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Synthesis, and Biological Studies of New Azo–azomethine Compounds Based on Sulfathiazole and Sulfadiazine

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Abstract

Two new series of azo-azomethine compounds based on sulfathiazole and sulfadiazine were synthesized. Condensation of sulfathiazole diazonium salt and sulfadiazine diazonium salt with 3-ethoxy-4-hydroxy benzaldehyde. These new azo compounds were reacted with sulfathiazole, sulfadiazine,3-hydroxy aniline, 2-amino-4,6-dimethyl pyridine, and 4-bromoaniline respectively, to form the new azo-azomethine compounds. All compounds were characterized by CHN analysis, IR, 1H NMR, and mass spectroscopic data. Antibacterial activities were studied for these compounds *in vitro* and showed some promising activity against *Escherichia coli and Staphylococcus* aureus. The antifungal activity against *Candida albicans* and *Aspergillus niger* was studied and showed good activity. The antioxidant activity was evaluated by using antioxidant tests *via* the free radical scavenging (DPPH) method and showed good antioxidant activities.

Keywords: Antibacterial; antioxidant; azo- azomethine; sulfathiazole; sulfadiazine.

1. Introduction

Antibacterial sulfonamides, such as sulfathiazole, and sulfadiazine are one of the most common functionalities in medicinal chemistry as they are incorporated into the structures of molecules of therapeutic value [1, 2]. In addition to being the first synthetic antibacterial agent, sulfonamides have been established as successful, and selectively antimicrobial drugs for many treatments such as drastic anti-inflammatory, respiratory infections, actinobacillosis, and antiepileptic [3]. They are effective against species of chlamydia genus and fungi and protozoa [4,5]. The first azo-dye sulfonamide drug is "prontosil (Prontosil rubrum) which used for the human body. The prontosil is metabolized forming sulfanilamide which is one of the main important types of sulfa drugs [6]. Azo compounds are known for their therapeutic significance and are reported to show a variety of applications in biological activities including, antifungal, pesticidal, antitumor, and anticancer [7, 8, 9]. On the other hand, Schiff bases are considered as an important intermediate for the synthesis of several bioactive compounds such as β-Lactams [10, 11], and they are used in analytical, industrial, clinical, and pharmaceutical processes [12]. It is worth noting that sulfathiazole is still used in commercial products in combination with other antibacterial agents, for the treatment of some infections [13]. Furthermore, both azo and Schiff bases are remarkable structures in the pharmaceutical and medicinal fields. Recently, many research and studies encouraged the synthesis of azo-azomethine compounds which achieved much application especially in biological activities and pharmacological action. It has been suggested that both azo moiety and azomethine linkage might be responsible for the biological activities of these compounds [4].

In continuation of our previous [14], which described the synthesis, antimicrobial and antioxidant properties of some new sulfa drug containing an azo-azomethine group, we report herein the synthesize new azo–azomethine compounds based on sulfathiazole and sulfadiazine to determine the effects of these modifications on the biological activity and

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antioxidant properties.

2. Experimental

Materials and reagents

All Solvents used of analytical grade, supplied from Fluka, without any further purification. 4-hydroxy-3-ethoxy benzaldehyde (ethyl vanillin), sulfathiazole, and sulfadiazine were purchased from Sigma-Aldrich 2-hydroxyaniline, 2-amino-4,6-dimethyl pyridine, 4- bromoaniline, sodium carbonate, sodium nitrate, hydrochloric acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied from Fluka company.

Physical measurements

FT-IR spectra were recorded for KBr discs with an FT-IR-8400s Shimadzu instrument in the range of 4000-400 cm^{-1}. The ^1H NMR spectra were measured in DMSO-d_6 at ambient temperature using a Brucker 400 MHz spectrometer. The chemical shifts, δ, are given in ppm with respect to tetramethylsilane (TMS) as the internal standard. The coupling constants (J) are reported in Hz. The elemental analysis (CHN) was performed with microanalyzer instrument Elemental Euro vector EA-3000 A. Mass spectra were recorded on a Technologies 5975c spectrometer at 70 eV. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The follow up of the reactions and the check of the purity of the compounds were made by the TLC on silica gel-protected aluminum sheets

Synthesis

4-((3-Ethoxy-5-formyl-2-hydroxyphenyl) diazenyl-N-(thiazol-2-yl)benzene sulfonamide (**1**)

A solution of sodium nitrite (0.41 g; 6 mmol) in distilled water (25 ml) was added slowly to a cold solution of sulfathiazole (0.51g; 2 mmol) in 25 ml of 2N hydrochloric acid. The mixture was cooled to 0-5ºC. To the resulting clear solution, a cold solution of 4-hydroxy-3-ethoxy benzaldehyde (0.33 g; 2 mmol) in 15 ml of an aqueous solution containing (1.06 g; 10 mmol) of sodium carbonate was added dropwise with stirring over 30 min. The reaction mixture was stirred for 2 h at 0–5◦C. A dark orange solid was formed, collected, washed several times with distilled water, recrystallized from ethanol, and dried over P_2O_5. The progress of the reaction was monitored on TLC using acetone and CH_2Cl_2 (3:7 v/v) as eluent.

Dark orange crystals were obtained in 76% yield. R_f= 0.63, m.p. 250-251ºC.

Anal. Calcd. for $C_{18}H_{16}N_4O_5S_2$: C, 49.99; H, 3.73; N, 12.96. Found: C, 50.42; H, 3.53; N, 12.91%.

IR (KBr) cm^{-1}: 3553(OH), 3421(NH), 3101(CH-Ar.), 2975(CH_{aliph}),1674(C=O),1589(N=N),1531($C=N_{sulfa}$), 1288(C-O), 1337, 1138(SO2), 1288(C-N), 1080 (C-O), 929 (S-N).

^1H NMR (DMSO-d_6); δ/ppm: 11.46 (s, 1H, NH); 9.88 (s,1H, HCO); 8.52 (s, IH, Ar-OH); 8.03 (dd, 2H, Ar-H), 7.96 (s,1H,Ar-H), 7.83 (dd, 2H, Ar-H), 7.47 (s,1H,Ar-H), 7.18 (d, 1H, Ar-H); 6.86 (d, 1H, Ar-H), 4.20 (q, 2H, OCH_2); 1.41 (t, 3H, CH_3).

4-((3-Ethyoxy-5-formyl-2-hydroxyphenyl)diazenyl)-N-(pyrimidin-2-yl)benzene sulfonamide (**2**)

This compound 2 was prepared by the same above method using sulfadiazine (0.50 g; 2 mmol) and 4-hydroxy-3-ethoxy benzaldehyde (0.33 g; 2 mmol).

Brown crystals. Yield 65%, R_f = 0.71, m.p. 180-181ºC.

Anal. Calcd. for $C_{19}H_{17}N_5O_5S$: C, 53.39; H, 4.01; N, 16.38. Found: C, 53.80; H, 4.05; N, 16.43%.

IR (KBr) cm^{-1}: 3564 (OH), 3421 (NH), 3093 (CH-Ar), 2985 ($CH_{aliph.}$), 1662 (C=O), 1581 (N=N), 1519 ($C=N_{sulfa}$), 1342, 1157 (SO2), 1265 (C-N), 1087 (C-O), 941 (S-N).

^1H NMR (DMSO-d_6): δ/ppm: 10.87 (s, 1H, NH), 9.79 (s,1H,HCO),8.57 (s, 1H, Ar-OH), 8.47 (d, 1H, Ar-H) ,8.07 (dd, 2H, Ar-H), 7.98 (s, 1H, Ar-H),7.88 (dd, 2H, Ar-H), 7.41 (s, 1H, Ar-H), 6.99 (t, 1H, Ar-H), 4.12 (q, 2H, OCH_2), 1.39 (t, 3H, CH_3).

Synthesis of sulfathiazole Schiff bases (**3a**–**3e**).

Compounds (**3a**–**3e**) were prepared by the following general procedure.

Equimolar of compounds 1 (0.43g, 1 mmol) and 1 mmol of the corresponding amino derivatives (*i.e.* sulfathiazole, sulfadiazine, 3-hydroxyaniline, 2-amino-4,6-dimethylpyridine, and 4-bromoaniline) were dissolved in 25 ml absolute ethanol. The resulting solution was refluxed for 24 h. The reaction was monitored by TLC. After cooling, the solution was poured into crushed ice. The precipitate of each compound was collected by filtration, washed several times with methanol, and recrystallized from ethanol to yield the corresponding compound (59-75%). The purity of each compound was checked by TLC.

4-((E)-(3-Ethoxy-2-hydroxy-5-((z)-((4-(N-thiazol-2-yl)sulfamayl)phenyl)-imino)methyl)phenyl)-diazenyl)-N-(thiazol-2-yl)benzene sulfonamide (**3a**)

Reddish purple crystals. Yield: 63%, R_f = 0.71, m.p. 260-262°C.

Anal. Calcd. for $C_{27}H_{23}N_7O_6S_4$: C, 48.42: H, 3.46; N,14.64. Found: C, 84.42; H, 3.48; N, 14.71%.

IR (KBr) cm^{-1}: 3515 (OH), 3360 (NH), 3109 (C-H-Ar.), 2975 (CH_{aliph}) 1631 (CH=N), 1593 (N=N), 1531 ($C=N_{sulfa}$), 1300, 1138 (SO_2), 1287 (C-N), 1080 (C-O).

^{1}HNMR(DMSO-d_6): δ/ppm: 10.50 (s, 2H, NH), 8.87(s, 1H, CH=N), 8.34 (s, 1H, OH), 8.27 (dd, 2H, Ar-H), 7.95 (dd, 2H, Ar-H), 7.88 (s, 1H, Ar-H), 7.81 (dd, 2H, Ar-H), 7.50 (s, 1H, Ar-H), 7.44 (dd, 2H, Ar-H), 7.10 (dd, 2H, Ar-H), 6.56 (dd, 2H, Ar-H), 4.12 (q, 2H, OCH_2),1.36 (t, 3H, CH3).

4-*((E)–(3-ethoxy-2-hydroxy-5-((z))-((4-N-(pyrimidin-2-yl)sulfamoyl)phenyl)imino)methyl)-phenyl)diazenyl)-N(thiazol-2-yl)benzene sulfonamide* (**3b**)

Light brown crystals. Yield:68%, Rf= 0.69,m.p. 228-230°C.

Anal. Calcd. for $C_{28}H_{24}N_8O_6S_3$: Calcd: C, 50.59; H, 3.64; N, 16.86. Found: C, 50.99; H, 3.61, N, 16,80.

IR (KBr) cm^{-1}: 3568 (OH), 3360 (NH), 3105 (C-HAr.), 2935(CH_{aliph}), 1693 (CH=N), 1589 (N=N), 1496 (C=Nsulfa), 1323, 1138 (SO2), 1265(C-N),1087 (C-O), 941 (S-N).

^{1}H NMR (DMSO-d_6): δ /ppm: 11.25 (s,1H, NH), 10.56 (s, 1H, NH), 8.51 (s, 1H, CH=N), 8.49 (s, 1H, OH), 8.47 (dd, 2H, Ar-H), 7.95 (dd, 2H, Ar-H), 7.90 (dd, 2H, Ar-H), 7.74 (dd, 3H, Ar-H), 7.47(s, 1H, Ar-H), 7.28 (dd, 2H, Ar-H), 7.01 (d, 1H, Ar-H), 6.97 (t, 1H, Ar-H), 6.57 (d, 1H, Ar-H), 4.11 (q, 2H, OCH_2), 1.36 (t, 3H, CH_3).

4-((E)-(3-ethoxy-2-hydroxy-5-((Z)-((3-hydroxyphenyl)imino)methyl)phenyl)-diazenyl)-N-(thiazol-2-yl)benzene sulfonamide (**3c**)

Dark brown crystals. Yield: 59%, R_f = 0.69, m.p. 300-302°C.

Anal. Calcd. for $C_{24}H_{21}N_5O_5S_2$: C, 55.06; H, 4.04; N, 13.38. Found: C, 55.46; H, 4.01; N, 13.33.

IR (KBr) cm^{-1}: 3448 (OH), 3356 (NH), 3085 (C-H-Ar.), 2935(C-H_{alph}), 1624 (CH=N), 1600 (N=N), 1508 ($C=N_{sulfa}$), 1350, 1136 (SO2), 1273 (C-N), 1080(C-O), 920 (S-N).

^{1}H NMR (DMSO-d_6): δ /ppm: 11.24 (s, 1H, NH), 10.56 (s, 1H, OH), 8.82 (s, 1H, CH=N), 8.49 (s, 1H, OH), 8.32 (dd, 2H, Ar-H), 7.96 (dd, 2H, Ar-H), 7.62(s, 1H, Ar-H), 7.44 (s, 1H, Ar-H), 7.16 (t, 1H, Ar-H), 7.14 (d, 1H, Ar-H), 6.87 (d, 1H, Ar-H), 6.79 (d, 1H, Ar-H), 6.73 (d, 1H, Ar-H), 6.55(s, 1H, Ar-H), 4.12 (q, 2H, OCH_2), 1.40 (t, 3H, CH_3).

4-((E)-(5-((Z)-((4),6-dimethylpyridin-2-yl)imino)-methyl)-3-ethoxy-2-hydroxyphenyl)diazenyl)-N-(thiazol-2-yl)benzene sulfonamide (**3d)**

Light brown crystals were obtained in 75% yield. Rf = 0.63, m.p. 254-256°C.

Anal. Calcd. for $C_{25}H_{24}N_6O_4S_2$: C, 55.96; H, 4.51; N, 15.66. Found: C, 55.68; H, 4.49; N, 15.61.

IR (KBr) cm^{-1}: 3448 (OH), 3352 (NH), 3105 (CH-Ar.), 2924 (C-H_{aliph}), 1631 (CH=N), 1593 (N=N), 1500 ($C=N_{sulfa}$), 1350, 1145 (SO_2), 1265 (C-N), 1080(C-O), 910 (S-N).

^{1}H NMR (DMSO-d_6): δ /ppm: 10.85 (s, 1H, NH), 9.87 (s, 1H, HC=N), 9.13 (s, 1H, OH), 7.95 (dd, 2H, Ar-H), 7.91 (dd, 2H, Ar-H), 7.75 (s, 1H, Ar-H), 7.50 (s, 1H, Ar-H), 7.14 (d, 1H, Ar-H), 7.06 (s, 1H, Ar-H), 6.73 (s, 1H, Ar-H), 6.59 (d, 1H, Ar-H), 4.19 (q, 2H, OCH_2), 2.91 (s, 3H, CH_3), 2.73 (s, 3H, CH_3), 1.40 (t, 3H, CH_3).

EI-MS(m/z): 536(M+), 452 $[C_{22}H_{22}N_5O_4S]^+$, 300 $[C_{16}H_{20}N_4O_2]^+$, 387 $[C_{22}H_{21}N_5O_2]^+$, 285 $[C_{16}H_{19}N_3O_2]^+$, 185 $[C_6H_7N_3O_2S]^+$, 107 $[C_6H_5NO]^+$, 80 $[C_5H_6N]^+$.

4-((E)-(5-((Z))-((4-bromophenyl)imino)methyl)3-ethoxy-2-hydroxyphenyl)diazenyl-N-(thiazole-2-yl)benzene sulfonamide (**3e**)

Reddish purple Crystals. Yield: 71%, Rf = 0.53, m.p. 242-244°C.

Anal. Calcd. for $C_{24}H_{20}BrN_5O_4S_2$: C, 49.15; H, 3.44; N,11.94. Found: C, 49.62, H, 3.41; N, 11.94.

IR (KBr) cm^{-1}: 3444 (OH), 3344 (NH), 3105 (CH-Ar.), 2925(C-H_{aliph}), 1627 (CH=N), 1593 (N=N), 1492 (C=Nsulfa), 1300, 1141 (SO_2), 1269(C-N), 1076(C-O), 914 (S-N).

^{1}H NMR (DMSO-d_6); δ /ppm: 10.31 (s, 1H, NH), 9.80 (s, 1H, HC=N), 9.11 (s, 1H, OH), 8.0 (dd, 2H, Ar-H), 7.95 (dd, 2H, Ar-H), 7.83 (s, 1H, Ar-H), 7.50 (dd, 2H, Ar-H), 7.29 (s,1H, Ar-H), 7.12 (dd, 2H, Ar-H), 7.11 (d, 1H, Ar-H), 6.57 (d, 1H, Ar-H), 4.09 (q, 2H, OCH_2), 1.41 (t, 3H, CH_3).

EI-MS (m/z): 586 (M+), 505 $[C_{24}H_{19}N_5O_4S_2]^+$, 427 $[C_{24}H_{21}N_5O_5S]^+$,382$[C_{22}H_{16}N_5S]^+$, 373 $[C_{16}H_{15}N_5O_2S_2]^+$, 225 $[C_{13}H_{13}N_4]^+$, 121$[C_6H_6N_3]^+$, 91$[C_6H_5N]^+$.

*Synthesis of sulfadiazine Schiff bases (***4a**–**4d***).*

Compounds **4a**-**4d** were then prepared by reaction of compound 2 (0.42g,1mmol) with 1 mmol of appropriate substituted aniline (*i.e*., sulfathiazole,

sulfadiazine, 3-hydroxyaniline, and 4-bromoaniline) under the previous conditions described for the preparation of compounds **3a**-**3e**.

4-((E)–(3-ethoxy-2-hydroxy-5-((z)-((4-(thiazol)-2-yl)sulfamoyl)phenyl)imino)methyl)phenyl)-diazenyl)-N-(pyrimidin)-2-yl)benzene sulfonamide (**4a**)

Light brown crystals. Yield: 65%, Rf = 0.53, m.p. 218-220^{o}C.

Anal. Calcd. for $C_{28}H_{24}N_8O_6S_3$: C, 50.59; H, 3.64; N, 16.86. Found: C, 50.92; H, 3.61; N, 16.82.

IR(KBr) cm^{-1}: 3568 (OH), 3363 (NH), 3105 (C-H-Ar.), 2980 (CH_{aliph}), 1681(CH=N), 1581 (N=N), 1523 ($C=N_{sulfa}$), 1338, 1145 (SO_2), 1265 (C-N), 1084 (C-O).

^{1}H NMR (DMSO-d_6); δ /ppm: 12.03 (s, 1H, NH), 10.69 (s, 1H, NH), 9.89 (s, 1H, CH=N), 8.46 (s, 1H, OH), 8.43 (dd, 2H, Ar-H), 7.98 (dd, 2H, Ar-H), 7.93 (dd, 2H, Ar-H), 7.88 (s, 1H, Ar-H), 7.82 (dd, 2H, Ar-H), 7.42 (s, 1H, Ar-H), 7.29 (dd, 2H, Ar-H), 7.14 (d, 1H, Ar-H), 6.93 (t, 1H, Ar-H), 6.71 (d, 1H, Ar-H), 4.11 (q, 2H, OCH_2), 1.41(t, 3H, CH_3).

4-((E)-(3-ethoxy-2-hydroxy-5-((z)-((4-(N-pyrimidin-2-yl)sulfamayl)phenyl)imino)methyl)phenyl)-diazenyl)-N-(pyrimidin-2-yl)benzene sulfonamide (**4b**)

Brown crystals. Yield: 52%, Rf = 0.74, m.p. 198-200^{o}C.

Anal. Calcd. for $C_{29}H_{25}N_9O_6S_4$: C, 52.80; H, 3.82; N, 19.11. Found: C, 52.30; H, 3.84; N, 19.16.

IR (KBr) cm^{-1}: 3559 OH), 3348 (NH), 3198 (C-H-Ar.), 2928 (CH_{aliph}), 1631(CH=N), 1581 (N=N), 1523 ($C=N_{sulfa}$), 1346, 1153 (SO_2), 1249 (C-N), 1087 (C-O).

^{1}H NMR (DMSO-d_6): δ /ppm: 10.02(s, 1H, NH), 8.56 (s, 1H, CH=N), 8.48 (s, 1H, OH) , 8.45 (dd, 4H, Ar-H), 7.98 (dd, 2H, Ar-H), 7.92 (dd, 2H, Ar-H), 7.81 (s, 1H, Ar-H), 7.79 (dd, 2H, Ar-H), 7.42 (s, 1H, Ar-H), 7.32 (dd, 2H, Ar-H), 7.10 (dd, 2H, Ar-H), 6.56 (dd, 2H, Ar-H), 6.96 (m, 2H, Ar-H), 4.11 (q, 2H, OCH_2), 1.38 (t, 3H, CH_3).

4-((E)-(3-ethoxy-2-hydroxy-5-((Z)-((3-hydroxyphenyl)imino)methyl)phenyl)-diazenyl)-N-(pyrimidin-2-yl)benzene sulfonamide (**4c**)

Dark brown crystals. Yield: 61%, Rf = 0.56, m.p. 200-202^{o}C.

Anal. Calcd. for $C_{25}H_{22}N_6O_5S_2$: C, 57.91 ; H, 4.28; N, 16.21. Found: C, 58.32; H, 4.29; N, 16.26.

IR (KBr) cm^{-1}: 3445 (OH), 3356 (NH), 3105 CH-Ar.), 2962 (C-H_{aliPh}), 1681 (CH=N), 1581 (N=N), 1523 ($C=N_{sulfa}$), 1346, 1156 (SO_2), 1248 (C-N), 1087(C-O), 920(S-N).

^{1}HNMR (DMSO-d_6): δ /ppm: 10.22 (s, 1H, NH), 9.77(s, 1H, OH), 8.63 (s, 1H, CH=N), 8.53 (d, 2H, Ar-H), 8.50 (s, 1H, OH), 8.04 (dd, 2H, Ar-H), 8.00 (dd, 2H, Ar-H), 7.92(s, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 7.42 (m, 1H, Ar-H), 7.10 (m, 1H, Ar-H), 6.97 (d, 1H, Ar-H), 6.92 (d, 1H, Ar-H), 6.87 (s, 1H, Ar-H), 4.12 (q, 2H, OCH_2), 1.40 (t, 3H, CH_3).

EI-MS (m/z): 518(M^+), 476 $[C_{23}H_{18}N_6O_4S]^+$, 423 $[C_{21}H_{19}N_4O_4]^+$, 421 $[C_{19}H_{16}N_6O_4S]$+, 456 $[C_{23}H_{17}N_6O_3S]^+$,407$[C_{21}H_{19}N_4O_3S]^+$, 361$[C_{21}H_{19}N_3O_3]^+$, 346 $[C_{21}H_{19}N_3O_2]^+$.

4-((E)-(5-((Z))-((4-bromophenyl)imino)methyl)3-ethoxy-2-hydroxyphenyl)diazenyl-N-(pyrimidin-2-yl)benzene sulfonamide (**4d**)

Light brown Crystals. Yield: 69%, Rf = 0.69, m.p. 180-182^{o}C.

Anal. Calcd. for $C_{25}H_{21}BrN_6O_4S_2$: C, 51.64; H, 3.64; N, 14.45. Found: C, 51.99; H, 3.46; N, 14.49.

IR (KBr) cm-1: 3430 (OH), 3383 (NH), 3093 ($CH_{Ar.}$), 2975(CH_{aliph}), 1681(CH=N), 1581(N=N), 1519 ($C=N_{sulfa}$), 1342, 1157 (SO_2), 1265 (C-N), 1087 (C-O).

^{1}HNMR (DMSO-d_6); δ /ppm: 10.19 (s, 1H, NH), 8.59 (s, 1H, HC=N), 8.47 (s, 1H, OH) ,8.53 (dd, 2H, Ar-H), 8.0 (dd, 2H, Ar-H), 7.97 (dd, 2H, Ar-H),7.85 (s, 1H, Ar-H),7.55 (dd, 2H, Ar-H), 7.40 (s, 1H, Ar-H), 7.15 (dd, 2H, Ar-H), 6.96 (d, 1H, Ar-H), 4.11 (q, 2H, OCH_2) ,1.42 (t, 3H, CH_3).

EI-MS (m/z): 581(M+), 332$[C_{15}H_{14}BrN_2O_2]^+$, 262 $[C_{10}H_8N_5O_2S]^+$, 211 $[C_{13}H_{11}N_2O]^+$,240 $[C_{15}H_{15}NO_2]^+$, 184 $[C_6H_6N_3SO_2]^+$, 151 $[C_9H_{13}NO]^+$,92$[C_6H_6N]^+$.

Antimicrobial activity

All newly synthesized compounds (**1**, **2**, **3a**- **3e**, and **4a**- **4d**) were screened in vitro for antimicrobial activity. They tested against Gram-positive *staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATTC 25922). The antifungal activity of all new compounds was tested against fungal Species, *Aspergillus nigen* and *Candida albicane* by agar diffusion method [15], using DMSO as a solvent and a negative control disc. The method involves uncovering the zone of inhibition towards the dispersal of microorganisms an agar plate. All synthesized compounds and the drugs that used as reference were dissolved in DMSO to

prepare a solution of 25 mg/ml concentration. The sterile discs were dipped in these prepared solutions and dried carefully, then placed on nutrient agar plates spreader with different microorganisms. Bacterial plates were incubated at (37±2 ºC), while fungal plates were incubated at (25±2 ºC) for 24 to 48 h, the diameters of zones of inhibition were measured in millimeter.

Antioxidant Activity

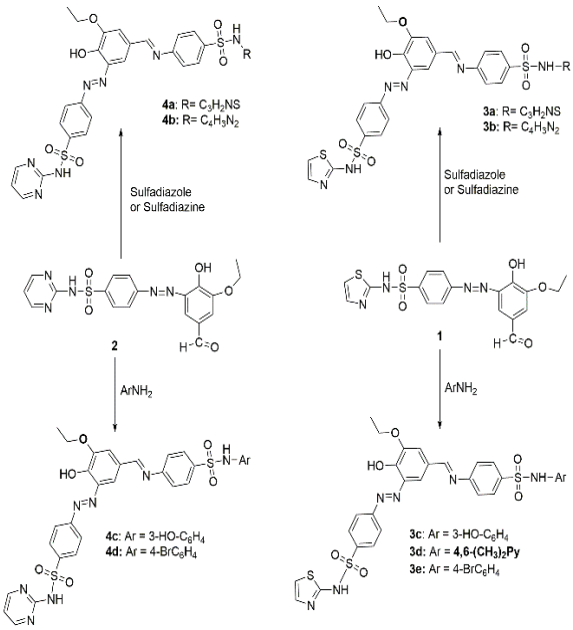
The radical scavenging activity of azo-azomethine compounds was determined by using DPPH free radical scavenging assay. 2,2-Diphenyl-1-picrylhydrazyl(DPPH) is a stable organic radical compound and its oxidative assay that used to detect the radical Scavengers capacity of samples. The activities of the sample were measured according to the method of Shoib Baba, and Malik [16]. A 200 µml of each compound of concentration 1000,2000,3000,4000,and 5000 µg/ml was added to 380 µml of (1000 µg/ml) DPPH. The solution mixture was incubated in the dark for 30 min at room temperature. The control included all reagents without the sample whilst methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm by using a UV-Vis spectrophotometer. Results are given IC_{50}. The reducing activities of azo-azomethine compounds were determined with some modifications [17]. Also, the DPPH radical scavenging activity of ascorbic acid [vitamin C] was examined for comparison. The percentage of inhibition was calculated by the following equation:

$$\text{Scavenging effects (\%)} = [(Ac-As)/Ac]\times 100$$

Where Ac is the absorbance of the control, and As is the absorbance of the sample.

3. Results and Discussion

Sulfathiazole and sulfadiazine were diazotized with $NaNO_2$ and HCl to form the corresponding diazonium salts. Treatment of these two salts with 3-ethoxy-4-hydroxy benzaldehyde at 0ºC, gave 4-((3-ethoxy-5-formyl-2-hydroxyphenyl)-N-(thiazol-2-yl)benzene sulfonamide (**1**)and 4-((3-ethoxy-5-formyl-2-hydroxyphenyl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide (**2**), respectively. Two series of azo-azomethine compounds were prepared by the reaction of equimolar quantities of compounds **1** and **2** with sulfathiazole, sulfadiazine, 2-hydroxyaniline, 2-amino-4,6-dimethyl pyridine, and 4-bromoaniline, respectively in ethanol, Scheme 1. Their elemental analyses are in agreement with the proposed formulae. All compounds are colored solid crystals and non-hygroscopic. The analytical and the spectroscopic data confirmed the structures of these compounds.



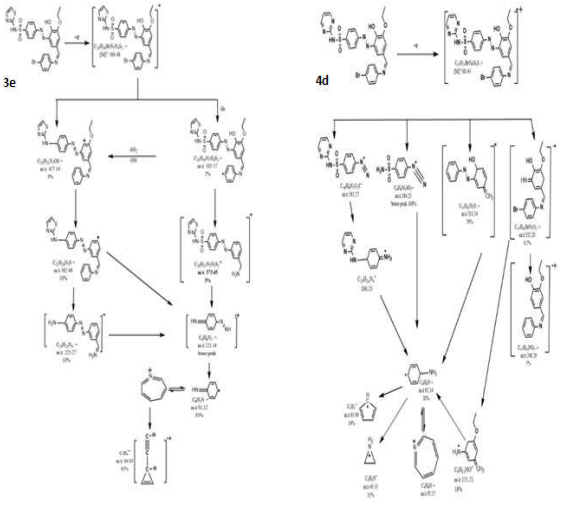
Scheme 1. Preparative methods for compounds **1**, **2**, **3a**-**3e**, and **4a**–**4d**

FT-IR spectra of all azo-azomethine compounds showed the absence of bands around 3250 due to υ (NH_2) group of aromatic amines and υ(C=O) of azo-aldehyde. New bands appeared at 1693-1624 cm^{-1} due to azomethine υ(CH=N) linkage which indicated the formation of azo-azomethine. The spectra of all compounds showed absorption bands between 1600-1581, 1531-1492, 1288-1248, and 1087-1076 due to υ(N=N), υ(C=N), υ(C-N), υ(C-O), respectively, which are in good agreement with the literature [18, 19]. Strong bands were observed in the IR spectra of compounds **1** and **2** showed bands at 1672 cm^{-1} and 1662cm^{-1} which were assigned to the υ(C=O). Furthermore, IR spectra of all compounds showed bands at a range of 1350-1300, 1157-1136, and 941-914 cm^{-1} which were assigned to the asymmetrical and symmetrical stretching vibration of SO_2 and υ(S-N) moiety, see Experimental Section and Figures S1 – S11 in the supplementary file.

In the ^{1}HNMR spectra of azo dyes (**1**, **2**) and azo-azomethine (**3a** - **3e** and **4a** - **4d**), the resonance of

NH groups attached to SO_2 moiety was observed in the range of 12.03 – 10.19 ppm [19-22]. The signal characteristic of the proton of the azomethine group for compounds **3a - 3e** and **4a - 4d** were observed in the range of 8.51-9.89 ppm. This is also supported by the disappearance of the signal at 9.88 and 9.79 ppm corresponding to the aldehyde proton of compounds **1** and **2**, respectively. The singlet signals around the range 8.34-9.13 ppm attributed to phenolic OH. Quartet signals at ranges of 4.09-4.20 ppm due to $–OCH_2$ group were observed in all ^1H NMR spectra, together with a singlet signal at range 1.36-1.41 ppm due to $–CH_3$ group. All protons of the aromatic ring are observed in their expected region, see Experimental Section. ^1H NMR spectra of all compounds give the correct ratio of aliphatic protons to aromatic protons, Experimental section and Figures S12 – S22 in the supplementary file.

The mass spectra of compounds **3d**, **3e**, **4c** and **4d** (Figures S23 – S26 in supplementary file) were reported here and data is presented in the Experimental section. The spectra of these compounds showed the molecular ion for compounds **3d**, **3e**, **4c** and **4d** at 536, 586, 518 and 581, respectively, Figure 1. It seems probable that we are observing a mass spectrum derived from the thermolysis of these compounds. The possible fragmentation pattern for compounds **3e** and **4d** is portrayed in Scheme 2



Scheme 2. Fragmentation of compounds **3e** and **4d**

Antioxidant Activity

DPPH assay is one of the most widely used methods for screening the antioxidant activity of various compounds. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability and hence it can be reduced primarily by more reactive reducing components such as phenolic substances [23, 24]. The antioxidant activity of all new compounds may be traced to the hydroxyl group of the phenolic group. All the compounds **(*i.e.,* 1, 2, 3a-3e and 4a- 4d)** containing phenolic hydroxyl moiety act as reducing agents by trapping free radicals. Figure 1 shows the free radical scavenging activity of all compounds in comparison with Vitamin C. Compounds **2** at a concentration of 1000, 2000, 3000, 4000 and 5000 µg/ml was able to scavenge the DPPH radical by 41.08, 50.31, 56.04, 58.48 and 59.78%, respectively so it can be concluded that the higher concentration showed the better antioxidant activity, Figure 2. The IC_{50} values together with the correlation coefficient (R^2) values for all compounds are presented in Table 1. The azo-azomethine compounds (*i.e.*,**3a - 3e** and **4a – 4d**) possess higher antioxidant potential (IC_{50}) than azo compounds **1** and **2** and lower than vitamin C. Therefore, these results suggest that the Azo-azomethine compounds can act as electron or proton donating groups to the DPPHradicals by breaking the free radical chain via hydrogen atom donation. Furthermore, the conjugation system may direct the resonance into the aromatic ring, causing the stability of free radicals [25, 26]. The obtained results indicated that the order antioxidant activity can be arranged as follow:

Vitamin C>3b>4d >4a>3c >3d >3a>3e>4c>4b

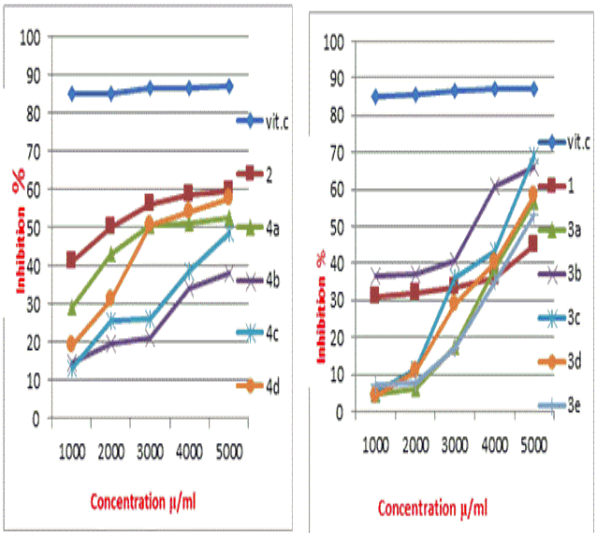


Figure 1. DPPH scavenging activity of all newly synthesized compounds

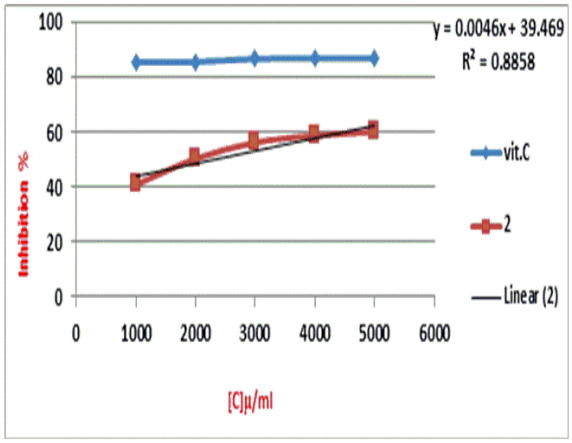


Figure 2. The plot of concentration and percentage of inhibition for compounds **2**

Biological activity

The synthesized new compounds **1**, **2**, **3a-3e** and **4a-4d** were biologically tested to investigate their ability to inhibit bacterial and fungal growth against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*),*Aspergillus niger* (*A.niger*)and*Candida albicans* (*C.albicane*) by a disc diffusion method. The compounds were screened at the concentration of 25 µg/ml in DMSO and compared with Sulfathiazole, Sulfadiazine, Amoxicillin and Nystatin. The results of the antimicrobial activity of the compounds are presented in Table 2. All compounds show a good to moderate activity towards gram-positive bacteria *staphylococcus aureus* and no activity toward gram-negative *Escherichia coli*. This may becorrelated to the outer membrane of *E.coli* and *P. aeruginosa*, which provides greater resistance to morphological changes caused by these compounds. The unique structural components in gram-negative bacteria essentially hinder access and prevent the bioactive compounds from disrupting the cell wall. In another word, the cell wall of gram-positive bacteria is less complicated and thickness than that of gram-negative bacteria, allowing the transmission of some compound through it [27]. Generally, compounds **3b** and **3d** showed a good activity while compounds **1**, **2**, **3a**, **3c**, **3e**, and **4d** show slight effect against *S. aureus* in comparison with standard antibiotics, Table 2.

The antifungal activity measurements of the synthetic compounds were performed by the disk diffusion method. Compounds **1**, **3a**, **3d**, and **3e** show a good to moderate antifungal activity, Table 2. The antimicrobial activity of these compounds may be attributed to their structure which contains sulfathiazole and sulfadiazine. It is worth noting that several studies demonstrated compounds containing azomethine groups in their structure showed their effectiveness for fighting pathogens and diseases caused by microorganisms [28].

4. Conclusion

The objective of the present study was to synthesize and evaluate two series of azo-azomethine compounds derived from sulfathaizal and sulfadiazine. In vitro biological evaluation of these compounds against two pathogenic bacterial strains reveals that all compounds exhibited good to moderate activity against *S. aureas*, and slightly efficiency as antifungal activity. The DPPH assay showed that the new compounds are capable of donating electrons or hydrogen atoms that react with free radicals to prevent cell damage.

5. Conflicts of interest

There are no conflicts to declare.

Table 1: DPPH scavenging capacities (IC_{50} µM) of the synthesized compounds

Comp.	Conc. µg/ml					R^2	IC_{50}
	1000	2000	3000	4000	5000		
1	30.86	31.85	33.47	36.03	44.95	0.814	7.58
2	41.084	50.311	56.047	58.48	59.788	0.885	2.28
3a	4.738	6.421	17.456	38.591	56.733	0.925	4.85
3b	36.40898	37.21945	40.77307	60.91022	65.71072	0.865	3.22
3c	4.925187	11.22195	35.97257	43.0798	69.07731	0.959	4.07
3d	4.36409	10.5985	28.99002	40.27431	58.04239	0.980	4.57
3e	6.982544	7.793017	17.26933	35.28678	53.17955	0.911	5.15
4a	29.05237	43.39152	50.43641	51.30923	52.74314	0.793	3.85
4b	14.77556	19.95012	21.07232	34.35162	38.02993	0.858	7.07
4c	13.02993	25.87282	26.30923	38.8404	48.81546	0.954	5.26
4d	19.07731	31.42145	50.62344	54.05237	57.66833	0.905	3.73
Vit. C	85.056	85.298	86.752	87.833	86.995	0.867	7.6×10^{-9}

IC_{50}: Inhibitory concentration; R^2=Correlation coefficient.

Table 2: The inhibition zones of the synthesized compounds against selected microbes

compounds	Bacteria		Fungal	
	E. coli	*S. aureas*	*C. albicane*	*A. niger*
1	0	13	21	0
2	0	17	0	0
3a	0	16	29	0
3b	0	21	13	0
3c	0	13	0	0
3d	0	20	33	14
3e	0	18	43	15
4a	0	0	0	0
4b	0	18	0	0
4c	0	0	0	0
4d	0	14	0	0
Sulfathiazole	0	0	-	-
Sulfadiazine	0	0	-	-
Amoxicillin	30	30.2	-	-
Nystatin	0	0	-	-

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