



Synthesis, Biological Evaluation and Study of the Effect of Various N-Substituents on the Thermal Stabilities of Some New Diethanolamine Derivatives

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Diethanolamine and its derivatives are of considerable interest in medicinal and other industrial products. A series of new diethanolamine derivatives has been synthesized and characterized by ¹H NMR, ¹³C NMR, FTIR and mass spectrometry. All synthesized compounds have been tested for their *in vitro* antibacterial activity against pathogenic microorganisms *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Pasteurella mutocida*, *Rhizopus oryzae* and *Salmonella typhi*. Among the tested compounds, compounds **2a**, **2b**, **2c**, **3a** and **4b** have been found to be most potent members, which inhibited most of the pathogens used in the assay. Diethanolamine must possess tosyl or trifluoro groups in order to have good antimicrobial activities. All synthesized derivatives exhibited average FRAP activity and considerably good metal chelating activity and compound **2c** showed excellent ABTS radical scavenging among all tested derivatives. Thermal stability and the effect of various N-protected groups on the thermal stability and degradation of selected diethanolamine derivatives (free flowing oils at room temperature and solid derivative **2a**) has been studied by TGA and DSC analysis.

Key Words: Diethanolamine, Antioxidant activity, Antibacterial, DSC, TGA.

INTRODUCTION

Diethanolamine and its derivatives have wide applications in pharmaceuticals, surfactants formation, polishers and cosmetics industries. Diethanolamine has been used as intermediate in the rubber chemicals industry and as an emulsifier and dispersing agent in various agricultural products^{1,2} and also used in adhesives, cements, coatings, electroplating, printing inks, paints, papers, petroleum, coal and polymer productions, textile finishing and lubricants³. The use of lubricants is crucial and plays an important role in our daily lives. The search for new lubricants particularly liquid and stable at high temperature is highly desired. TGA/DSC have successfully been utilized for determination of physical properties and study of chemical reactions⁴⁻⁸ and for investigation of thermal stabilities of compounds^{9,10}. Diethanolamine derivatives have previously been in

high concern due to their fuel lubricity view point. However there are limited TGA and DSC analysis performed for diethanolamine and its derivatives to predict their thermal stabilities. They have essential contribution to maintain required lubricating properties¹¹.

The increasing resistance of pathogens to current antimicrobial agents is a dilemma and fungal infections are also serious issue of concern¹². Antioxidants are widely used and have been investigated for direct and indirect prevention of diseases such as cancer, diabetes mellitus, coronary heart disease and even altitude sickness. Antioxidants have also been used in food industry to prevent deterioration, nutritional losses and as flavoring agents in various foods¹³. Diethanolamine and its derivatives constitute an important class of heteroatom compounds as they have attracted significant interest in medicinal chemistry. Diethanolamine derivatives possess vibrant

spectrum of biological activities including antibacterial¹⁴, anticancer¹⁵, local anesthetic¹⁶, antiplatelet aggregation¹⁷ and antioxidant properties¹⁸ antituberculosis¹⁹. Such promising biological potentials of diethanolamine and its derivatives have prompted us to devise, synthesize and biologically evaluate a new series of compounds with potent antimicrobial and antioxidant potential and also to test their thermal properties.

EXPERIMENTAL

All the solvents were used as obtained, except THF and Et₂O were dried over sodium in benzophenone. TLC was performed on silica coated aluminium plates (6F₂₅₄, 0.2 mm). IR spectra were recorded on a JASCO A-302 IR spectrophotometer. ¹H and ¹³C NMR spectra were recorded on Bruker NMR 500 MHz and chemical shifts were calculated with reference to CDCl₃ (7.25). Mass spectra were recorded on a Varian MAT 312 double focusing spectrometer, connected to an IBM-AT compatible PC computer system. Standard antioxidants such as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), iron(II) sulphate, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), ferrozine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Fluka (UK). HPLC grade ethanol was purchased from Rathburn Chemicals Ltd. (Walkerburn, Peebleshire, Scotland). Spectrophotometric measurements were made on UV-1700 PharmaSpec. UV-visible spectrophotometer, Shimadzu, Japan equipped with temperature control device. All the solutions were made in triplicate and experiments were performed three times. The results obtained were averaged. Thermogravimetric analysis (TGA) and differential scanning microscopy (DSC) analyses of all derivatives were carried out from 15-450 °C at a heating rate of 20 °C min⁻¹ in nitrogen atmosphere using SDT Q-600. Dried alumina pan was used as a reference material and alumina sample holder (90 µL) was employed for taking curves.

N,N-Bis(2-hydroxyethyl)-4-methylbenzenesulfonamide (2a): A solution of tosyl chloride (7.56 g, 0.03 mmol) in CH₂Cl₂ (20 mL) was added slowly to a stirred solution of diethanolamine (5 g, 0.04 mmol) in triethylamine (12.1 g, 0.12 mmol) at room temperature. The reaction mixture was further stirred at room temperature for 4 h. After completion of reaction, water (15 mL) was added in reaction mixture and extracted with CH₂Cl₂ (3 × 15 mL). Organic layers were combined, dried over anhydrous magnesium sulphate and filtered. The filtrate was concentrated under vacuum which afforded the titled **2a** as white crystalline solid (90 %); m.p. 92-94 °C; R_f, 0.13 (1:1; *n*-hexane and ethyl acetate); λ_{max} (neat, ν_{max}, cm⁻¹): 3523-3241 (O-H), 3030-2925 (C-H, ar. and aliph.), 1595 (C-C Ar.), 1162 (S=O), 1087 (C-O), 714 (ar. C-S); ¹H NMR (500 MHz, CDCl₃), 7.70 (2H, d, *J* = 8.2, Ar-H), 7.33 (2H, d, *J* = 8.2, Ar-H), 3.86 (4H, t, *J* = 4.8, CH₂OH), 3.26 (4H, t, *J* = 4.8, CH₂-N), 2.43 (3H, s, CH₃-Ar); ¹³C NMR (500 MHz; CDCl₃) 143.7 (C), 135.2 (C), 129.83 (CH), 127.2 (CH), 62.2 (CH₂O), 52.94 (CH₂), 52.86 (CH₂), 21.48 (CH₃).

2,2,2-Trifluoro-N,N-bis(2-hydroxyethyl)acetamide (2b): Trifluoroacetic anhydride (4.39 g, 20.94 mmol) was added drop wise to a stirred solution of diethanolamine (2 g, 19.04 mmol) in THF (10 mL). The reaction mixture was further stirred at room temperature for 6 h. The solvent was evaporated

under vacuum, then water (15 mL) was added to residue and extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried over anhydrous magnesium sulphate and filtered. After evaporation of ethyl acetate under vacuum, **2b** was obtained as colourless liquid (58 %); R_f 0.11 (1:1; *n*-hexane and ethyl acetate); ¹H NMR (500 MHz, CDCl₃); 3.85 (4H, br s, CH₂-O), 3.19 (4H, br s, CH₂-N); ¹³C NMR (500 MHz; CDCl₃) 157.4 (C), 115.8 (C), 49.7 (CH₂), 49.5 (CH₂), 59.4 (CH₂), 59.0 (CH₂).

Phenyl bis(2-hydroxyethyl)carbamate (2c): Diethanolamine (1.0 g, 9.52 mmol) was added to a stirred solution of NaHCO₃ (0.79 g, 10.47 mmol) in water (10 mL). The solution was further stirred at room temperature for 15 min. Then phenoxylchloroformate (1.48 g, 9.52 mmol) was added drop wise. The reaction mixture was further stirred for 5 h at room temperature. The reaction mixture was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried over anhydrous magnesium sulphate and filtered. On evaporating solvent, **2c** was obtained as colourless liquid (51 %); R_f 0.16 (1:1; *n*-hexane and ethyl acetate); ¹H NMR (500 MHz, CDCl₃) 7.08-7.36 (5H, m, ArH), 3.81 (4H, t, *J* = 4.5, CH₂O), 3.58 (4H, t, *J* = 4.5, CH₂N); ¹³C NMR (500 MHz; CDCl₃) 155.0 (C), 150.9 (C), 129.3 (CH) 125.4 (CH), 121.0 (CH), 60.8 (CH₂), 60.3 (CH₂), 52.2 (CH₂), 52.1 (CH₂).

N,N-Bis(2-hydroxyethyl)acetamide (2d): Acetic anhydride (1.06 g, 10.47 mmol) was added to a stirred solution of diethanolamine (1.0 g, 9.52 mmol) in water (10 mL). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was extracted with ethyl acetate (3 × 30 mL). The organic layers were combined, dried over anhydrous magnesium sulphate and filtered. On evaporating solvent, **2d** was obtained as yellowish liquid (47 %); ¹H NMR (500 MHz, CDCl₃); 4.23 (2H, t, *J* = 6.0, CH₂-O), 4.21 (2H, t, *J* = 6.0, CH₂-O), 3.62 (2H, t, *J* = 2.0, CH₂-N), 3.61 (2H, t, *J* = 4.5, CH₂-N), 2.15 (3H, s, CH₃).

2,2'-(Tosylazanediy)bis(ethane-2,1-diy)bis(4-methylbenzenesulfonate) (3a): Sodium hydride (154 mg, 3.85 mmol) was added to a stirred solution of N,N-bis(2-hydroxyethyl)-4-methylbenzene sulfonamide (**2a**) and tosyl chloride (0.6 g, 3.24 mmol, 2.11 equiv.) in anhydrous CH₂Cl₂ (10 mL). The reaction mixture was stirred at room temperature for 3 h. Then reaction mixture was quenched with water (15 mL) and extracted with CH₂Cl₂ (3 × 15 mL). The organic layers were combined, dried over anhydrous MgSO₄ and concentrated under vacuum, **3a** was obtained as white solid (65 %); R_f 0.20 (7:3; *n*-hexane and ethyl acetate); m.p. 81-82 °C; (neat, ν_{max}, cm⁻¹): 3030-2923 (C-H, ar. and aliph.), 1595 (C-C Ar.), 1361 (S-O), 1162 (S=O), 1095 (C-O), 720-815 (ar. C-S); ¹H NMR (500 MHz, CDCl₃) 7.26-7.76 (12H, m, ArH), 4.11 (4H, t, *J* = 6.0, CH₂-O), 3.37 (4H, t, *J* = 6.0, CH₂-N), 2.44 (9H, s, CH₃-Ar); ¹³C NMR (500 MHz; CDCl₃) 144.9 (C), 144.8 (C), 143.8 (C), 135.0 (C), 132.1 (CH), 132.1 (CH), 129.7 (CH), 129.6 (CH), 129.4 (CH), 127.6 (CH), 126.9 (CH), 65.9 (CH₂), 65.8 (CH₂), 48.1 (CH₂), 48.1 (CH₂), 21.3 (CH₃), 21.2 (CH₃), 21.1 (CH₃).

2-(N-(2-Hydroxyethyl)-4-methylphenylsulfonamido)-ethyl 2-nitrobenzenesulfonate (3b): N-(2-Hydroxyethyl)-N-(hydroxymethyl)-4-methylbenzene sulphonamide (**2a**) (100 mg, 0.386 mmol) was dissolved into dichloromethane (4 mL), then

aqueous solution of sodium hydroxide (8 mL, 10 M) and 2-nitrobenzene sulfonylchloride (25 mg, 0.11 mmol) were added to the reaction mixture. Then reaction mixture was refluxed for 12 h, cooled to room temperature, quenched with water (10 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were passed over the anhydrous magnesium sulphate, filtered and concentrated in vacuum to afford the 2-(N-(2-hydroxyethyl)-4-methylphenylsulfonamido) ethyl 2-nitrobenzene sulfonate as white solid (86.5 %), was washed with *n*-hexane to remove the excess benzene sulfonyl chloride; R_f 0.37 (1:1; *n*-hexane and ethyl acetate); m.p. 91-93 °C; (neat, ν_{\max} , cm^{-1}): 2852-3400 (C-H, ar. and aliph.), 1595.6 (C-C Ar.), 1365 (S-O), 1162 (S=O), 1087 (C-O), 731-795 (ar. C-S); $^1\text{H NMR}$ (500 MHz, CDCl_3) 7.30-8.15 (8H, m, ArH), 4.40 (2H, t, $J = 6.0$, $\text{CH}_2\text{-O}$), 3.74 (2H, t, $J = 4.5$, $\text{CH}_2\text{-O}$), 3.5 (2H, t, $J = 6.0$, $\text{CH}_2\text{-N}$), 2.98 (2H, t, $J = 4.5$, $\text{CH}_2\text{-N}$), 2.43 (3H, s, CH_3); MS m/z (EI) 444; HRMS calcd. (%) for $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_8\text{S}_2$ (M^+) 444.0661, obs. 444.0556.

2,2'-(Tosylazanediyl)bis(ethane-2,1-diyl) dibenzene-sulfinate (3c): N-(2-Hydroxyethyl)-N-(hydroxymethyl)-4-methylbenzene sulphonamide (2a) (200 mg, 0.77 mmol), was dissolved in dichloromethane (5 mL). Then benzene sulphonyl chloride (0.33 mL, 1.62 mmol) and aqueous solution of sodium hydroxide (4 mL, 10 M) was added and the reaction mixture was refluxed for 7 h. Then reaction was quenched with water (20 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic layers were passed over anhydrous magnesium sulphate, filtered and concentrated in vacuum to afford 2,2'-(tosylazanediyl)bis(ethane-2,1-diyl) dibenzene sulfinate (3c) as white solid (96 %); R_f 0.65 (1:1; *n*-hexane and ethyl acetate); m.p. 107-108 °C; (neat, ν_{\max} , cm^{-1}): 3411-2924 (C-H), 1596 (C-C), 1358 (S-O), 1169 (S=O), 1095 (C-O), 730-810 (C-S); $^1\text{H NMR}$ (500 MHz, CDCl_3) 7.56-7.89 (12H, m, ArH), 4.14 (4H, t, $J = 6.0$, $\text{CH}_2\text{-O}$), 3.38 (4H, t, $J = 6$, $\text{CH}_2\text{-N}$), 2.43 (3H, s, CH_3); MS m/z (EI) 539; HRMS calcd. (%) for $\text{C}_{23}\text{H}_{25}\text{NO}_8\text{S}_3$ (M^+) 539.0742, obs. 539.0734.

2,2'-(2,2,2-Trifluoroacetylazanediyl)bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (3d): Tosyl chloride (2.0 g, 10.9 mmol) was added slowly to a stirred solution of 2,2,2-trifluoro-N,N-bis(2-hydroxyethyl)acetamide (2b) (1.0 g, 4.97 mmol) in CH_2Cl_2 (15 mL). Then aqueous NaOH solution (10 mL, 10 M) was added and refluxed for 5 h. Reaction mixture was quenched with water (25 mL) and extracted with dichloromethane (3 × 20 mL). The organic layers were combined, dried over anhydrous magnesium sulphate, filtered and concentrated in vacuum to afford 3d as white solid (60 %); R_f 0.21 (7:3; *n*-hexane and ethyl acetate); m.p. 47-49 °C; (neat, ν_{\max} , cm^{-1}): 2927-3400 (C-H), 1767 (C=O), 1598 (C-C), 1357 (S-O), 1167 (S=O), 1095 (C-O), 734-815 (C-S); $^1\text{H NMR}$ (500 MHz, CDCl_3) 7.30-7.77 (8H, m, ArH) 4.11 (4H, t, $J = 6$, CH_2O), 3.37 (4H, t, $J = 6$, $\text{CH}_2\text{-N}$), 2.46 (3H, s, CH_3), 2.43 (3H, s, CH_3); MS m/z (EI) 509; HRMS calcd. (%) for $\text{C}_{23}\text{H}_{25}\text{NO}_8\text{S}_3$ (M^+) 509.0790, obs. 539.0779.

2,2'-(Acetylazanediyl)bis(ethane-2,1-diyl)bis(4-methylbenzene sulfonate) (3e): Tosyl chloride (3.23 g, 17 mmol) was added slowly to a stirred solution of N,N-bis(2-hydroxyethyl)acetamide (2d) (1.0 g, 6.8 mmol) in CH_2Cl_2 (10 mL). Then aqueous solution of sodium hydroxide (10 mL,

10 M) was added and refluxed for 5 h. Reaction mixture was quenched with water (20 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and concentrated in vacuum to afford 3e as white solid (53 %); R_f 0.20 (7:3; *n*-hexane and ethyl acetate); m.p. 61-63 °C; (neat, ν_{\max} , cm^{-1}): 3428-2926 (C-H), 1770 (C=O), 1597 (C-C), 1353 (S-O), 1176 (S=O), 1094 (C-O), 723-814 (C-S); $^1\text{H NMR}$ (500 MHz, CDCl_3) 7.31-7.76 (8H, m, ArH), 4.11 (4H, t, $J = 5.5$, $\text{CH}_2\text{-O}$), 3.37 (4H, t, $J = 5.5$, $\text{CH}_2\text{-N}$), 2.46 (3H, s, CH_3), 2.43 (3H, s, CH_3); MS m/z (EI) 455; HRMS calcd. (%) for $\text{C}_{20}\text{H}_{25}\text{NO}_7\text{S}_2$ (M^+) 455.1072, obs. 455.1061.

2,2,2-Trifluoro-N,N-bis(2-(trifluoromethoxy)ethyl)acetamide (4b): 2,2,2-Trifluoro-N,N-bis(2-hydroxyethyl)acetamide (2b) (0.50 g, 2.49 mmol) was added to a stirred solution of trifluoroacetic acid (1.44 mL, 0.01 mmol) in THF (10 mL). The reaction mixture was stirred at room temperature for 6 h. After the completion of reaction solvent was evaporated on rotary evaporator, quenched with water and extracted with ethyl acetate (3 × 20 mL). The obtained organic layers were combined obtained, dried over anhydrous magnesium sulphate, concentrated in vacuum to get 2,2,2-trifluoro-N,N-bis(2-(trifluoromethoxy)ethyl)acetamide (4b) as clear yellowish liquid of fuming nature (90 %); R_f 0.58 (1:1; *n*-hexane and ethyl acetate); $^1\text{H NMR}$ (500 MHz, CDCl_3) 4.4 (4H, m 2 × $\text{CH}_2\text{-O}$), 3.65 (2H, t, $J = 5.5$, $\text{CH}_2\text{-N}$), 3.49 (2H, t, $J = 5.5$, $\text{CH}_2\text{-N}$); MS m/z (EI) 393; HRMS calcd. (%) for $\text{C}_{10}\text{H}_8\text{F}_9\text{NO}_5$ (M^+) 393.0259, obs. 393.0247.

N,N-Bis(2-azidoethyl)-4-methylbenzenesulfonamide (5a): 2,2'-(Tosylazanediyl)bis(ethane-2,1-diyl)bis(4-methylbenzenesulfonate) (3a) (2.0 g, 0.17 mmol) was dissolved in DMF (5 mL). Then sodium azide (24.07 mg, 0.37 mmol) was added in the solution and reaction mixture was heated at 80 °C for 2 h. After this reaction mixture was quenched with water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over anhydrous magnesium sulphate and concentrated under vacuum to afford N,N-bis(2-azidoethyl)-4-methylbenzenesulfonamide (5a) as white solid (82 %); R_f 0.52 (1:1; *n*-hexane and ethyl acetate); m.p. 75-77 °C; (neat, ν_{\max} , cm^{-1}): 3395-2924 (C-H), 2121 ($\text{N}\equiv\text{N}$), 1593 (C-C), 1160 (S=O), 707-812 (C-S); $^1\text{H NMR}$ (500 MHz, CDCl_3) 7.26-7.72 (4H, m, Ar-H), 3.5 (2H, t, $J = 6.0$, $\text{CH}_2\text{-N}$), 3.30 (4H, t, $J = 6.0$, $\text{CH}_2\text{-N}$), 2.44 (3H, s, $\text{CH}_3\text{-Ar}$); $^{13}\text{C NMR}$ (500 MHz; CDCl_3) 144 (C), 135.7 (C), 129.9 (CH), 127.2 (CH), 50.7 (CH_2), 48.9 (CH_2), 21.5(CH_3), 21.4 (CH_3).

2-(N-(2-Azidoethyl)acetamido)ethyl 4-methyl benzene sulfonate (5e): 2,2'-(Acetylazanediyl)bis(ethane-2,1-diyl)bis(4-methylbenzene sulfonate) (3e) (200 mg, 0.43 mmol) was dissolved in DMF (5 mL). Then sodium azide (62.45 mg, 0.96 mmol, 2.2 equiv.) was added to the solution and reaction mixture was heated at 80 °C for 2 h. After this reaction mixture was quenched with water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over anhydrous magnesium sulphate and concentrated under vacuum to afford N,N-bis(2-azidoethyl)acetamide (5e) as white solid (71 %); m.p. 117-120 °C; (neat, ν_{\max} , cm^{-1}): 3388-2915 (C-H), 2124 ($\text{N}\equiv\text{N}$), 1627 (C=O); $^1\text{H NMR}$ (500 MHz, CDCl_3)

7.64-7.39 (4H, m, ArH), 3.73(2H, t, $J = 5.0$, CH₂-O), 2.99 (2H, t, $J = 5.0$, CH₂-N), 2.44 (3H, s, CH₃), 1.256 (2H, t, $J = 5.0$, CH₂-N), 0.92 (2H, t, $J = 5.0$, CH₂-N=N); MS m/z (EI) 438; HRMS calcd. (%) for C₁₈H₂₂N₄O₅S₂ (M⁺) 438.1032, obs. 438.1025.

Antioxidant activity

ABTS assay: ABTS radical cation was produced by a reaction between ABTS and potassium persulphate (7 and 2.45 mM final concentrations, respectively) and before its use the mixture was allowed to stand in the dark at room temperature for 12-16 h. To study the antioxidant activity of samples, the ABTS stock solution was diluted with PBS buffer (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. After addition of 10 μ L of sample to 2.99 mL of diluted ABTS^{•+} solution ($A = 0.700 \pm 0.020$), the absorbance was measured at 30 °C with exactly 1 min intervals for 6 min. Appropriate solvent blanks were run in each assay for accurate readings. The percentage inhibition of absorbance was calculated by the following formula.

$$\text{Inhibition (\%)} = \left(\frac{1 - A}{A_0} \right) \times 100 \quad (1)$$

where, A_0 is the absorbance at 734 nm of the negative control and A is the absorbance at 734 nm of the mixture with sample.

Ferric reducing power (FRAP) assay: The reducing capacity of derivatives was measured according to the reported method²⁰. Fresh FRAP solution was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM tripyridyl triazine (TPTZ) solution in 40 mM HCl solution and 2.5 mL of 20 mM ferric chloride solution. The mixture was incubated at 37 °C throughout the monitoring period. 3000 μ L of FRAP reagent was mixed with 100 μ L of sample and 300 μ L of distilled water. Absorbance was measured at 593 nm after 6 min.

$$\text{Inhibition} = \frac{A_0}{0.025} \quad (2)$$

where, A_0 is the absorbance taken at 593 nm.

Metal chelating assay: For metal chelating activity we followed the method of Denis *et al.*²¹. Sample solution (10 mg/mL) was prepared. Then 100 μ L of sample solution was added to a solution of 50 μ L FeSO₄. The reaction was initiated by the addition of ferrozine (200 μ L). The total volume of mixture was adjusted to 3650 μ L with ethanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of absorbance was calculated by the following formula.

$$\text{Inhibition (\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (3)$$

where, A_0 is the absorbance of the solution without sample at 562 nm and A_1 is the absorbance in the presence of the sample. EDTA was used as a reference compound.

Antimicrobial activity

Test-microorganisms: Pathogenic microorganisms *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*,

Pseudomonas aeruginosa, *Bacillus subtilis*, *Pasteurella mutocida Rhizopus oryzae* and *Salmonella typhi* were used for *in vitro* antibacterial activity. These bacterial strains were obtained from Pakistan Council of Scientific and Industrial Research (PCSIR) Lahore.

Antibacterial assay: Agar well diffusion method was used to determine the antimicrobial activity²². The plates were autoclaved for 5 h. We used nutrient agar 28 g/L of distilled water. The media was poured in the petri dishes and allowed to solidify. The well was dug in the media using a sterile borer. The surface of the nutrient agar was inoculated by microbes using a sterile cotton swab. The samples were introduced into respective wells. For each treatment, three replicates were maintained. Dichloromethane and the reference antibacterial and antifungal drug served as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 24 h. The activity was determined by measuring the diameter of the inhibition zone in millimeter. Growth inhibition was calculated with reference to the positive control. Standard deviation was also calculated.

The percentage inhibition was calculated as follows;

$$\text{Inhibition (\%)} = \left(\frac{A}{A_0} \right) \times 100 \quad (4)$$

A = Area of inhibition of sample compound, A_0 = Area of inhibition of standard drug (positive control) $\times 100$.

RESULTS AND DISCUSSION

Diethanolamine was used as a precursor which contains two hydroxyl groups and an amine group. All these groups are susceptible to acylation, alkylation and sulfonamide formation. Under mild reaction conditions in first step we were able to selectively attach only one acyl or sulfonyl group to nitrogen to synthesize **2a-2d**, following acylation on two hydroxyl groups of compound **2b** gave **4b**. Derivatives **3a-3e** were prepared from **2a-2d** by reacting with different sulfonyl chlorides in the presence of excess NaOH and 1:1 mixture of dichloromethane and water. Compounds **3a** and **3e** yielded azides **5a** and **5e** by reacting with sodium azide in the presence of DMF. The reactions with azides were clean and completed in 2 h (**Scheme-I**). Derivatives **2b-2d** and **4b** were isolated as free flowing oils. We were able to easily synthesize 6 new compounds (**3b-3e**, **4b** and **5e**).

Thermogravimetric analysis (TGA): One of the aim of this study was to synthesize new potential lubricants from diethanolamine. We were successful to prepare four free flowing oils (**2b-2d**, **4b**) which could be used as lubricants. Thermal stabilities of compounds **2a-2d** and **4b** were studied by TGA at heating rate of 20 °C min⁻¹ under nitrogen atmosphere. For illustration, TGA curves of **2a-2d** and **4b** at heating rate of 20 °C min⁻¹ are shown in Fig. 1.

It was observed that different N-substituents of diethanolamine has great influence on the thermal stabilities of products. Compound **2a** showed better thermal stability as compared to other samples there was almost no weight loss until 200 °C. This is presumably due to the greater stability of tosyl group. It is expected that tosyl group cleaves from nitrogen at 200 °C and then gradual thermal decomposition started as shown in Fig. 1.

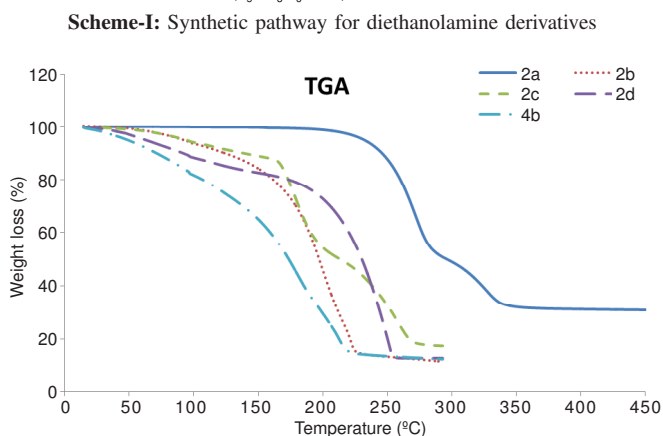
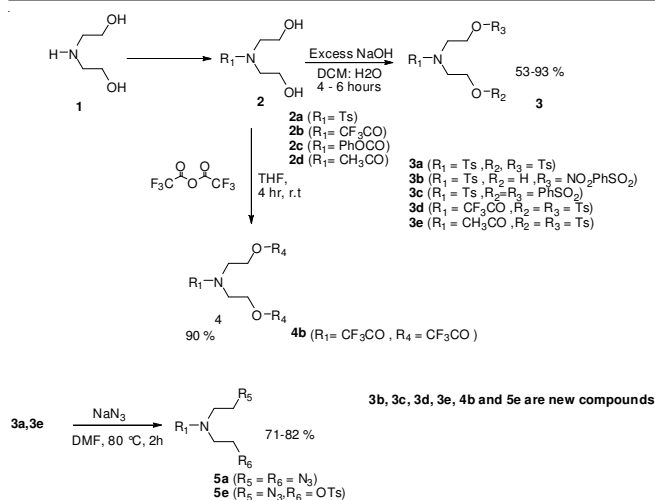


Fig. 1. Thermal decomposition as weight per cent loss (W %) and heat flow (mW) versus temperature (T/°C)

Thermal stability of **2b-2d** was almost similar in which initially presumably N substituent detaches, followed by the decomposition of diethanolamine as shown in Fig. 1. Final decomposition temperatures (225-300 °C) were high that could be due to loss of hydrogen bonding among hydroxyl groups of diethanolamine²³. The compound **4b** have no hydroxyl groups, therefore it was comparatively less stable among the derivatives studied. In case of **4b** significant thermal decomposition occurred at 40 °C and completed almost at 225 °C. For comparative studies, activation energies, enthalpies and entropies of derivatives **2a-2d** and **4b** were calculated (Table-1) using graphical data obtained from thermogravimetric analysis incorporated with curve-fitting software- the Battery program²⁴.

TABLE-1
ACTIVATION ENERGY, ENTHALPY, ENTROPY
AND ORDER OF REACTION OF **2a-2d** AND **4b**

Samples	Activation energy (J/mol)	Enthalpy (J/mol)	Entropy (J/mol.K)
2a	258727.88	257065.08	2691.64
2b	9238.56	7575.76	-193.25
2c	12898.57	11235.77	-145.07
2d	82219.33	80556.53	686.86
4b	4040.54	2377.74	-268.69

Statistical analysis: Using one way ANOVA system, the analysis of variance for thermal analysis was performed for

diethanolamine derivatives **2a-2d** and **4b** to observe the effect of temperature. It was observed by ANOVA results that except **4b** and **2a** there is insignificant difference among these derivatives. Compound **2a** showed comparatively greater stability among these derivatives. However in case of derivative **4b**, decomposition became significant in temperature range 15-200 °C.

Differential scanning calorimetry (DSC): The DSC thermograms of **2a-2d** and **4b** are compared in Fig. 2. All derivatives show first broad endothermic peak in their thermograms at 50-100 °C which could be due to their phase change.

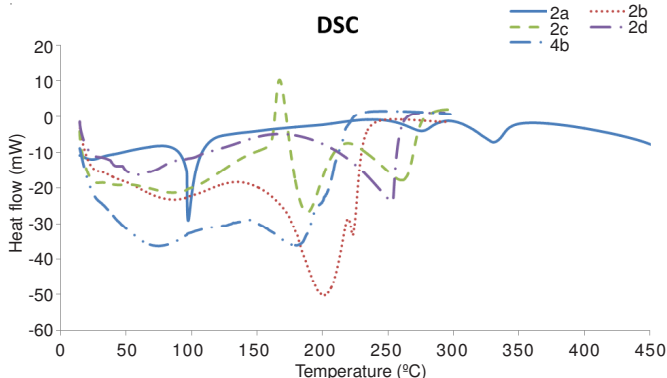


Fig. 2. DSC thermograms as heat flow (mW) versus temperature (°C)

The compound **2a** showed a glass transition at temperature range T_g (20-60 °C) and characteristic endothermic peak of melting at T_m (95 °C). There was no endothermic or exothermic transition in temperature range of 115-250 °C. This might be due to the heat absorbed by **2a** to increase its internal energy that leads to an endothermic peak of boiling at 270 °C followed by final glass transition of decomposition at 325 °C. The compounds **2b**, **2d** and **4b** showed their endothermic peak of boiling at 200, 175 and 70 °C respectively. The distinguished endothermic peak at 185 °C for compound **2c** could be due to the intermolecular cyclization and subsequent removal of phenoxy group as shown in Fig. 3. It is important to note there is no such intramolecular cyclization possibility present in other studied compounds.

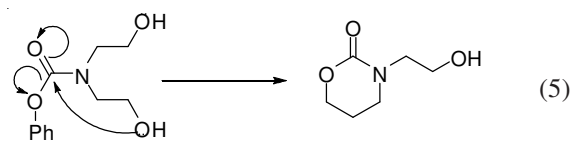


Fig. 3. Intermolecular cyclization of derivative **2c**

Heat capacity C_p of **2a-2d** and **4b** was calculated by following formula.

$$C_p = \frac{[E \times H \times 60]}{\{M \times H_r\}} \quad (6)$$

where C_p is heat capacity, E is cell calibration constant (dimensionless) at a temperature of interest, H is heat flow (mW), 60 is conversion constant for minute to seconds, M is mass of sample and H_r is heating rate (°C/min). The values of C_p of **2a-2d** and **4b** have been measured for temperatures from 50-298 °C (Fig. 4).

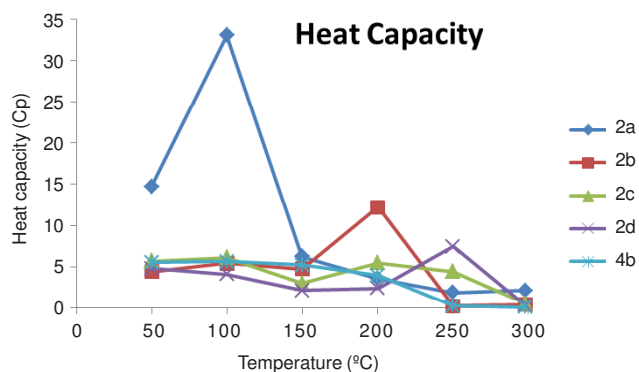


Fig. 4. Heat capacity of samples

Antioxidant activity

ABTS^{•+} radical cation decolorization assay: Antioxidant ability of synthesized compounds was studied in comparison with reference compound, Trolox²⁵.

Diethanolamine derivatives (**2a-5e**) showed better ABTS radical scavenging than reactant diethanolamine as shown in Fig. 5. These derivatives have TEAC value between 2.5-16 while precursor diethanolamine exhibited 2.3 TEAC value. By definition, Trolox has a TEAC of 1. As the TEAC of **2c** is 16, this shows that one molecule of **2c** scavenges on average 16 times as much ABTS as Trolox. Among all derivatives **2c** showed excellent antioxidant activity that may be due to phenolic moiety. Derivatives other than **2a** and **3b** showed average scavenging of ABTS radical cations while **2a** and **3b** showed comparatively poor scavenging.

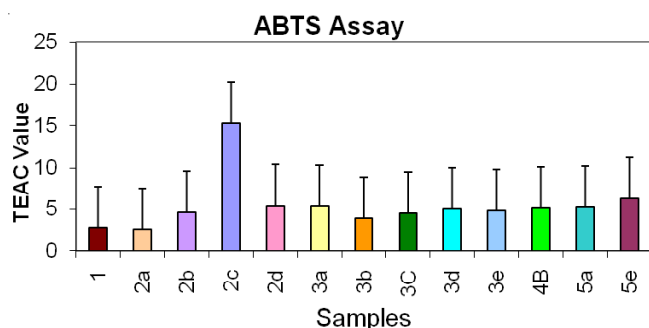


Fig. 5. ABTS radical scavenging

FRAP Assay: The FRAP assay was carried out following method developed by Benzie and Strain²⁰. Any antioxidant species with lower reduction potential than that of Fe(III) TPTZ salt (0.7 V) can reduce Fe³⁺-TPTZ to Fe²⁺ TPTZ. This reduction is monitored spectrophotometrically at 593 nm. If a sample contains reducing components intense blue colour is appeared. The original method of Benzie and Strain uses a 4 min interval but we noted that the reaction/colour change is in progress even after 4 min interval. Therefore absorbance was measured at a 6 min interval after addition of sample to TPTZ reagent allowing the reaction to reach a steady state. The FRAP values of the samples were measured in comparison with a calibration curve obtained using iron(II) sulphate as the standard reductant.

Diethanolamine and its derivatives showed FRAP values in the range of 4-6. As graphical data showed that **2a**, **2c**, **3c**,

4b and **5e** showed comparatively higher FRAP values than other derivatives, may be due to the presence of high electron density as delocalisation in substituents of the derivatives. While **2b**, **2d** and **3f** exhibited comparatively lower FRAP values that can be attributed to the low electron density or absence of aromaticity (Fig. 6).

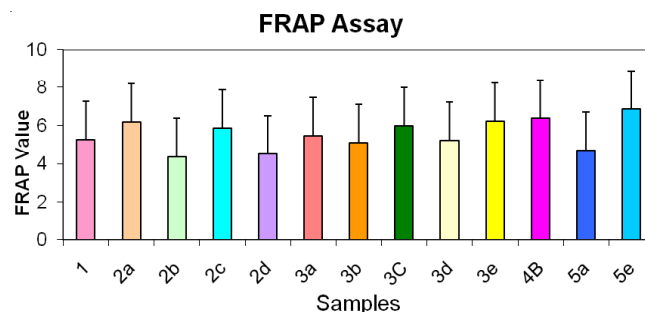


Fig. 6. FRAP result

Metal chelating activity: An important advantage of antioxidants is their ability to chelate/deactivate transition metals²⁶, which possess the ability to catalyze hydroperoxide decomposition and Fenton-type reactions. Chelating agents may also serve as secondary antioxidants since they reduce redox potential, thereby stabilizes the oxidized forms of metal species. Therefore, the ion(II) chelating capacities of the samples were screened (Fig. 7).

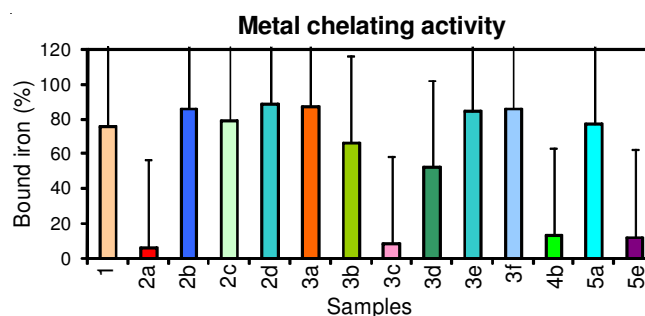


Fig. 7. Metal chelating activity

Ferrozine forms complexes with Fe²⁺. In the presence of chelating agents, complex formation between ferrozine and Fe²⁺ is disrupted, as a result red colour of the complex is reduced which allows estimation of the chelating activity of the co-existing chelator. Interestingly, all derivatives were found to have metal chelating activity.

As the reactive oxygen species (ROS) generation is associated with redox active metal catalysis which involves chelating of metal ion. Diethanolamine derivatives showed good result in metal chelating activity as compared to ABTS assay and FRAP assay. Diethanolamine derivatives considerably interfered the formation of ferrozine complex by capturing ferrous ions. All derivatives except **2a**, scavenged ferrous ions before its formation to ferrozine complex which may be due to the presence of OH, protected alcohols and protected amine groups.

Comparitively poor metal chelating activity of **2a** may be due to bulky group (mention group here in bracket) attached with nitrogen which hindered chelate formation with ferrous ions.

Conclusion

We have described herein synthesis, biological and thermal evaluation of a series of diethanolamine derivatives. Order of thermal stabilities of free flowing oils **2b-2d** and solid derivative **2a** was **2a** > **2c** > **2d** > **2b** > **4b**. Compounds **2a** and **4b** had significant difference in thermal decompositional behaviour while **2b-2d** exhibited insignificant difference. The activation energies of oils **2b-2d** increased as function of thermal stability of N-substitution at diethanolamine. All the compounds were tested for their *in vitro* antimicrobial and antioxidant activities. Regarding antioxidant activity all derivatives showed moderate-to-good FRAP and ABTS result and considerably excellent metal chelating activity and derivative **2c** having phenoxy group exhibited excellent ABTS radical scavenging among all derivatives. Studies showed that compounds **2a**, **2b**, **3a** and **4b**, having tosyl and trifluoro group as a substituent were found to be the most potent member against used microorganisms and pathogenic microbes as compared to others.

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