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SYNTHESIS, CHARTERIZATION AND EVALUATION OF IRON CHELATION AND ANTIOXIDANT ACTIVITY OF NOVEL HETEROCYCLIC COMPOUNDS CONTAINING 1, 2, 4-TRIAZOLE RING

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ABSTRACT
A novel iron chelation agents 2, 2'-(1H-1, 2, 4-triazole-3, 5-diyl) diphenol derivatives has been synthesized and chelation properties has been demonstrated. This molecule showing iron chelation activity and chelation activity is comparable with Deferasirox. The antioxidant activity was conducted and comparable with Ascorbic acid. The antibacterial activity in the series of triazole based on heterocycle derivatives. The synthesized compounds were evaluated by FTIR, UV-Vis, ^{1}H- NMR, ^{13}C –NMR, Mass spectrometry and elemental analysis.

KEYWORDS
Antioxidant activity, Chelating agents, hexadentate ligand, Iron chelation Triazole, 1,3-oxazin-4-one, Tridentate ligand.

INTRODUCTION
Deferasirox **API** molecule is an orally active tridentate compounds, which is **FDA** approved (**NOV 2005**) and is marketed under the trade name of **exjade®** for the treatment of transfusion dependent chronic iron over load. Commercially available of deferasirox under the brand name of **exjade®** and supplied as dispersible tablet with different strengths [i, xvii]. Without treatment for the anemia every year **300000-500000** children's are born with serve hemoglobin disorder, which including sickle cell anemia and thalassemias, they die in the first few year of the life. Chronic iron overload associated with red cell disorders (i.e., sickle cell anemia and thalassemia) and other myelodysplastic syndromes affect a significantly larger number of individual's [ii, xxii, xxiii, xx, xxi]. Normal human body iron stores in humans in 3-4g; however, an excess of iron of 20g or more lead to organ damage such as heart, liver, anterior pituitary, pancreas and other diseases, such as diabetes and arthritis [iii, xv, xxi]. Iron stored inside cell in two major forms i.e., ferritin and hemosiderin[iv]. Doctor can identify in the human body blood carries little or too much of the iron by conducting the Total iron

binding capacity test (TIBC). As there is no natural mechanism for the human body eliminate the excess iron [v, vi, xiii, xviii]. Therefore treatment with an iron chelation becomes inevitable, which lead to the discovery of Deferasirox. The excess iron removed by using deferasirox chelating agents. It is used for the treatment of iron chelation, liver diseases and friedreich ataxia. Deferasirox is a tridentate chelator that mobilizes iron stores by binding selectivity to the ferric (Fe^{3+}) form of iron. The selectivity of iron is high, with low affinity trace metal such as Zn or Cu. Tridentate chelator require two molecules to bind the one iron atom [vii, xiv, xv]. In literature found many substituted deferasirox are known and biological activities as anticancer, iron chelation.

The anticancer activity mainly due to its iron chelation nature. Chelation excess iron in the biological systems. Iron is essential for many proteins in the body to maintain normal cellular function. The excess iron level in cells can cause toxicity since it generate highly reactive oxygen species (ROS), hydroxyl radical ($HO^{\cdot}$) or ferryl by **Fenton's reaction** which can damage the cells [viii].

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\cdot}OH + {}^{\ominus}OH$$

It can also act as a cofactor in many biological pathways such as synthesis of ATP, DNA and neuratransmittiers. It is found in hemoglobin, iron is an essential metal. Deferasirox also reported Mo^{VI} high valent, VO, Mg, Ca, Cu, Zn and Al metal chelating complexes [ix, x]. Recently deferasirox has been tested as anti cancer drug for many cancer cell lines. Addition to this reported the cytotoxicity of deferasirox in normal liver cell line was less than that of liver cancer cell line. Deferasirox and its derivatives including methoxy deferasirox (mDef), imidazole modified deferasirox (idef) encapsulated polymeric micelles as PH-responsive iron chelating nano cancer for cancer chemotherapy reported. The cytotoxicity deferasirox in various types of cancer cell lines have been reported. Deferasirox also shows synergistic in vitro anticancer effect with may well know drugs such as docetaxel, sorafenib and benvacizumab [xi]. Initially the dose of deferasirox drug is started at 20mg/kg/day orally once a day. The dose is gradually increased in 5-10 mg/kg/day every 3-6month depending upon the serum ferritin, LIC values [xvi].

The reported process for the synthesis of deferasirox involve the condensation of salicylic acid or salicyloyl chloride with salicylamide to an elevated temperature of 140-170ºC to obtain 2-(2-hydroxyphenyl)-4H-benzone[e][1,3]oxazin-4-one.further react with 4-hydrazino benzoic acid at 80ºC to give **deferasirox**[iii].

Deferasirox derivatives were successfully synthesized and displayed good chelation activity, anti oxidant activity and anti bacterial activity. The deferasirox based on triazole molecule were synthesized various methods and captured in literature. Currently few studied have been reported where deferasirox has been use to treat in cancer human.Triazole based on heterocycle is known for its iron chelating activities / chelating agents. In this series we have synthesized novel way of Deferasirox derivatives belongs to new class of oral tridentate chelator. N and O substituted of phenyl 1, 2, 4-triazole ring based on iron chelation agent and its chelating activity along with antioxidant activity and antibacterial activity described in this paper.

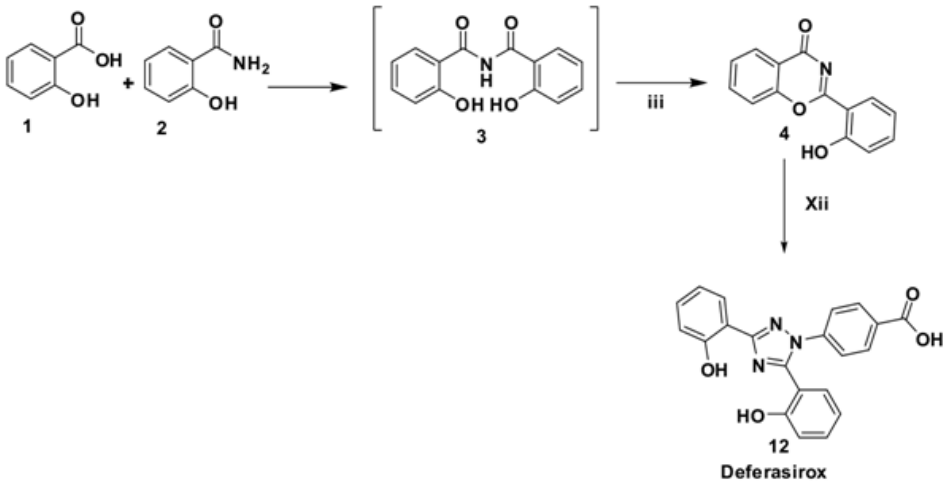
RESULT AND DISCUSSION

The preparation of 3, 5'-bis (2-(tert-butyl dimethyl silyoxy)phenyl)-1H-1,2,4-triazole can achieved effectively in two steps procedure given by **Ryabukhin (1983)**. In the first step, salicylic acid and salicylamide are condensed yielding 2-(2-hydroxyphenyl)-4H-benzone[e][1,3]Oxzin-4-one reacting in the second step with a suitably substituted hydrazine

to give desired 3, 5'-bis(2-(tert-butyl dimethyl silyoxy)phenyl)-1H-1,2,4-triazole derivative [xxii].

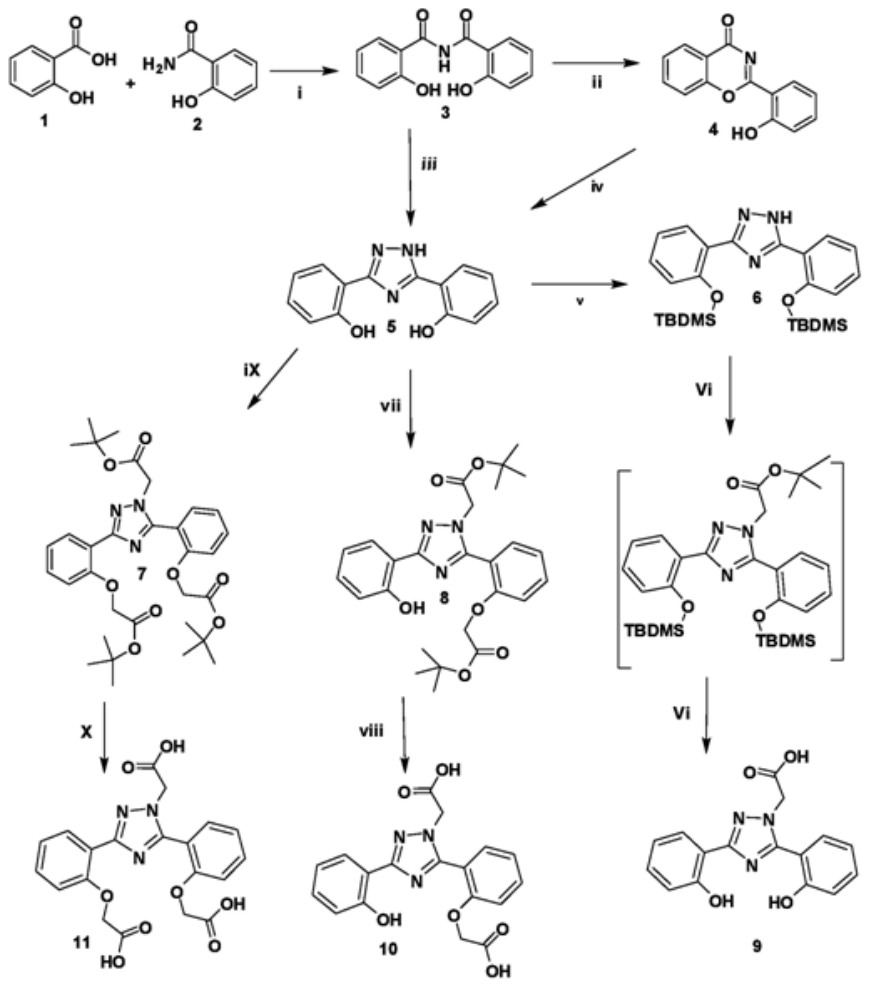
The Salicylic acid and salicylamide reacts with thionyl chloride, catalytic amount of DMF and TBAB, toluene at 140°C to give intermediate followed by cyclised product (**4**) and react with 4-hydrazino benzoic acid and IPA to give deferasirox product (**12**) (Scheme**1**) known compound of **Deferasirox (Standard)** comparable with **deferasirox** series compounds.

4-(3,5-bis(2-hydroxyphenyl)-1H-1,2,4-triazol-1yl)benzoic acid (deferasirox,12) [iii, xx]
LC-MS: m/z 374.4 [+ve mode, M+H]; ^{1}H-NMR (400MHz, DMSO-d_6) δ : 10.83 (s, OH), 10.05 (s, OH), 8.04 (dd, *J* = 6.36 Hz, *J* = 1.36 Hz, 1H), 7.94 (d, *J* = 8.56 Hz, 2H), 7.47-7.528 (m, 3H), 7.34-7.40 (qd, *J* = 1.6 Hz 2H), 6.94-7.03 (m, 3H), 6.87 (d, *J* = 8.24 Hz, 1H) (^{1}H patterns match with the literature value) [iii, xx]



Scheme-1 synthetic scheme **12** of reagent and conditions; (i)Toluene,$SOCl_2$,catalytic amount of DMF, and TBAB at 140°C.(ii) IPA,4-hydrazino benzoic acid at 80°C

Scheme (2). To a solution of 2-hydroxy-N-(2-hydroxy benzoyl) benzamide (**3)** in toluene, triethylamine was added followed by addition of hydrazine monohydrate to give the product (**5**), from this we are prepared the N-alkylation of mono acid by using TBDMS protection, Tert-butyl bromo acetate followed by TFA and TBAF. Similarly N-alkylation and one O-alkylation of the diacid and N-alkylation and two O-alkylation of triacid. In this paper discussed the synthetic triazole compounds are comparable with deferasirox of iron chelation activity, Antioxidant activity of standard Ascorbic acid and antibacterial studies. The anti bacterials of two gram positive of Staphylococeus aureus, Bacillus Subtilis and two Gram negative of pseudomonas aeruginose, Escherichinacoli.



Scheme-2- Synthetic scheme of 9,10,11 Reagent and conditions: (i) $SOCl_2$, Toluene, catalytic amount of DMF at 80-85°C; (ii) Toluene, TBAB at 140°C; (iii) NH_2-NH_2.H_2O, Toluene, TEA at 60°C; (iv) NH_2-NH_2.H_2O, toluene, TEA at 50°C; (v) TBDMSCl, DCM, TEA at 0-5°C; (vi) DMF, tert-butyl bromo acetate, K_2CO_3 at 0-10°C;DCM,TFA at 10°C; DCM ,TBAF at 20-25°C; (vii) tert-butyl bromoacetate, DMF at 65°C; (viii) tert-butyl bromoacetate, DMF, K_2CO_3 at 20-25°C; (ix) TFA,DCM at 20-25°C (x) TFA, DCM at 20-25°C.

Step-1: Synthesis of 2-hydroxy-N-(2-hydroxybenzoyl)Benzamide (3) [vii]

To a suspension of 2-hydroxy benzoic acid **1** (10 g, 0.053 mol), 2-hydroxybenzamide **2** (11.8g, 0.086 mol) in toluene (60 ml) was simultaneously added catalytic amount of DMF and thionyl chloride (6.34 ml, 1.2 eq) under nitrogen atmosphere. The rate of addition was maintained between 0-5°C. After complete addition, reaction temperature was raised to 80-85°C and stirring was continued 3 h at 80-85°C.The progress of the reaction was monitor by TLC. Thionyl chloride and toluene was concentrated completely under reduced pressure at 60°C. The residue was diluted with ice cold water (100 ml) and stirred 20 min at 20-25°C. Solid was filtered. The crude product **3** was purified with (50 ml) of isopropyl alcohol

heating at 60°C stirred for 1h and Cool the mass 5-10°C stirred for 30 min. Filtered the solid, dried at 50°C to result in compound **3** as a white solid. Yield: 14 g (75%).

LC-MS: m/z 255.9 [-ve mode, M-H]. IR (KBr, ν cm^{-1}): 3246 (N-H, OH, str.), 3050 (aryl-CH str.), 1711.76 (C=O, str.), 1317.34 (C-N, str.), 1603.96 (C=C, str.), 794.42 (aryl-CH out of plane bend); ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 12.1 (s, NH), 11.5 (s, 2OH), 7.84 (d, J = 8 Hz, 2H), 7.47 (d, J = 8Hz, 2H), 6.98-7.07 (m, 4H); ^{13}C NMR (100 MHz DMSO-d_6) δ : 165.02, 157.30, 134.80, 131.14, 120.20, 119.44, 117.55. UV-vis (CH_3OH) (λ_{max}/nm (log ε)) 367(0.644). Anal.Calcd. (%) for $C_{14}H_{11}NO_4$: C, 65.37; H, 4.31; N, 5.44. Found C, 65.39; H, 4.31; N, 5.46; (^{1}H patterns match with the literature value) [vii]

Step-2: Synthesis of 2-(2-hydroxyphenyl)-4H-benzone[e][1,3]Oxzin-4-one (4) [ix]

To a suspension of 2-hydroxy –N -(2- hydroxybenzoyl) benzamide **3** (5 g, 0.019 mol) and TBAB (0.62 g, 0.001 mol) in toluene (30 ml) with dean-stark apparatus heat the reaction mixture at 120°C for 5h. The progress of the reaction was monitor by TLC. Concentrate the reaction mixture at 80°C under reduced pressure and cool the reaction mixture to 5-10°C. Diluted with chilled ice water stirred for 30 min at 0-5°C. Filter the solid then dried at 55°C to obtain in compound **4** as a Yellow colors solid. Yield: 3.5 g (76%). The product used without further purification.

LC-MS: 239.94 [+ve mode, M+H]. IR (KBr, ν cm^{-1}) 3380 (Ar-OH), 1696.51 (C=O, str.), 1240.47 (C=N, str.), 3010 (C-H, str.), 1539.60 (C=C, str.), 10150.22 (C-O, str.). 752.90 (aryl-CH out of plane bend); ^{1}HNMR (400MHz, DMSO-d_6) δ : 12.88 (s, OH), 8.15-8.17 (m, 1H), 8.04 (dd, J = 8Hz, 2Hz, 1H), 7.89-7.93 (m, 1H), 7.76 (dd , J = 8.4 Hz, 0.8 Hz, 1H), 7.55-7.62 (m, 2H) 7.03-7.083 (m, 2H); ^{13}CNMR (100MHz, DMSO) δ : 164.81, 163.44, 161.84, 153.89, 136.72, 136.02 ,128.99, 127.20, 126.76, 119.58, 177.97,117.83, 117.49, 111.4. UV-vis (CH_3OH) (λ_{max}/nm (logε)) 408 (0.455), 803 (0.187). Anal.Calcd. (%) for $C_{14}H_9NO_3$ C, 70.29; H, 3.79; N, 5.86. Found C, 70.32; H, 3.82; N, 5.86. (^{1}H and ^{13}C patterns match with the literature value) [ix]

Step-3: Synthsesis of 2,2'-(1H-1,2,4-triazole-3,5-diyl)Diphenol (5)[ix]

To a suspension of 2-hydroxy-N-(2-hydroxy benzoyl) benzamide **3** (5 g, 0.019 mol) in toluene (30 ml), TEA (3.47 g, 0.038 mol) was added at 0-10°C, followed by addition of hydrazine monohydrate (10 ml). The rate of addition was maintained between 0-10°C. After complete addition the reaction temperature was raised to 65°C and stirring was continued 4h at 65°C. The progress of the reaction was monitor by TLC. The reaction mass was diluted with water (90 ml) and stirred for 30 min. then filter the solid. The crude product **5** was slurry with ethyl acetate: methanol (1:1, 25ml). Filtered the solid and dried at 50°C to result in compound **5** as an off white solid. Yield: 3.8 g (77.2%).

LC-MS: m/z 254 [+ve mode, M+H]. IR (KBr, ν cm^{-1}): 3397.43 (Aryl-OH, str.), 2922.84 (C-N, str.), 1249.85 (C=N, str.), 745.2, 794.15 (aryl-CH out of plane bend); ^{1}H-NMR (400MHz, DMSO-d_6) δ : 14.75 (s, NH), 11.91 (s, 2OH), 8.02 (d, J = 7 Hz 2H), 7.34 (t, J = 7.8Hz, 2H), 6.97-7.04 (m, 4H); ^{13}CNMR (400MHz, DMSO) δ : 157.30, 156.90, 132.0, 128.02, 120.28, 117.33, 114.30. UV-vis (CH_3OH) (λ_{max}/nm (logε)) 307(0.523), 373(0.5423). Anal. Calcd. (%) for $C_{14}H_{11}N_3O_2$ C, 66.40; H, 4.38; N, 16.59. Found C, 66.39; H, 4.39; N, 16.58. (^{1}H and ^{13}C patterns match with the literature value) [ix]

Step-4: Synthesis of 2,2'-(1H-1,2,4-triazole-3,5-diyl)Diphenol (5) [ix]

To a solution of 2-(2-hydroxy phenyl)-4H-benzo[e][1,3]oxazin-4-one (3.5 g, 0.014 mol) in toluene (21 ml) and TEA (3 ml, 0.021 mol) was added at 0-5°C. Followed by addition of hydrazine monohydrate (7 ml) stir for 10 min. The rate of addition was maintained between 0-5°C. After complete addition temperature was raised to 50°C and stirring was continued 3h at 50°C. The progress of the reaction was monitor by TLC. The reaction mass was diluted with water (65 ml) and stirred for 30 min at 20-25°C then filters the solid. The crude product

5 was slurry with ethyl acetate: methanol (1:1, 30 ml). Filtered the solid and dried at 50°C. The solid was slurry with ethyl acetate (10.5 ml) then filtered and dried at 50°C to result in compound **5** as a White solid. Yield: 2.9 g **(**80.0%). (^{1}H and ^{13}C patterns match with the step-3 value) [ix]

Step-5: Synthesis of 3, 5'-bis(2-(tert-butyl dimethyl silyoxy)phenyl)-1H-1,2,4-triazole (6).

To a solution of 2,2'-(1H-1,2,4-triazole-3,5-diyl)Diphenol **(**2 g, 0.078 mol) in DCM (14 ml) and triethylamine (3 ml, 0.021 mol) was added at 0-5°C. Followed by addition of TBDMSCl (2.97 g, 0.0197 mol) at 0-5°C. After complete addition temperature was raised to 20-25°C and stirring was continued 3h at 20-25°. The progress of the reaction was monitor by TLC. The reaction mass was quenching with water (10 ml) and stirred for 10 min. at 20-25°C then extracted with DCM. The organic layers wash with (10 ml) of water. The organic layer dried at 45°C to result in compound 6 as a Pale yellow liquid **6.** Yield: 3.5 g (92.1.0%).

LC-MS: 399 [+ve mode, M+H]. ^{1}H-NMR (400MHz, DMSO-d_6) δ : 13.7 (s, NH), 7.65-7.76 (dd, *J* =7.6 Hz, 3.8 Hz, 2H), 7.34 (dt, J = 40.8Hz, 7.4Hz, 2H), 6.96-7.14 (m, 4H, Ar-H), 0.848 (s, 18H), -0.036 (s, 12H); ^{13}CNMR (400MHz, DMSO-d_6) δ : 155.94, 152.99, 131.06, 130.66, 120.40, 119.452, 116.59, 25.78, 17.78, -3.23.

Step-6: Synthesis of 2-(3,5-bis(2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl)acetic acid (9).

To a solution of 3,5'-bis(2-(tert-butyl dimethyl silyoxy)phenyl)-1H-1,2,4-triazole (3 g, 0.0062 mol) in DMF (21 ml) under nitrogen atmosphere was added potassium carbonate (1.72 g, 0.0124 mol) at 0-10°C. Followed by addition of tertiary-butyl bromoacetate (1.45 g, 0.0074 mol) at 0-10°C. After complete addition temperature was raised to 20-25°C and stirring was continued 1h at 20-25°C during which TLC showed the completion of the reaction. The reaction mass was diluted with water (30 ml) and stirred for 10 min at 20-25°C then extracted with DCM. The organic layers wash with (25 ml) of water. The organic layer dried over anhydrous sodium sulphate.

To a solution of tert-butyl 2-(3,5-bis(2-tert-butyldimethylsilyloxy) phenyl)-1H-1,2,4-triazol-1yl)acetate (3 g, 0.005 mol) in DCM (21 ml). TFA (15 ml) was added at 0-10°C. After complete addition temperature was raised to 20-25°C and stirring was continued 3h at 20-25°C. The progress of the reaction was monitor by TLC. The reaction mass was concentrated under reduced pressure at 60°C. Add TBAF (9 ml) into the reaction mass. Stir the reaction mass at 25-30°C 1h. Quenching with water (15 ml) and stirred for 10 min. at 20-25°C then Extracted with DCM. The organic layer wash with (15 ml) of water. The organic layer dried at 45°C to result in compound **7** as a White solid. Yield: 1.3 g (83.3%).

LC-MS: m/z 311.9 [+ve mode, M+H]**.** IR (KBr, ν cm$^{-1)}$: 2982.52 (aryl-CH, str.), 3613.80 (aryl-OH, str.), 1636.43 and 1749.57 (C=O, str.), 1226.87 (C-O, str.), 1510.24 (C=C, str.), 1084.97 (C-N, str.), 1226.87 (C-O, str.), 744.03 (aryl-CH out plane bending); ^{1}H -NMR (400MHz, DMSO-d_6) δ : 13.10 (s, COOH), 10.93 (s, OH), 10.51 (s, OH), 7.95 (dd, *J* = 7.6, *J* = 1.6, 1H), 7.39-7.43 (m, 2H), 7.03-7.34 (m, 1H), 7.03-7.05 (d, 2H, *J* = 8Hz), 6.94-7.03 (m, 3H), 5.05 (s, 2H); ^{13}CNMR (400MHz, DMSO) δ : 168.15, 159.21, 156.15, 154.87, 153.29, 132.34, 131.25, 130.97, 126.292, 119.527, 119.527, 119.461, 116.869, 116.327, 113.831, 113.57, 50.573. Anal. Calcd. (%) for $C_{14}H_{13}N_3O_4$ C, 61.73; H, 4.21; N, 13.50. Found C, 61.74; H, 4.22; N, 13.49.

Step-7: Synthesis of tert-butyl 2-(3-(2-(2-tert-butoxy-2-oxethoxy) phenyl-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-1yl) acetate (8).

To a solution of 3,5'-bis(2-(tert-butyl dimethyl silyoxy)phenyl)-1H-1,2,4-triazole **6** (2 g, 0.0078 mol) in DMF (16 ml) under nitrogen atmosphere was added potassium carbonate (2.92 g, 0.0211 mol) at 0-10°C. Followed by addition of tertiary-butyl bromoacetate (3.38 g, 0.0.0173 mol) at 0-10°C. After complete addition temperature was raised to 20-25°C and stirring was continued 1h at 20-25°C. The progress of the reaction was monitor by TLC. The

reaction mass was diluted with water (20 ml) and stirred for 10 min. at 20-25°C then extracted with DCM. The organic layers wash with (20 ml) of water. The organic layer dried at 45ºC to result compound **7** as a white solid. Yield: 3.4 g (89.4%).

LC-MS: 482.2 [+ve mode, M+H]**.** IR (KBr, ν cm^{-1}): 3224.64 (aryl-OH, str.), 3060.70 (aryl-CH, str.), 1746.24 (C=O, str.), 1588.12 (C=C, str.), 1154.05 (C-N, str.), 747.0 (aryl-CH out plane bending); ^{1}H-NMR (400MHz, DMSO-d_6) δ : 10.85 (OH), 7.97 (dd, *J* = 7.76 Hz, 1.64 Hz 1H), 7.52-7.61 (m, 1H), 7.33-7.37 (m, 1H), 7.18(q, *J* = 11.68 Hz, 4.24 Hz,1H), 7.09-7.12 (d, *J* = 8.4 Hz, 1H), 6.9-7.01 (m, 2H), 5.14 (s, 2H, CH_2), 4.79 (s, 2H, CH_2); ^{13}CNMR (400MHz, DMSO-d_6) δ : 167.41, 165.70, 159.43, 156.11 ,154.56, 152.68, 132.49, 131.71, 131.03, 126.32, 121.39, 119.48, 116.87, 152.25, 113.71, 112.34, 82.14, 81.91 64.69, 51.20, 27.57, 27.38, 27.33. (UV-Vis (CH_3OH) (λ max/ nm (logε)) 284 (1.190). Anal. Calcd. (%) for $C_{26}H_{31}N_3O_6$; C, 64.85; H, 6.49; N, 11.37. Found. C, 64.83; H, 6.47; N, 8.73.

Step-8: Synthesis of 2-(5-(2-(caroxymethoxy)phenyl)-3-(2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl)acetic acid (10)

To a solution of tert-butyl 2-(5-(2-(2-tert-butoxy-2-oxethoxy) phenyl-3-(2-hydroxyphenyl)-1H-1,2,4-triazol-1yl)acetate (**9**) (2 g, 0.0041 mol) in DCM (20 ml). TFA (10 ml) was added at 0-10°C. After complete addition temperature was raised to 20-25°C and stirring was continued 3h at 20-25°C. The progress of the reaction was monitor by TLC. The reaction mass was concentrated under reduced pressure at 60°C. Diluted with water (20 ml) and stirred for 10 min. at 20-25 then extracted with DCM (20 ml). The organic layers wash with (10 ml) of water. The organic layer dried at 45°C to yield in compound **10** as a white solid Yield: 1.2 g, (81.69%).

LC-MS: 369.0 [+ve mode, M+H]**.** IR (KBr, ν $cm^{-1)}$: 2898.24 (aryl-CH str.), 288.98 (aryl-OH, str.), 2829.82 (OH, Acid OH-str.), 1736.12 and 1616.65 (C=O, str.), 1469.69 (C=C, str.), 1201.33 (C-N, str.), 1074.16 (C-O, str.), 750.76 (aryl-CH out plane bending); 1H-NMR (400MHz, DMSO-d_6) δ : 13.8 (s, 2COOH), 10.90 (s, OH), 7.96 (d, *J* = 7.2 Hz, 1H), 7.51-7.57 (q, *J* = 16, *J* = 8.4 Hz, 2H), 7.33 (t, *J* = 6.8 Hz, 1H), 7.10-7.16 (m, 2H), 6.95-6.98(q, *J* = 13.2 Hz, *J* = 5.2 Hz, 2H), 5.12(s, 2H, $CH_{2)}$, 4.788(s, 2H, CH_2) ; ^{13}CNMR (400MHz, DMSO-d_6) δ : 170.39, 168.93, 159.85, 156.65, 155.25, 153.172, 132.95, 132.21, 131.52, 126.83, 121.73 , 120.02, 117.41, 115.85, 114.33, 112.88, 64.89, 51.284. (UV-Vis (CH_3OH) (λ max/ nm (logε)) 288 (0.943). Anal.Calcd. (%) for $C_{18}H_{15}N_3O_6$ C, 58.54; H, 4.09; N, 11.38.Found. C, 58.56; H, 4.07; N, 11.37.

Step-9: Synthesis of tert-butyl 2, 2'-(1-(2-tert-butoxy-2-oxoethyl)-1H-1,2,4-triazole-3,5-diyl)bis(2,1- phenylene)Bis(oxy) diacetate (7).

To a solution of 3,5'-bis(2-(tert-butyl dimethyl silyoxy)phenyl)-1H-1,2,4-triazole **6** (2g, 0.0078 mol) in DMF (16 ml) under nitrogen atmosphere was added potassium carbonate (4.36 g, 0.0315 mol) at 0-10°C. Followed by addition of tertiary-butyl bromoacetate (3.38 g, 0.0260 mol) at 0-10°C. After complete addition temperature was raised to 60-65°C and stirring was continued 3h at 60-65°C during which TLC showed the completion of the reaction. The reaction mass was quenching with water (20 ml) and stirred for 10 min. at 20-25°C then Extracted with DCM (20 ml). The organic layers wash with (20 ml) of water. The organic layer dried at 45ºC to result in compound 11 as a Brown color liquid. Yield: 4.2 g, (89.3%).

LC-MS: 596.3 [+ve mode, M+H]**.** IR (KBr, ν cm^{-1}); 297.860 (aryl-CH, str.), 1627 and 1746.94 (C=O, str.), 1463.94 (C=C str.), 1156.49 (C-N, str.), 752.2 (Aryl C-H out plane bending); ^{1}H-NMR (400MHz, DMSO-d_6) δ : 7.79 (dd, *J* = 7.6 Hz, J = 1.6 Hz, 1H), 7.45-7.53 (m, 2H), 7.35-7.40 (m, 1H), 6.97-7.14 (m, 4H), 5.02(s, 2H, CH_2), 4.76 (s, 2H, CH_2), 4.68 (s, 2H, $CH_{2,}$), 1.41 (d, 18H, t-butyl), 1.27 (s, 9H, t-butyl); ^{13}CNMR (400MHz, DMSO-d_6)

δ : 167.84, 167.56, 166.10, 158.89, 155.63, 154.36, 152.99, 131.99, 131.811, 130.48, 130.08, 121.16, 120.71, 116.60, 114.08, 112.06, 81.90, 81.76, 66.13, 64.87, 51.05, 27.62, 27.36. Anal.Calcd. (%) for $C_{32}H_{41}N_3O_8$; C, 64.52; H, 6.94; N, 7.05. Found. C, 64.50; H, 6.95; N, 7.07.

Step-10 Synthesis of 2,2'-(2,2'-(1-(2-carboxymethyl)-1H-1,2,4-triazole-3,5-diyl)bis(2,1-phenylene)bis(oxy)diacetic acid (11).

To a solution of tert-butyl2,2'-(1-(2-tert-butoxy-2-oxoethyl)-1H-1,2,4-triazole-3,5-diyl)bis(2,1-phenylene)bis(oxy)diacetate (**11**) (2 g, 0.003 mol) in DCM (20 ml). TFA (10ml) was added at 0-10°C. After complete addition, temperature was raised to 20-25°C and stirring was continued 3h at 20-25°C. The progress of the reaction was monitor by TLC. The reaction mass was concentrated under reduced pressure at 60°C. Quenching with water (20 ml) and stirred for 10min at 20-25°C then extracted with DCM (20 ml). The organic layers wash with (10 ml) of water. The organic layer dried at 45°C. The crude material was purified with EA (5 ml): hexanes (20 ml) to result compound **12** as a White solid 1.25 g (83.9%).

LC-MS: 425.8 [-ve mode, M-H]**.** IR (KBr, ν cm^{-1}): 3059.40 (aryl-CH, str.), 2577.41 (OH, str.), 1734.28 (C=O, str.), 1485.16 and 1434.99 (aryl-C=C, str.), 1248.62 (C-O, str.), 1183.47 (C-N, str.), 837.27 (aryl-CH out of plane bend); ^{1}H-NMR (400MHz, DMSO-d_6) δ : 12.098 (s, 3COOH), 7.912 (dd, *J* = 8 Hz, *J* = 1.6 Hz, 4H), 7.519-7.562(m, 1H), 7.486 (dd, *J* = 6.4 Hz, *J* = 1.2 Hz,1H), 7.410-7.453(m, 1H), 7.090-7.163(m, 4H), 5.092 (s, 2H, CH_2), 4.815 (s, 4H, $2CH_2$); ^{13}CNMR (400MHz, DMSO-d_6) δ : 170.80, 170.39, 168.95, 158.60, 156.12, 155.127, 153.586, 132.766, 132.388, 131.315, 130.193, 132.296, 121.719, 119.99, 116.10, 115.53, 112.765, 67.123, 64.698, 51.017. (UV-Vis (CH_3OH) (λ max/ nm (logε)) 300 (0.8). Anal.Calcd. (%) for $C_{20}H_{17}N_3O_8$ C, 56.21; H, 4.01; N, 9.83. Found. C, 56.22; H, 4.02; N, 9.82.

EXPERIMENTAL

DPPH Free radical Scavenging Assay [xii] Antioxidant activities of deferasirox derivatives of (3, 5, 9, 10, and 11) were established by using DPPH free radical assay. The DPPH assay was determined by oyedemi et al., (2011).

Principle: The method described by Oyedemi et al., (2011) was used to determine DPPH scavenging activity of the plant extract. The solution of (0.135 mM) DPPH was prepared in methanol. Different concentration of extract (0.5 ml) was mixed with (2.5 ml) of DPPH solution. The reaction mixture was vortexed thoroughly and left in the dark are room temperature for 30 min. The absorbance of the mixture was measured at 517 nm by spectrophotometer. Ascorbic acid was used as the reference drug which compared with the corresponding absorbance of standard ascorbic acid concentration (5 µg / ml), (10 µg / ml), (20 µg / ml), (50 µg / ml), (100 µg / ml), (200 µg / ml) (OD 0.887).

The ability of plant extract to scavenge DPPH radical was calculated from the following formula:

%DPPH inhibition= [(OD of control - OD of test)/ (OD of control)] x100

DPPH radical activity is one of the most widely used for the screening the anti oxidant activity of synthesis deferasirox derivatives of (3, 5, 9, 10 and 11). At first, 6 test tubes were taken to make aliquots of 6 concentrations (5 µg / ml), (10 µg / ml), (20 µg / ml), (50 µg / ml), (100 µg / ml), (200 µg / ml) with samples. Sample and ascorbic acid were weighed accurately and dissolved in methanol to make the required concentration by dilution

technique. Here ascorbic acid was taken as positive control. DPPH was weighed and dissolved in methanol to make the solution (0.135 mM).To dissolve homogenously magnetic stirrer was used. After making the desired concentration (2.5 ml) of (0.135 mM) DPPH solution was applied on each test tube by pipette. The room temperature was recorded and kept the test tube for 30min. in the dark to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only methanol was taken as blank. After 30min, the absorbance of each test tube was taken by a UV spectrophotometer. The effect of antioxidant on DPPH radical scavenging on DPPH radical Scavenging was thought to be due to their hydrogen donating ability DPPH is stable free radical and accepts an electron(or) hydrogen radical become a stable diamagnetic ability.

The highest level of DPPH radical scavenging activity of (BZ) 96.62 % and ascorbic acid 96.59 % was found at (200 µg / ml), followed by (TZ) 96.42 %, (MA) 95.70 %, (DA) 94.25 %, (TA) 92.59 %. The antioxidant activities have been listed as order of decreasing activity BZ > TZ > MA > DA > TA.

The results were summarized in **table 1** and graphically represented in **Figure 1**. The IC_{50} value were summarized in **table 2** and graphically represented in Figure 2. The anti oxidant activity of standard Ascorbic acid is graphically represented in **figure 3.**

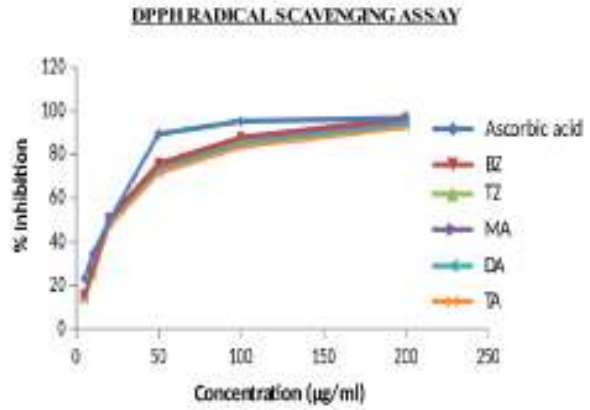


Figure 1. Free radical Scavenging Activity of Triazole compounds using **DPPH.**

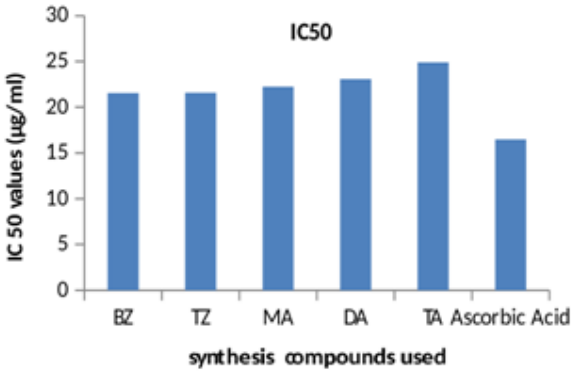


Figure 2. IC_{50} of Triazole compounds using **DPPH**

conc. (µg/ml)	Ascorbic acid	BZ	TZ	MA	DA	TA
5	23.28±0.33	15.65±0.10	15.39±0.07	14.92±0.09	14.69±0.16	13.40±0.18
10	34.43±0.33	27.62±0.20	26.67±0.09	26.27±0.11	25.76±0.09	24.55±0.27
20	49.53±0.29	50.54±0.21	50.57±0.37	50.14±0.12	49.66±0.28	47.38±0.15
50	89.33±0.34	75.68±0.06	75.43±0.28	74.89±0.14	73.59±0.28	71.31±0.09
100	95.15±0.51	87.64±0.09	87.45±0.21	87.42±0.13	85.450±0.18	83.53±0.30
200	96.95±0.19	96.62±0.08	96.42±0.16	95.70±0.05	94.25±0.35	92.59±0.32

Table 1. Free radical Scavenging activity of Triazole compounds using **DPPH**
Values are expressed as mean ± **SD** of triplicates

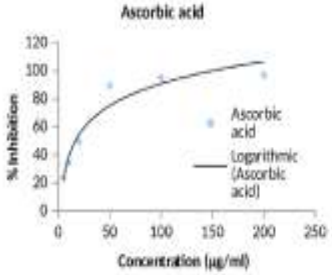


Figure 3. Free radical Scavenging Activity of Ascorbic acid using **DPPH**

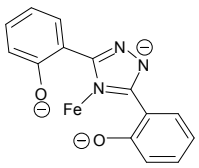
Sample Name	BZ	TZ	MA	DA	TA	Ascorbic Acid
IC50	21.54	21.58	22.22	23.06	24.89	**16.48**

Table 2. IC_{50} of triazole compounds using **DPPH**

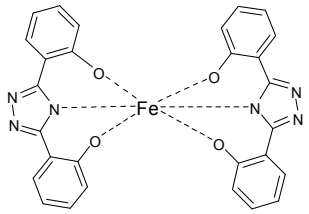
Metal chelating activity [viii]

Metal chelating activities of modified deferasirox (3, 5, 9, 10 and 11) was established by using Dinis et al. Ferrozine can quantitatively chelate with Fe^{2+} and form a complex with a purple color. This reaction is limited in the presence of other chelating agents and results in a decrease of the purple color of the ferrozine-Fe^{2+} complexes. Measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions. The chelation of ferrous ions is estimated using the method of Dinis et al. (1994). (1 ml) of the various concentrations of compounds is added to a solution of (1 ml) ferrous chloride (0.2 mM). The reaction is initiated by the addition of 1ml of ferrozine (5 mM) and incubated at room temperature for 10 min. and then the absorbance is measured at 562 nm. Na_2-EDTA or was used as a positive control. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as [(Ao -As)/ As]*100, were Ao was the absorbance of the control, and As was the absorbance of the extract/ standard. Na_2-EDTA or was used as a positive control.

The highest level of iron chelation activity of (BZ) 97.93 % and Deferasirox 97.80 % was found at (5000 µg/ml), followed by (TZ), 96.81 %; (MA), 96.38 %; (DA), 95.99 %; (TA), 94.81%. The metal chelation activities have been listed as order of decreasing activity BZ > TZ > Deferasirox >MA >DA> TA. The results were summarized in **table 3** and graphically represented in **Figure 4**. The IC_{50} value were Summarized in **table 4** and graphically represented in **Figure 6**. The iron chelation activity of Deferasirox (standard) is graphically represented in **figure 5**. The tridentate ligand one molecule chelate with iron. The hexadentate ligand two molecules coordinate with one metal. The general iron chelation activity of the compound represented below structure [x].



Iron chelation Activity of Tridentate ligand complex with iron.



Iron chelation Activity of hexadentate ligand complex with iron[xiv]

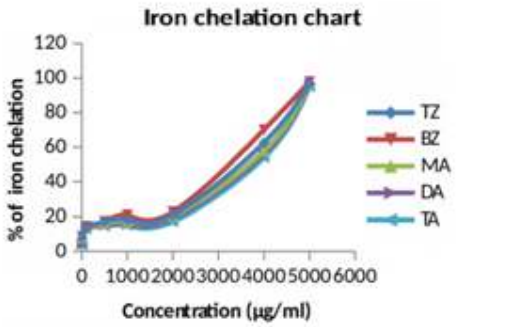


Fig 4. Iron chelation activity of triazole compounds

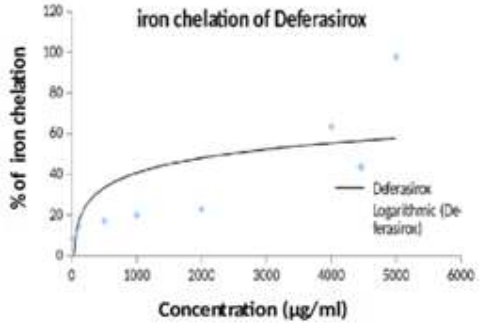


Fig 5. Iron chelation activity of Deferasirox

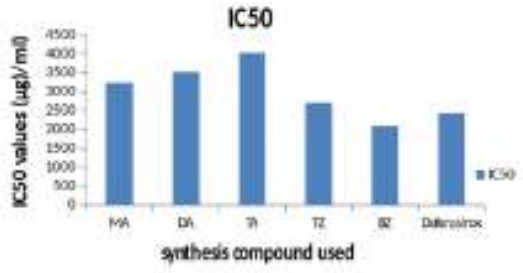


Fig 6. IC_{50} values of triazole synthesis compounds.

Sample name	MA	DA	TA	TZ	BZ	Deferasirox
IC50	3227.18	3508.04	4033.26	2697.28	2093.91	2424.86

Table 4. IC_{50} values of triazole synthesis compounds

Conc. (µg/ml)	Deferasirox	TZ	BZ	MA	DA	TA
10	8.464628±0.37	8.001236±0.14	8.576381±0.89	7.568736±0.14	4.016064±0.38	3.555048±0.46
100	14.51962±0.61	13.43837±0.09	14.48872±0.19	13.16033±0.18	12.66605±0.23	12.86228±0.14
500	17.02193±0.29	17.14551±0.24	17.26908±0.14	15.94069±0.64	15.0319±0.18	14.42694±0.32
1000	19.89496±0.86	18.13407±0.29	20.84705±0.38	17.05283±0.18	15.56997±0.09	14.98301±0.14
2000	22.89157±0.37	20.91443±0.29	22.67532±0.28	20.23479±0.14	18.99907±0.18	17.08372±0.23
4000	63.45382±0.28	62.58882±0.28	70.06487±0.18	57.76954±0.23	56.10133±0.35	53.93883±0.18
5000	97.80661±0.41	96.81804±0.23	97.93018±0.14	96.38554±0.18	95.99011±0.03	94.87179±0.43

Table 3. Metal chelation activity of triazole compounds
Values are expressed as mean ± SD of triplicates

Antibacterial activity

All the Deferasirox series synthetic compounds were screened for their antibacterial activity by disc diffusion technique. Compounds are screened *in vitro* for their anti-microbial activity against *E. coli, S. aureus, P. aeruginosa* and *B. subtilis* are compared with standard drug Streptomycin (10µg). The zones of inhibition formed for the compounds against organisms were calculated.

Disc-diffusion assay

The antibacterial activities of all test compounds were carried out by disc diffusion method. The concentrations of the test compounds 1000 µg, 2000 µg and standard drug Streptomycin 10 µg / disc. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain.

Disc made of Whatman No.1, diameter 6 mm was presterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug Streptomycin (10 µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial activity. All the synthesis compounds were evaluated for their Antimicrobial activity using Disc. Diffusion assay method were tested against two gram positive and two gram negative bacterial strains namely S. *aureus* , *B. subtilis and E. coli, P. aeruginosa.* The results were summarized in **table 5** and photo copy of represented in **Figure 7, 8, 9 & 10**.

COMPOUNDS	Zone of Inhibition (mm)							
	S. aereus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
	1000µg	2000 µg	1000µg	2000 µg	1000µg	2000 µg	1000µg	2000 µg
MA	14	19	14	17	14	17	14	18
DA	16	20	15	19	15	19	16	20
TA	13	18	13	16	12	17	13	17
TZ	-	-	-	-	-	-	-	-
Streptomycin (10 µg)	24		24		23		22	

Table 5. Antibacterial activities of MA, DA, TA and TZ compounds.

The Antibacterial activates have been listed as order decreasing activity. Two gram positive and two gram negative bacterial strains namely S. aureus, B. subtilis and E. coli, P. aeruginosa. DA > MA > TA compared with standard streptomycin. The TZ compound there is no anti bacterial activity observed.

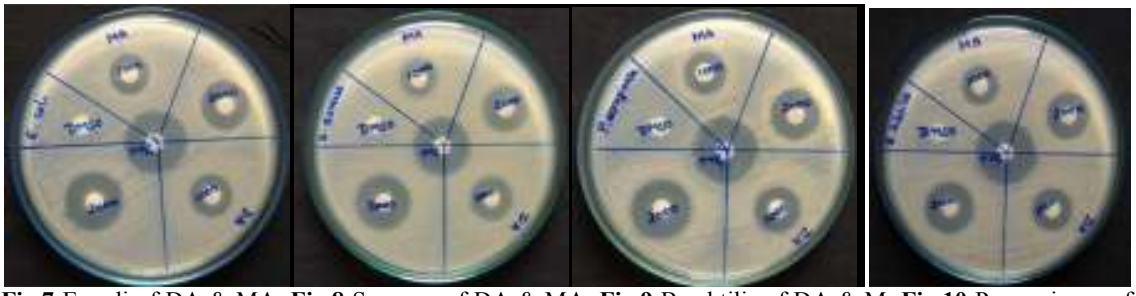


Fig.7 E. coli of DA & MA, **Fig.8** S.aureus of DA & MA, **Fig.9** B subtilis of DA & M, **Fig.10** P.aeruginosa of DA & MA

LIST OF ABBREVIATIONS

DMF: N, N-Dimethyl formamide, TBAB: Tetra butyl ammonium bromide, TEA: Triethylamine, TBDMSCl: Tert-butyl dimethylsilyl chloride, DCM: Dichloro methane, TFA: Trifuloroacetic acid. **BZ:** 2-hydroxy-N-(2-hydroxy benzoyl) benzamide (**3**)**; TZ:** 2,2'-(1*H*-1,2,4-triazole-3,5-diyl)diphenol) (**5**)**; MA:** 2-(3,5-bis(2hydroxyPhenyl)-1*H*-1,2,4-triazol-1-yl)aceticacid (**9**)**; DA:** 2-(3-(2(carboxymethoxy)Phenyl)-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-1yl)aceticacid (**10**)**; TA:** 2,2'-(2,2'-(1-carboxymethyl)-1H-12,4-triazole-3,5-diyl)bis (2,1-phenylene)bis(oxy)diacetic acid (**11**)**.**

CONCULSION

The synthesis of deferasirox series compounds having higher metal chelation, antioxidant activity and antibacterial activity. The highest level of iron chelation activity of (BZ) 97.93 % and Deferasirox 97.80 % was found at (5000 µg / ml), followed by (TZ) 96.81 %, (MA) 96.38 %, (DA) 95.99 %, (TA) 94.81 %. The metal chelation activities have been listed as order of decreasing activity BZ > TZ > Deferasirox > MA > DA > TA.

The highest level of DPPH radical scavenging activity of (BZ) 96.62% and ascorbic acid 96.59% was found at (200 µg / ml), followed by (TZ) 96.42 %, (MA) 95.70 % , (DA) 94.25 %, (TA) 92.59 %. The antioxidant activities have been listed as order of decreasing activity BZ > TZ > MA > DA > TA. The antibacterial activates have been listed as order decreasing activity. Two gram positive and two gram negative bacterial strains namely S. *aureus*, *B.* subtilis and E. coli, P. aeruginosa. DA > MA > TA. The TZ compound there is no anti bacterial activity observed.

MATERIALS AND METHOD

All the reagents purchased were purchased from Merck and Aldrich and used without further purification. The NMR spectra were recorded on a Bruker Advance DPX 400 MHz instrument. The spectra were measured in DMSO-D_6 relative to TMS (0.00 ppm). Elemental analysis was performed on a Elementar Vario EL III. TLC was performed on silica gel Polygram SIL G/UV 254 plates. The FTIR spectra were recorded on a Thermo Nicolet, Avatar 370. UV-Vis spectra were recorded on a Varian, Cary 5000.

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CONFLICT OF INTEREST
The authors confirm that this article content has no conflicts of interest.

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