4.27 (s, 36H; Cp), 5.06 (s, 8H), 5.14 (s, 4H), 5.28 (s, 8H), 5.39 (s, 4H), 6.46 (s, 4H), 6.53 (s, 3H), 6.56 (s, 2H), 7.51 (s, 4H), 7.59 (s, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$): δ_C 50.1, 54.1, 54.6, 62.0, (68.6, 68.8, 68.9, 69.1, Cp), 80.7, 101.7, 102.0, 107.5, 122.4, 123.1, 133.5, 136.8, 137.8, 143.3, 144.1, 159.6, 159.8. MS (ES): *m/z* = 1687 [M^+]. Elemental Anal. Calcd for $C_{83}H_{77}Fe_4N_{21}O_6$: C, 50.06, H, 4.60, N, 17.43%. Found: C, 49.99, H, 4.44, N, 17.37%.

Bis(epoxy methyl) resorcinol 11

Bis(epoxy methyl) resorcinol **11** (0.95 g) was obtained as gummy solid by the reaction of resorcinol (1 g, 1.4 mmol) with epichlorohydrin(0.18 g, 2.8 mmol) in presence of NaH followed by elution from the column with $CHCl_3$.Hexane (3:2). Yield : 85% ^{1}H NMR :(300 MHz, $CDCl_3$): δ_H 2.72- 2.74 (dd , J =5.2 ,4.1 Hz , 2H) 2.89 - 3.31 (dd, J = ,5.3 ,4.2 Hz , 2H) 3.31-3.36 (m, 2H) 3.87- 3.92 (dd, J = 9.3, 4.2Hz , 2H) 4.18- 4.23 (dd, J = 9.3, 4.2Hz , 2H) 6.51-7.19 (m , 4H);^{13}C NMR : (75 MHz, $CDCl_3$): δ_C 44.6, 50.1, 68.8, 101.8, 107.3, 130.0, 159.7.*m/z*= 222 [M^+]. Elemental Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85, H, 6.35, O, 28.80%. Found: C, 53.80, H, 6.25, O, 28.15%.

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β –hydroxypropargyl ether 12.

β–Hydroxypropargyl ether **12** (0.70 g) was obtained by the reaction of from bis(epoxy methyl) resorcinol **11** (1 g, 1.4 mmol) with propargyl alcohol (0.18 g, 2.8 mmol) dry DMF (20 ml) followed by elution from the column with $CHCl_3$: MeOH (25:1) Yield: 72% ^{1}H NMR : (300 MHz, $CDCl_3$): δ_H 2.46-2.60 (s ,2H), 2.61 (d, J = 2.6 ,2H), 3.65-3.77 (m , 2H), 3.76 (d , J= 2.8, 2H), 4.04-4.17 (d , J = 3.4 ,4H) 4.17-4.23 (s, 4H), 6.52 (q , 2H), 6.55 (d , J = 6.2 , 2H), 7.17 (d, J = 7.1 , 1H) 7.27 (s , 1H,)^{13}C NMR : (75 MHz, $CDCl_3$): δ_C 58.7, 69.0, 69.1, 70.8, 75.0, 79.28, 101.8, 107.33, 130.01, 159.76.*m/z*= 334[M^+]. Elemental Anal. Calcd for $C_{18}H_{22}O_6$: C, 64.66, H, 6.63, O, 28.71%. Found: C, 64.60, H, 6.57, O, 29.98%.

Ferrocenyldendrimar 1:

Following the general procedure A, the ferrocenyl dendrimer **1** (0.95 g) was obtained as light yellow solid from the ferrocenyl azide **4** (1 g, 2.1 mmol) and β –hydroxypropargyl ether **12** (1.7g, 1.0 mmol) and after eluting from the column with $CHCl_3$: MeOH (25:1). Yield : 96% M. P. : 134-136 ^{o}C ^{1}H NMR : 300 MHz, $CDCl_3$): δ_H 3.20 (s, 2H), 3.66 (d, *J* = 2.1 4H), 3.95 (d, J = 2.1, 4H), 4.14 (s, 2H), 4.17- 4.25 (m, 18H), 4.63 (s, 4H), 5.24 (s, 4H), 6.46 (t ,3H) 7.13(t , 1H) 7.42 (S, 2H).^{13}C NMR :(75 MHz, $CDCl_3$): δ_C 30.1, 50.1, 64.7, 68.9, 69.0, 69.1, 71.4, 80.6, 101.7, 107.2, 121.9, 129.9, 144.6, 159.8.MS (MALDI-TOP): *m/z* = 839.25 [M+Na^+]. Elemental Anal. Calcd for $C_{40}H_{44}Fe_2N_6O_6$: C, 58.84, H, 5.43, N, 10.29%. Found: C, 58.80, H, 5.47, N, 10.24%.

Ferrocenyldendrmar 2:

Following the general procedure A, the ferrocenyl dendrimer **2** (0.95 g) was obtained as light yellow solid from the ferrocenyl dendritic azide **7** (1.7g, 1.0 mmol) and β–hydroxypropargyl ether **12** (1 g, 1.4 mmol) and after eluting from the column with $CHCl_3$:

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MeOH (25:2) Yield: 86%^{1}H NMR : (300 MHz, $CDCl_3$): δ_H 3.68 (s, 4H), 3.90 (d, J = 2.1 4H), 4.01 (d, J = 2.1 2H), 4.11- 4.65(m, 36H), 4.93 (s, 43H) , 5.04 (s, 8H), 5.34 (s, 8H), 5.45 (s ,4H) 6.44 -6.53 (m ,9H) 7.48(t , 1H) 7.56-7.72 (s, 6H)^{13}C NMR : (75 MHz, $CDCl_3$): δ_C 50.2, 58.7, 62.1, 64.8, 68.6, 68.8, 68.9, 69.0, 69.1, 70.8, 75.0, 76.6, 77.0, 77.4, 80.7, 101.6, 101.8, 102.1, 107.4, 122.5, 122.9, 130.0, 133.5, 136.9, 143.3, 145.2, 159.9.MS (MALDI-TOP): *m/z* = 1803.39 [M+Na^+]. Elemental Anal. Calcd for $C_{88}H_{88}Fe_4N_{18}O_{10}$: C, 59.3, H, 4.98, N, 14.16%. Found: C, 59.1, H, 4.85, N, 14.26%.

Ferrocenyl dendrimer 3:

The ferrocenyl dendrimer **3** (0.99 g) was obtained as light brown solid by the reaction of β –hydroxypropargyl ether **12** (0.05 g, 0.14 mmol) with ferrocenyl dendritic azide **9** (1.16 g, 0.69 mmol) under click chemistry condition and after eluting from the column with $CHCl_3$-MeOH (25:2) Yield: 81% M.P 165-167 ^{o}C ^{1}H (300 MHz, $CDCl_3$): δ_H 3.57(s, 4H), 3.66 (d, J = 2.1 4H), 3.84- 4.07(m, 72H), 4.18 (s, 16H), 4.52 (s,2H), 4.91(s, 24H), 5.17- 5.25 (s, 16H), 6.33 (m , 12H) 6.43 (s, 9H), 6.98 (s, 1H), 7.45-7.49 (s, 14H)^{13}C NMR : (75 MHz, $CDCl_3$): δ_C 49.1, 52.9, 57.2, 60.9, 67.7, 67.9, 68.0, 68.1, 68.7, 72.4, 74.1, 74.4, 75.6, 76.1, 76.5, 79.8, 100.8, 101.0, 106.2, 106.4, 106.6, 121.49, 122.5, 129.8, 142.2, 144.2, 148.8, 158.7, 163.6, 167.5, 175.7. (MALDI-TOP): *m/z* = 3733.67 [M+Na^+]. Elemental Anal. Calcd for $C_{184}H_{176}Fe_8N_{42}O_{18}$: C, 59.56; H, 4.78; N, 15.85; Found: C, 59.38, H, 4.71, N, 15.52%.

Minimum Inhibitory Concentration

The minimum inhibitory concentrations of the compounds against the human pathogens were analyzed by resazurin reduction assay described by Sarkar *et al*.[23].

Method to Prepare Resazurin dye solution

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The Resazurindye solution was made by dissolving a 270 mg tablet in 40 mL of sterile distilled water. The instrument vortex mixer was used to ensure that the resazurinsolution was well-dissolved and form homogenous solution.

Preparation of the activity plates

96 wells plates were prepared under aseptic conditions. 200µL of the compound (1mg/mL) in 5% (v/v) dimethyl sulfoxide was pipetted into the first row of the sterile 96 wells plate.[30] To all other remaining wells 100µL of Sabouraud dextrose broth was added. The serial dilutions were performed with sterile pipette tips such that each well had 100µL of the test material in serially descending concentrations. To all these wells 10µL of resazurin dye solution was added. 10µL of fungal suspension (5×10^6 cells/mL) was added to each well to achieve a concentration of 5×10^5 cells/mL. The commercial antibiotic streptomycin and amphotericin-B were used as positive controls in the assay plate. The plates were then placed in an incubator at 37 °C for 18–24 h. The colour change was then observed visually. The colour changes from purple to pink or colourless were recorded as reduction of dye by the viable bacteria. The lowest concentration at which no colour change occurred was taken as the MIC value.

MTT Assay:

The stock solution of MTT dye (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) 5 mg per ml, was made in sterilized phosphate buffered saline. The lysis solution was prepared by mixing 0.6 ml of acetic acid and 99.4 ml of dimethyl sulphoxide. The MCF-7 cells were incubated with the appropriate concentrations of sample for 24 and 48 h. After incubation the consumed medium was carefully removed from all the wells of the assay plate and was replaced with freshly prepared Dulbecco's Modified Eagle Medium (DMEM). The MTT dye (100µl per ml of stock solution) was added to each well and the culture plates were incubated for 3 h in CO_2 incubation chamber. The

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supernatant was aspirated carefully, taking care not to remove formazan crystals formed with in the cells. The lysis solution in amounts equal to that of the DMEM added before incubation was added and the cells were lysated over 5 minutes and mixed thoroughly. 200 µl of the lysate from each of the 24 well culture plate was transferred to the pre-marked 96 well plate and then the optical density (OD) of the lysate was measured at 570 nm. The percentage of OD value (OD value of Test/OD value of control x 100%) was calculated. A larger OD value represented higher cell viability and thus adhesion.

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Synthesis, characterization, optical, electrochemical properties and antifungal, anticancer activities of ferrocenyl conjugated novel dendrimers

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Graphical abstract

New class triazolo ferrocenyl conjugates were prepared by copper (I) catalyzed Click chemistry, which shows good antifungal activity against the fungal pathogens viz Candida albicans, Candida glabrata and Candida crusi and also shows excellent anticancer activity against the MCF-7 cells.