Egypt.J.Chem. **Vol. 63**, No. 1. pp. 171 - 184 (2020)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Synthesis, Antimicrobial, and Docking Investigations of Remarkably Modified Sulfathiazole Derivatives

Samir T. Gaballah [1*], H. Amer [2, 3], A. Hofinger-Horvath [4], M. Al-Moghazy[5], M. I. Hemida [6]

[1]*Photochemistry Department, Division of Chemical Industries, National Research Centre, El-Buhoth St, Dokki 12622, Giza, Egypt*
[2]*Department of Chemistry of Natural and Microbial Products, National Research Centre, El-Buhoth St, Dokki 12622, Giza, Egypt*
[3]*Department of Chemistry, University of Natural Resources and Life Sciences, UFT Campus Tulln, Konrad-Lorenz-Straße 24, A-3430 Tulln, Austria*
[4]*Department of Chemistry, University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria*
[5]*Food Sciences & Industry Division,National Research Centre*
[6]*Chemistry Department, Faculty of Science, Benha University, Benha-Egypt.*

SOME new sulfathiazole derivatives were synthesized. The sulfathiazole starting material was reacted with ethyl bromoacetate and gave unpredictably an ester product 2. The substitution occurred selectively at the tautomeric proton of the NH thiazolyl nitrogen rather than the aromatic NH_2 protons. The ester was further hydrazinolysed followed by condensation with several aldehydes to establish hydrazones (4a-h). Hantzesch thiazole synthesis was also applied to build antimicrobial agents containing multi-thiazole moieties. The structures of the synthesized compounds were confirmed by ^1H, ^{13}C, 2D ^1H NMR, MS, and microanalyses. The synthesized compounds were tested for their antimicrobial activity towards Gram-positive and Gram-negative bacteria, and fungi strains. Some of the investigated compounds showed prominent high potency. The docking study revealed the mode of action between the modified sulfathiazole ligands and the binding site of DHPS.

Keywords: Sulfathiazole; Antimicrobial; Molecular Docking; Dihydropteroate Synthase (DHPS)

Introduction

Antibiotics or antimicrobial agents are different families of chemical compounds that are characterized by the presence of distinctive kinds of functional groups or chemical structures which are responsible of the bacterial inhibition. Historically, ancient civilizations utilized antimicrobial substances such as zinc and copper in promoting wound healing and water disinfection, respectively [1]. The research during the period between 1877 and 1939 on the antibiotics discovery has produced a great number of potential antimicrobial substances. Continued research for new antibiotics was molded and improved by new technologies through the period 1900-1950 [2]. Penicillin, which was discovered in 1929 by Fleming, was selected by Florey and coworkers for further investigation in 1938 after being proven to destroy staphylococci. Research on sulfonamide began in Bayer AG Laboratories in 1932 and the research team successfully

*Corresponding author e-mail: samir.gaballah@gmail.com
Received 23/6/2019; Accepted 8/8/2019
DOI: 10.21608/ejchem.2019.13909.1862

introduced a new sulfa drug ever under a brand name Prontosil that had a strong protecting effect against streptococci and could excellently treat a variety of internal bacterial diseases [3]. Afterwards, a group of sulfa drugs as sulfanilamide and sulfathiazole has been advanced and widely recommended as antimicrobial agents .

Since the discovery of sulfanilamide a comprehensive number of applications have developed [4]. Continuous endeavors with industrial support have encouraged the research and development of sulfa drugs to introduce different and wide range of applications. {Ballagi-Pordány, #4}Carbutamide, glibenclamide, gliquidone, glyclopyramide, and glimepiride are chemically classified as sulfonylurea and used as antidiabetic drugs [5-8]. Many other pharmacological activities of sulfonamides have been recently reported that include *anti*-inflammatory, endothelin receptor, and 5-HT6 receptor antagonism [9, 10]. Acetazolamide is a sulfa drug that usually sold under the trade name Diamox is a reversible carbonic anhydrase inhibitor [11].

The research in this area has presented a reasonable explanation on the pharmacological properties of sulfa drugs and the results showed that they inhibit the bacterial growth via a competitive inhibition of a key enzyme known as dihydropteroate synthase (DHPS) [12-17]. The latter is involved in the biosynthesis of tetrahydrofolic acid which is an essential growth factor for bacteria [18]. Sulfathiazole, one of the family members of sulfonamides, is a sulfa drug and used as a short-acting antibiotic. Formerly, it was a common oral and topical antimicrobial such as sulfathiazole ointment that was used in the treatment of pyogenic dermatoses [19]. Sulfathiazole is still sporadically used, sometimes in combination with sulfabenzamide and sulfacetamide, and in aquariums. Despite their versatile applications in the treatment of several diseases, the commercial distribution of the sulfa drugs has been restricted due to severe toxicity and immunological reactions that causes abdominal discomfort, vomiting, diarrhea, breathing distress, fever, headache, skin rashes, kidney damage etc. in case of continued treatment [20-22]. Research in this area has to face a double challenge in designing new antimicrobial agents that have minimal side effects on one side and high potency on the other side; taking in mind the increased microbial resistance. In view of these annotations and continuing our previous work on antimicrobial candidates [23], it was believed worthy to synthesize some novel sulfathiazole derivatives and test and evaluate their antimicrobial properties against some strains of Gram-positive and Gram-negative bacteria as well as fungi hoping to obtain new antimicrobial agents with minimal side effects and potent activity.

Experimental

Materials and reagents

All chemicals were purchased from common commercial suppliers and used without further purification. Melting points (M.p.) were determined on a Gallenkamp melting point apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker spectrometer at 600 and 150 MHz, respectively, in DMSO-d_6 as a solvent. Mass spectra were recorded on Thermo Finnigan SSQ 7000 Advantage spectrometer in EI ionization mode. Microanalyses were performed at the Microanalytical Center in Cairo University. All reactions were performed in air. The reaction progress was monitored using thin layer chromatography (TLC) which was performed on silica gel 60 F_{254} aluminum plates (E.Merck, layer thickness 0.2 mm). 4-Amino-*N*-(thiazol-2-(3*H*) ylidene)benzenesulfonamide (4) was prepared according to published procedure [24, 25].

Synthesis

N-(3-acetylthiazol-2(3H)-ylidene)-4-aminobenzenesulfonamide (1a).

Sulfathiazole 1 (2.55 g, 10 mmol) and acetyl chloride (1.4 mL, 19.7 mmol) were added to pyridine (15 mL, 186.2 mmol) as a solvent and stirred at room temperature (4 h). The mixture was poured on crushed ice/water. The solid compound obtained was filtered and dried in air to obtain product 1a without further purification. M.p.: 267-270 °C; yield 2.048 g (69%); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 7.73-7.69 (m, 4H, Ar), 7.23 (d, J = 4.6 Hz, 1H, Ar), 6.80 (d, J = 4.6 Hz, 1H, Ar), 2.06 (s, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) = 168.81, 142.40, 136.11, 126.87, 124.35, 118.39, 108.01, 24.05. FTIR (KBr disk) ν/cm^{-1}: 3352, 3296, 3190, 3111, 2995, 2957, 1686, 1589, 1493, 1404, 1271, 1134, 1081, 926, 827, 690; Anal. Calcd. for $C_{11}H_{11}N_3O_3S_2$ (297.02): C, 44.43; H, 3.73; N, 14.13; S, 21.56; Found: C, 44.25; H, 3.95; N,

14.35; S, 21.73.

Ethyl 2-(2-(((4-aminophenyl)sulfonyl)imino) thiazol-3(2H)-yl)acetate (2).

Sulfathiazole 1 (5.11 g, 20 mmol) and potassium carbonate (5.53 g, 40 mmol) in acetone (30 mL) were refluxed for 1 h then ethyl bromoacetate (3 mL, 27 mmol) was added and the mixture was refluxed further until complete consumption of the starting material as indicated by TLC (5 h). The mixture was poured upon crushed ice/water. The precipitate was filtered, washed with water, and finally dried in air to afford 2 as a pale yellow solid and used in the next step without further purification. M.p.: 188-189 °C; yield 6.37 g (93%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 7.39 (d, J = 8.8 Hz, 2H, Ar), 7.32 (d, J = 4.5 Hz, 1H, Ar), 6.82 (d, J = 4.5 Hz, 1H, Ar), 6.54 (d, J = 8.8 Hz, 2H, Ar), 5.84 (s, 2H, NH_2), 4.74 (s, 2H, CH_2), 4.07 (q, J = 7.2 Hz, 2H, CH_2), 1.13 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 166.97, 165.92, 152.32, 128.20, 127.70, 127.27, 112.31, 105.94, 61.24, 48.24, 13.83; FTIR (KBr disk) ν_{max}/cm^{-1}: 3467, 3428, 3117, 2996, 1758, 1690, 1592, 1493, 12275, 1134, 1082, 928, 689; Anal. Calcd. for $C_{13}H_{15}N_3O_4S_2$ (341.40): C, 45.74; H, 4.43; N, 12.31; S, 18.78. Found: C, 45.91; H, 4.25; N, 12.64; S, 18.53.

2-(2-(((4-Aminophenyl)sulfonyl)imino) thiazol-3(2H)-yl)acetic acid (2a).

Ethyl 2-(4-amino-*N*-(thiazol-2-yl) phenylsulfonamido) acetate (2) (0.342 g, 1 mmol) and potassium hydroxide (0.056 g, 9.98 mmol) in methanol (10 mL) was stirred at room temperature (1 h) then heated on water bath (2 h). Excess potassium hydroxide (0.050 g, 8.9 mmol) was added and heated on water bath again for 1.5 h. The solvent was removed *in vacuo* and the residue was dissolved in water and finally acidified with HCl (10%) to produce the free acid (2a). M.p.: 214-216 °C; yield 0.303 g (96%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 7.40 (d, J = 5.0 Hz, 2H, Ar), 7.32 (d. J = 4.5 Hz, 1H, Ar), 6.81 (s, br, 2H, Ar), 6.54 (d, J = 4.5 Hz, 1H, Ar), 5.84 (s, br, 2H, NH_2), 4.65 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 168.30, 165.99, 152.22, 128.40, 127.75, 112.35, 105.76, 48.19; FTIR (KBr disk), ν_{max}/cm^{-1}: 3469, 3343, 3121, 2993, 2956, 1700, 1594, 1499, 1275, 1134, 1082, 933, 829, 684 Anal. Calcd. for $C_{11}H_{11}N_3O_4S_2$ (313.35): C, 42.16; H, 3.54; N, 13.41; S, 20.46. Found: C, 41.92; H, 3.77; N, 13.68; S, 20.18.

4-Amino-N-(3-(2-hydrazinyl-2-oxoethyl) thiazol-2(3H)-ylidene)benzenesulfonamide (3).

Hydrazine hydrate (1 mL, 31.9 mmol) was added to 2 (1.5 g, 4.3 mmol) in absolute ethanol (25 mL) then the mixture was refluxed until complete consumption of the starting material as indicated by TLC (4 h) to furnish 3 which separated by gravity filtration. M.p.: 212-213 °C; yield 1.372 g, (97%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 9.34 (s, br, 1H, NH), 7.41 (d, J = 8.6 Hz, 2H, Ar), 7.26 (d, J = 4.5 Hz, 1H, Ar), 6.78 (d, J = 4.5 Hz, 1H, Ar), 6.54 (d, J = 8.6 Hz, 2H, Ar), 5.82 (s, 2H, NH_2), 4.53 (s, 2H, CH_2), 4.25 (s, br, 2H, NH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 165.86, 165.16, 152.21, 128.95, 127.77, 112.35, 105.38, 47.81; FTIR (KBr disk), n_{max}/cm^{-1}: 3346, 3301, 3197, 3151, 3111, 2994, 2958, 1690, 1627, 1588, 1489, 1406, 1270, 1133, 1080, 927, 689; Anal. Calcd. for $C_{11}H_{13}N_5O_3S_2$ (327.38): C, 40.36; H, 4.00; N, 21.39; S, 19.59. Found: C, 40.12; H, 3.73; N, 21.61; S, 19.75.

4-Amino-N-(3-((4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl)thiazol-2(3H)-ylidene)benzenesulfonamide (3a).

A mixture of hydrazide 3 (0.172 g, 0.52 mmol) and phenyl isothiocyanate (0.075 g, 0.55 mmol) in ethanol (15 mL) was refluxed for 72 h until complete consumption of the starting material as indicated by TLC. The precipitate was filtered and dried in air to afford (3a). M.p.: 293-294 °C (dec.); yield 0.142 g (58%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 12.10 (s, br, 1H, NH), 8.25-8.31 (m, 2H, Ar), 8.11-813 (m, 1H, Ar), 7.96-8.02 (m, 2H, Ar), 7.38-7.42 (m, 2H, Ar), 7.34 (d, J = 4.7 Hz, 1H, Ar), 6.84 (d, J = 4.7 Hz, 1H, Ar), 6.50-6.55 (m, 2H, Ar), 5.86 (s, br, 2H, NH_2), 5.18 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 167.60, 166.35, 152.31, 147.85, 144.92, 142.02, 140.10, 127.98, 127.85, 127.30, 124.06, 112.37, 48.63, 48.61; FTIR (KBr disk), ν_{max}/cm^{-1}: 3468, 3343, 3214, 3125, 2992, 2957, 1700, 1627, 1591, 1495, 1393, 1273, 1132, 1081, 929, 828, 689; Anal. Calcd. for $C_{18}H_{16}N_6O_2S_3$ (444.55): C, 48.63; H, 3.63; N, 18.91; S, 21.64. Found: C, 48.77; H, 3.92; N, 18.69; S, 21.79.

General procedure for the synthesis of compounds 4a-h.

2-Amino-*N*-(3-(2-hydrazinyl-2-oxoethyl) thiazol-2(3H)-ylidene)benzenesulfonamide (3) (0.5 mmol) and the appropriate aldehyde (0.5 mmol) were reacted in boiling absolute ethanol (15 mL) until complete consumption of the starting

material as indicated by TLC (5-8 h) to furnish the hydrazone derivatives 4a-h. The reaction mixture was allowed to cool to room temperature and the product was filtered and dried in air.

4-Amino-N-(3-(2-(2-(3,5-dimethoxybenzylidene)hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene)benzenesulfonamide (4a).

Compound 3 reacted with 3,5-dimethoxybenzaldehyde and gave 4a; M.p.: 237-238 °C; yield 0.181 g, (82%); major *E*-imine isomer *anti*-imide bond rotamer (82%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.79 (s, 1H, NH), 7.94 (s, 1H, =CH), 7.38-7.43 (m, 2H, Ar), 7.34 (d, *J* = 4.8 Hz, 1H, Ar), 6.86-6.88 (m, 2H, Ar), 6.82 (d, *J* = 4.8 Hz, 1H, Ar), 6.50-6.57 (m, 3H, Ar), 5.82 (s, 2H, NH_2), 5.14 (s, 2H, CH_2), 3.78 (s, 6H, 2 OMe); minor *E*-imine isomer *syn*-imide bond rotamer (18%) ^{1}H NMR (DMSO-d_6, 150 MHz): δ (ppm) = 11.79 (s, 1H, NH), 8.13 (s, 1H, =CH), 7.38-7.43 (m, 2H, Ar), 7.34 (d, *J* = 4.8 Hz, 1H, Ar), 6.86-6.88 (m, 2H, Ar), 6.82 (d, *J* = 4.8 Hz, 1H, Ar), 6.50-6.57 (m, 3H, Ar), 5.82 (s, 2H, NH_2), 4.72 (s, 2H, CH_2), 3.78 (s, 6H, 2 OMe); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 167.14, 166.29, 160.65, 152.20, 144.05, 135.77, 127.79, 127.39, 112.34, 105.53, 104.69, 102.31, 55.32; FTIR (KBr disk, n_{max}/cm^{-1}): 3465, 3365, 3295, 3149, 3111, 2994, 2956, 1697, 1626, 1590, 1491, 1405, 1327, 1274, 1247, 1133, 1081, 929, 827, 687; Anal. Calcd. for $C_{20}H_{21}N_5O_5S_2$ (475.54): C, 50.52; H, 4.45; N, 14.73; S, 13.48. Found: C, 50.26; H, 4.28; N, 14.99; S, 13.74.

4-Amino-N-3-(2-(2-(4-cyanobenzylidene)hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene)benzenesulfonamide (4b).

Compound 3 reacted with 4-cyanobenzaldehyde and gave 4b; M.p.: 251-253 °C; yield 0.164 g (74.3%); major *E*-imine isomer *anti*-imide bond rotamer (82%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 12.00 (s, 1H, NH), 8.07 (s, 1H, =CH), 7.89-7.91 (m, 4H, Ar), 7.40-7.41 (m, 2H, Ar), 7.33 (d, *J* = 4.8 Hz, 1H, Ar), 6.81 (d, *J* = 4.8 Hz, 1H, Ar), 6.51-6.554 (m, 2H, Ar), 5.82 (br, s, 2H, NH_2), 5.16 (s, 2H, CH_2); minor *E*-imine isomer *syn*-imide bond rotamer (18%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 12.00 (s, 1H, NH), 8.26 (s, 1H, =CH), 7.89-7.91 (m, 4H, Ar), 7.40-7.41 (m, 2H, Ar), 7.33 (d, *J* = 4.8 Hz, 1H, Ar), 6.81 (d, *J* = 4.8 Hz, 1H, Ar), 6.51-6.554 (m, 2H, Ar), 5.81 (br, s, 2H, NH_2), 4.74 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 167.44, 166.27, 152.21, 142.37, 138.22, 132.66, 128.79, 127.75, 127.53, 127.38, 118.58, 112.33, 111.86, 105.58, 48.53; FTIR (KBr disk, ν_{max}/cm^{-1}): 3450, 3424, 3361, 3298, 3149, 3109, 2995, 2958, 2923, 2853, 2218, 1703, 1592, 1496, 1404, 1372, 1327, 1250, 1128, 1082, 934, 829, 692; Anal. Calcd. for $C_{19}H_{16}N_6O_3S_2$ (440.50): C, 51.81; H, 3.66; N, 19.08; S, 14.56. Found: C, 51.56; H, 3.91; N, 18.74; S, 14.29.

4-Amino-N-3-(2-(2-((E)-2-bromobenzylidene)hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene)benzenesulfonamide (4c).

Compound 3 reacted with 2-bromobenzaldehyde and gave 4c; M.p.: 254-257 °C; yield 0.180 g (72.8%); major *E*-imine isomer *anti*-imide bond rotamer (81%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.97 (s, 1H, NH), 8.37 (s, 1H, =CH), 8.0 (dd, *J* = 7.7, 1.8 Hz, 1H, Ar), 7.70 (dd, *J* = 8.0, 1.0 Hz, 1H, Ar), 7.44-7.48 (m, 1H, Ar), 7.40-7.48 (m, 2H, Ar), 7.34-7.39 (m, 1H, Ar), 7.32 (d, *J* = 4.7 Hz, 1H, Ar), 6.82-6.83 (m, 1H, Ar), 6.52-6.54 (m, 2H, Ar), 5.82 (s, 2H, NH_2), 5.15 (s, 2H, CH_2); minor *E*-imine isomer *syn*-imide bond rotamer (19%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.97 (s, 1H, NH), 8.56 (s, 1H, =CH), 7.93 (dd, *J* = 7.9, 1.7 Hz, 1H, Ar), 7.70 (dd, *J* = 8.0, 1.0 Hz, 1H, Ar), 7.44-7.48 (m, 1H, Ar), 7.40-7.48 (m, 2H, Ar), 7.34-7.39 (m, 1H, Ar), 7.32 (d, *J* = 4.7 Hz, 1H, Ar, 6.82-6.83 (m, 1H, Ar),), 6.52-6.54 (m, 2H, Ar), 5.82 (s, 2H, NH_2), 4.72 (s, 2H, CH_2), ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 167.24, 166.25, 152.20, 142.66, 133.13, 132.52, 131.70, 128.07, 127.76, 127.29, 123.30, 112.33, 105.55, 48.49; Anal. Calcd. for $C_{18}H_{16}BrN_5O_3S_2$ (494.38): C, 43.73; H, 3.26; Br, 16.16; N, 14.17; S, 12.97. Found: C, 43.46; H, 3.49; Br, 15.87; N, 14.17; S, 13.23.

4-Amino-N-(3-(2-(2-(2,4-dihydroxybenzylidene)hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene)benzenesulfonamide (4d).

Compound 3 reacted with 2,4-dihydroxybenzaldehyde and gave 4d; mp 165-168 °C; yield 0.185 g (83%); major *E*-imine isomer *anti*-imide bond rotamer (62%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.49 (s, 1H, NH), 9.93 (s, 1H, OH), 9.79 (s, 1H, OH), 8.20 (s, 1H, =CH), 7.52 (d, *J* = 8.3, 1H, Ar), 7.38-7.42 (m, 2H, Ar), 7.31-7.33 (m, 1H, Ar), 6.80-6.82 (m, 1H, Ar), 6.51-6.55 (m, 2H, Ar), 6.33-6.35 (m, 1H, Ar), 6.29-6.31 (m, 1H, Ar), 5.82 (s, 2H, NH_2), 5.06 (s, 2H, CH_2); minor *E*-imine isomer *syn*-imide bond rotamer (38%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.80 (s, 1H, NH), 11.06 (s, 1H, OH), 9.94 (s, 1H, OH), 8.28 (s, 1H, =CH), 7.52 (d, *J*

= 8.3, 1H, Ar), 7.38-7.42 (m, 2H, Ar), 7.31-7.33 (m, 1H, Ar), 6.80-6.82 (m, 1H, Ar), 6.51-6.55 (m, 2H, Ar), 6.33-6.35 (m, 1H, Ar), 6.29-6.31 (m, 1H, Ar), 5.82 (s, 2H, NH_2), 4.70 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 166.24, 166.00, 161.75, 160.52, 157.98, 152.17, 142.38, 139.97, 135.43, 128.92, 127.77, 112.39, 107.87, 48.39; EIMS m/z (Rel. Int. %) 447 ([M]$^+$, 0.24), 355 (2), 255 (3), 156 (27), 108.10 (49), 92 (79); FTIR (KBr disk, ν_{max}/cm^{-1}): 3467, 3343, 3217, 3155, 3122, 2991, 2958, 1697, 1628, 1591, 1499, 1336, 1274, 1131, 1079, 929, 827, 689, 549; Anal. Calcd. for $C_{18}H_{17}N_5O_5S_2$ (447.48): C, 48.31; H, 3.83; N, 15.65; S, 14.33. Found: C, 48.09; H, 3.68; N, 15.88; S, 14.14.

4-Amino-N-(3-(2-(2-(2-chlorobenzylidene) hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene) benzenesulfonamide (4e).

Compound 3 reacted with 2-chlorobenzaldehyde and gave 4e; M.p.: 229-230 °C; yield 0.132 g (58.7%); major *E*-imine isomer *anti*-imide bond rotamer (61%) ^1H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.95 (s, 1H, NH), 8.41 (s, 1H, =CH), 8.02 (d, *J* = 8.0 Hz, 1H, Ar), 7.53-7.54 (m, 1H, Ar), 7.40-7.45 (m, 4H, Ar), 7.32-7.33 (m, 1H, Ar), 6.82-6.83 (m, 1H, Ar), 6.53-6.54 (m, 2H, Ar), 5.82 (s, 2H, NH_2), 5.16 (s, 2H, CH_2); minor *E*-imine isomer *syn*-imide bond rotamer (39%) ^1H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 12.01 (s, 1H, NH), 8.60 (s, 1H, =CH), 7.95 (d, *J* = 8.0 Hz, 1H, Ar), 7.53-7.54 (m, 1H, Ar), 7.40-7.45 (m, 4H, Ar), 7.32-7.33 (m, 1H, Ar), 6.82-6.83 (m, 1H, Ar), 6.53-6.54 (m, 2H, Ar), 5.82 (s, 2H, NH_2), 4.73 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 168.39, 149.29, 140.83, 131.91, 127.91, 112.81, 105.88, 49.03; EIMS m/z (Rel. Int.%) 449 ([M]$^+$, 8), 450 (2.6), 451 (3.7), 418 (3.7), 312 (14.9), 296 (77.6), 157 (7), 156 (44), 141 (13), 140 (43), 113 (100), 92 (72); Anal. Calcd. for $C_{18}H_{16}ClN_5O_3S_2$ (449.93): C, 48.05; H, 3.58; Cl, 7.88; N, 15.57; S, 14.25. Found: C, 47.79; H, 3.81; Cl, 8.19; N, 15.79; S, 14.42.

N-(3-(2-(2-((1H-indol-3-yl)methylene) hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene)-4-aminobenzenesulfonamide (4f).

Compound 3 reacted with 3-1H-indole-3-carbaldehyde and gave 4f; M.p.: 260-263 °C; yield 0.132 g (58.6%); major *E*-imine isomer *anti*-imide bond rotamer (86%) ^1H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.60 (s, 1H, NH), 11.48 (s, 1H, NH, indole), 8.20 (s, 1H, =CH), 8.12 (d, *J* = 7.7 Hz, 1H, Ar), 7.83 (d, *J* = 2.8 Hz, 1H, Ar), 7.35-7.46 (m, 4H, Ar), 7.12-7.23 (m, 2H, Ar), 6.83-6.85 (m, 1H, Ar), 6.53 (d, *J* = 8.7 Hz, 2H, Ar), 5.85 (s, 2H, NH_2), 5.19 (s, 2H, CH_2); minor *E*-imine isomer *syn*-imide bond rotamer (14%) ^1H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.60 (s, 1H, NH), 11.44 (s, 1H, NH, indole), 8.36 (s, 1H, =CH), 8.12 (d, *J* = 7.7 Hz, 1H, Ar), 7.83 (d, *J* = 2.8 Hz, 1H, Ar), 7.35-7.46 (m, 4H, Ar), 7.12-7.23 (m, 2H, Ar), 6.83-6.85 (m, 1H, Ar), 6.53 (d, *J* = 8.7 Hz, 2H, Ar), 5.85 (s, 2H, NH_2), 4.71 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 166.35, 166.21, 152.31, 152.27, 141.68, 137.10, 130.86, 129.17, 127.85, 127.41, 124.01, 122.70, 121.70, 120.73, 112.39, 111.93, 111.19, 105.48, 48.65; Anal. Calcd. for $C_{20}H_{18}N_6O_3S_2$ (454.52): C, 52.85; H, 3.99; N, 18.49; S, 14.11. Found: C, 52.69; H, 4.18; N, 18.70; S, 13.88.

4-Amino-N-(3-(2-(2-(3-bromobenzylidene) hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene) benzenesulfonamide (4g).

Compound 3 reacted with 3-bromobenzaldehyde and gave 4g; M.p.: 203-205 °C; yield 0.174 g (68.2%); major *E*-imine isomer *anti*-imide bond rotamer (82%) ^1H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.87 (s, br, 1H, NH), 7.99 (s, 1H, =CH), 7.94 (t, *J* = 1.7 Hz, 1H, Ar), 7.71-7.72 (m, 1H, Ar), 7.62 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H, Ar), 7.39-7.42 (m, 3H, Ar), 7.32 (d, *J* = 4.8 Hz, 1H, Ar), 6.82-6.83 (m, 1H, Ar), 6.52-6.54 (m, 2H, Ar), 5.82 (s, 2H, NH_2), 5.15 (s, 2H, CH_2); minor *E*-imine isomer *syn*-imide bond rotamer (18%) ^1H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 11.87 (s, br, 1H, NH), 8.17 (s, 1H, =CH), 7.94 (t, *J* = 1.7 Hz, 1H, Ar), 7.71-7.72 (m, 1H, Ar), 7.62 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H, Ar), 7.39-7.42 (m, 3H, Ar), 7.32 (d, *J* = 4.8 Hz, 1H, Ar), 6.82-6.83 (m, 1H, Ar), 6.52-6.54 (m, 2H, Ar), 5.82 (s, 2H, NH_2), 4.73 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 167.94, 166.96, 152.19, 142.58, 136.24, 132.56, 130.92, 129.00, 128.85, 127.77, 127.40, 126.14, 122.20, 112.34, 105.51, 103.58, 48.59; Anal. Calcd. for $C_{18}H_{16}BrN_5O_3S_2$ (494.38): C, 43.73; H, 3.26; Br, 16.16; N, 14.17; S, 12.97. Found: C, 43.90; H, 3.05; Br, 15.89; N, 14.36; S, 13.15.

4-Amino-N-(3-(2-(2-(4-nitrobenzylidene) hydrazinyl)-2-oxoethyl)thiazol-2(3H)-ylidene) benzenesulfonamide (4h).

Compound 3 reacted with 4-nitrobenzaldehyde and gave 4h; M.p.: 288-290 °C; yield (0.186 g, 76.9%); major *E*-imine isomer *anti*-imide bond

rotamer (64%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 9.91 (s, br, 1H, NH), 8.44 (s, 1H, =CH), 7.34-7.35 (m, 2H, Ar), 7.27-7.29 (m, 2H, Ar), 7.21 (d, *J* = 4.8 Hz, 1H, Ar), 7.16-7.18 (m, 2H, Ar), 6.75 (d, *J* = 4.8 Hz, 1H, Ar), 6.54-6.56 (m, 2H, Ar), 5.86 (s, 2H, NH_2), 5.21 (s, 2H, CH_2); minor *E*-imine isomer *syn*-imide bond rotamer (36%) ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 9.91 (s, br, 1H, NH), 8.73 (s, 1H, =CH), 7.34-7.35 (m, 2H, Ar), 7.27-7.29 (m, 2H, Ar), 7.21 (d, *J* = 4.8 Hz, 1H, Ar), 7.16-7.18 (m, 2H, Ar), 6.75 (d, *J* = 4.8 Hz, 1H, Ar), 6.54-6.56 (m, 2H, Ar), 5.86 (s, 2H, NH_2), 4.53 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 167.85, 165.41, 152.37, 152.20, 147.48, 134.98, 128.61, 127.70, 127.10, 126.72, 112.37, 112.34, 106.53, 45.88; Anal. Calcd. for $C_{18}H_{16}N_6O_5S_2$ (460.48): C, 46.95; H, 3.50; N, 18.25; S, 13.92. Found: C, 47.19; H, 3.31; N, 18.06; S, 13.74.

N-(Thiazol-2-yl)-4-thioureidobenzenesulfonamide (5).

To sulfathiazole (1, 2.55 g, 10 mmol) suspension in water (25 mL) HCl (5 mL) was added with stirring. Ammonium thiocyanate (0.837 g, 10.1 mmol) was dissolved in distilled water (25 mL) was gradually added to the previously prepared solution with vigorous stirring. The reaction mixture was refluxed for 3 h, cooled to room temperature, and finally the product was filtered and dried to give the solid product 5; M.p.: 218-220 °C; yield 1.3 g (41.3%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 12.71 (s, br, 1H, NH), 10.02 (s, br, 1H, NH), 8.10-7.30 (m, 6H, overlapped NH_2 and Ar,), 7.24 (d, *J* = 4.8 Hz, 1H, Ar), 6.81 (d, *J* = 4.8 Hz, 1H, Ar); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 181.15, 142.64, 136.90, 126.43, 121.60, 108.06; EIMS m/z (Rel. Int.%) 314 ([M]$^+$, 0.19), 297 (48), 255 (5), 235 (9), 234 (16), 233 (91), 232 (53), 198 (26), 191 (13), 150 (23), 134 (100), 108 (36), 99 (50), 92 (41), 90 (54), 76 (19), 59 (15). Anal. Calcd. for $C_{10}H_{10}N_4O_2S_3$ (314.40): C, 38.20; H, 3.21; N, 17.82; S, 30.59. Found: C, 38.39; H, 3.01; N, 17.60; S, 30.37.

4-((4-(4-Chlorophenyl)thiazol-2-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (6).

N-(Thiazol-2-yl)-4-thioureidobenzenesulfonamide (5) (0.16 g, 0.5 mmol) and 2-bromo-1-(4-chlorophenyl)ethan-1-one (0.11 g, 0.5 mmol) in ethanol (20 mL) was refluxed for 7 h. The reaction mixture was cooled to room temperature, filtered, and dried in air to achieve 6 as a pale yellow powder; M.p.: 178-180 °C; yield 0.16 g (71%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 12.61 (s, br, 1H, NH), 10.69 (s, 1H, NH), 7.96 (dd, *J* = 8.4, 1.1 Hz, 2H, Ar), 7.82-7.87 (m, 2H, Ar), 7.76-7.80 (m, 2H, Ar), 7.45-7.50 (m, 3H, Ar), 7.23 (dd, *J* = 4.6, 1.2 Hz, 1H, Ar), 6.80 (dd, *J* = 4.6, 1.2 Hz, 1H, Ar); EIMS m/z (Rel. Int.%) 448 ([M]$^+$, 35), 450 (16), 449 (9), 386 (30), 385 (19), 384 (71), 287 (41), 286 (35), 285 (100), 150 (4), 149 (26), 133 (12), 123 (5), 122 (13), 111 (3), 99 (8), 89 (22). Anal. Calcd. for $C_{18}H_{13}ClN_4O_2S_3$ (448.96): C, 48.16; H, 2.92; Cl, 7.90; N, 12.48; S, 21.42. Found: C, 48.37; H, 2.75; Cl, 8.17; N, 12.66; S, 21.26.

4-((4-Phenylthiazol-2-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (7).

N-(Thiazol-2-yl)-4-thioureidobenzenesulfonamide (5) (0.16 g, 0.5 mmol) and 2-bromo-1-phenylethan-1-one (0.10 g, 0.5 mmol) in of ethanol (20 mL) was refluxed for 3 h and cooled to room temperature. The solid product was filtered off and dried in air to give a pale yellow powder 7; M.p.: 258-260 °C (dec.); yield 0.108 g (86%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 10.81 (s, 1H, Ar) 10.69 (s, 1H, NH), 7.92-7.95 (m, 2H, Ar), 7.74-7.78 (m, 4H, Ar), 7.49 (d, *J* = 8.8 Hz, 2H, Ar), 7.43 (d, *J* = 8.8 Hz, 2H, Ar), 7.23 (d, *J* = 4.8 Hz, 1H, Ar), 6.80 (d, *J* = 4.8 Hz, 1H, Ar); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 168.55, 161.12, 147.29, 144.06, 143.38, 134.57, 133.24, 128.65, 128.39, 128.33, 128.00, 127.34, 125.75, 116.44, 116.07, 107.96, 91.64; EIMS m/z (Rel. Int.%) 414 ([M]$^+$, 21), 350 (43), 251 (100), 149 (33), 134 (62), 122 (16), 99 (25), 90 (36), 77 (23), 64 (26), 55 (56). Anal. Calcd. for $C_{18}H_{14}N_4O_2S3$ (414.52): C, 52.16; H, 3.40; N, 13.52; S, 23.20. Found: C, 52.39; H, 3.57; N, 13.25; S, 23.38.

4-((4-Oxothiazolidin-2-ylidene)amino)-N-(thiazol-2-yl)benzenesulfonamide (8).

N-(Thiazol-2-yl)-4-thioureidobenzenesulfonamide (5) (0.16 g, 0.5 mmol) and 2-bromo-*N*-(thiazol-2-yl)acetamide (0.11 g, 0.5 mmol) in ethanol (20 mL) was refluxed for 7 h and left to cool to room temperature. The reaction mixture was filtered off and the solid product was dried in air to give a pale yellow powder 8; M.p.: 239-241 °C; yield 0.07 g (39%); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 12.68 (s, 1H, NH), 9.95 (s, 1H, NH), 7.79-7.70 (m, 2H, Ar), 7.62-7.65 (m, 2H, Ar), 7.23-7.25 (m, 1H, Ar), 6.81-6.83 (m, 1H, Ar), 4.02 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) = 168.25, 161.07, 147.24, 143.32, 134.58, 133.17, 128.53, 127.90, 127.59, 127.22, 107.84, 28.33; FTIR (KBr disk)

ν/cm^{-1}: 3468, 3343, 3216, 3155, 3122, 2993, 2957. 1696, 1591, 1499, 1405, 1336, 1274, 1131, 1080, 929, 828, 690, 547 Anal. Calcd. for $C_{12}H_{10}N_4O_3S_3$ (354.42): C, 40.67; H, 2.84; N, 15.81; S, 27.14. Found: C, 40.90; H, 2.66; N, 15.63; S, 27.35.

Antimicrobial Activity

Two Gram negative bacteria; *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* (ATCC 8739), two Gram positive *Staphylococcus aureus* (25923) and *Bacillus cereus* (ATCC 33018), and two yeasts; *Candida albicans* (ATCC10231) and *Saccharomyces cereviseae* microbial strains were generously given by Microbiology Dep., Faculty of Agriculture, Cairo University and Microbiology Dep., Faculty of Agriculture, University of Modena and Reggio Emilia. Different Microbial strains were preserved on BHI agar and YPD agar slants for bacteria and yeast strains respectively, and kept at 4 ˚C and regularly transferred each 2 months. For experiment a loop full of bacteria or yeast was added to 10 mL of BHI or YBD broth and incubated at 37 or 32 ± 2 ˚C overnight for bacteria and yeasts respectively.

Screening of antimicrobial activity was performed using agar diffusion method. Nutrient agar plate for bacteria and YPDA plates for yeasts were over layered with approximately 2 mL soft agar inoculated with 10^5-10^6 cfu/mL of overnight activated microbial cultures, then wells of 6 mm diameter were holed by cork borer, 40 µL of each tested compound were injected in every well. Negative control was performed using dimethyl sulfoxide (DMSO). Plates were incubated for 24 h at 37 ˚C and 32 ± 2 ˚C for bacteria & yeasts respectively. Diameters of inhibition clear zones; without microbial growth were measured using graded ruler.

Molecular Docking

Docking simulations were performed to predict the binding mode of the sulfathiazole derivatives with active site of DHPS from Yersinia pestis, (PDB ID: 5*J*Q9) using its crystal structure which was downloaded from RCSB protein data bank (http://www.pdb.org) which is resolved at 2.10 Å using X-ray diffraction. The PDB file was retrieved from the Protein Data Bank and chain B was deleted. Structure of chain A was processed using the Structure Preparation application in MOE [26]. Subsequently, the missing hydrogen atoms were added and the charges were assigned properly. The resultant model was further refined by energy minimization to a gradient of 0.01 kcal/mol/Å keeping atoms tethered within 0.5 Å from their crystal structure positions. The default procedure in the MOE Dock application was used to find the favorable binding configurations of the studied ligands. Initial placement poses generated by the Alpha Triangle matcher were rescored and filtered using the London dG Scoring method to pick those exhibiting maximal hydrophobic, ionic, and hydrogen-bond contacts to the protein. This was followed by a refinement stage. The generated poses were energy minimized using the MMFF94x force field. Docking poses were visually inspected and interactions with binding pocket residues were analyzed.

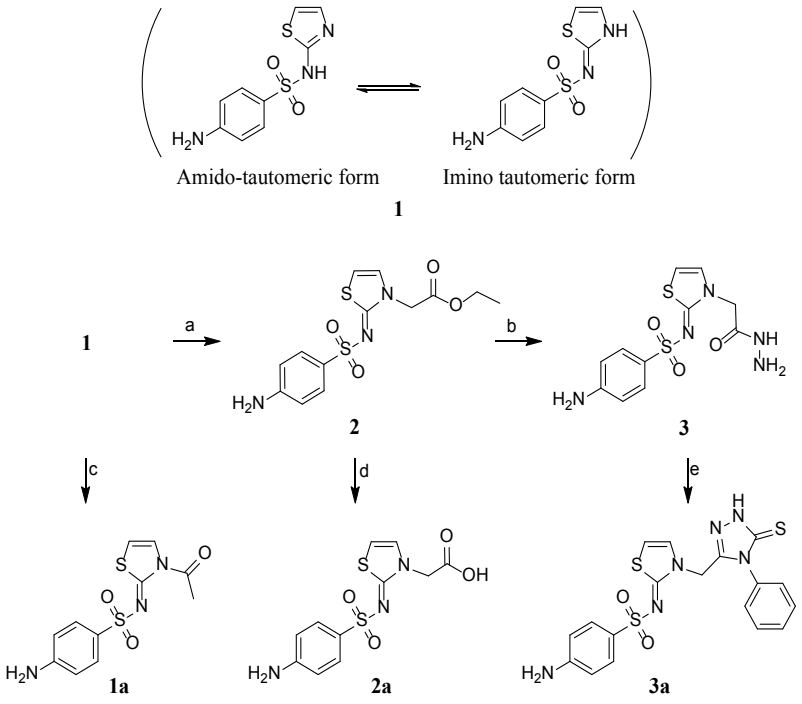
Results and Discussion

Synthesis

The synthesis of sulfathiazole derivatives starting with aniline has been described in the literature previously [27]. However, another benign and advantageous procedure that was used is depicted below in Scheme 1 [28]. Accordingly, 4-acetamidobenzenesulfonyl chloride, prepared by chloro-sulfonating *N*-phenylacetamide using warm chlorosulfonic acid, was reacted with 2-aminothiazole in refluxing acetonitrile in the presence of dry K_2CO_3 to afford *p*-acetamido-protected 1. Acid hydrolysis of the *p*-acetamido group of the latter by employing aqueous HCl followed by neutralization with Na_2CO_3 afforded 1. Unexpectedly, direct acetylation of 1 using acetyl chloride in dry pyridine at ambient temperature to obtain the precursor *p*-acetamido-protected 1 afforded 1a via the replacement of the acetyl group of for the active hydrogen atom of the NH of the thiazole ring. Sulfathiazole 2, the key compound, was synthesized via reacting sulfathiazole 1 in boiling acetone with ethyl bromoacetate in the presence of dry powdered K_2CO_3 as a heterogeneous base. Sulfathiazole 2 was treated with an ethanolic hydrazine hydrate at reflux temperature to afford 3 in a quantitative yield. The free acid 2a was obtained by nonaqueous saponification of the ester 2 using KOH with heating at 80 °C for 1 h followed by neutralization with HCl and filtration. The reaction of acyl hydrazide 3 with phenyl isothiocyante under reflux for 72 h afforded 3a in a quantitative yield. The reaction of the hydrazide 3 with a series of aromatic aldehydes in absolute ethanol at reflux temperature afforded acyl hydrazones 4a-h, Scheme 2. The structures of the compounds presented in Schemes 1-3 were characterized

using ^{1}H, ^{13}C, HSQC, and HMBC NMR, FTIR, mass spectrometry, and microanalyses. The ^{1}H NMR of 2 showed the characteristic triplet-quartet signals for the ethyl group besides the methylene spacer singlet. A signal appeared at 5.84 (2H) was assigned to the free NH_2. The HMBC spectrum of 2 showed a cross peak at 5.84, 111.41 ppm due to the ^{2}J coupling between the carbons of the phenyl ring and the NH_2 group. Additionally, a cross peak appeared at 4.74, 128.20 ppm due to the ^{3}J coupling interaction between the thiazole carbons and the methylene protons. This assignment is in a good agreement with the literature analysis of the sulfathiazole structure which exists in two tautomeric forms in various states (amido and the imino tautomeric forms; Scheme 1). Both the amido and the imino tautomeric forms exist in a state of equilibrium in the liquid state, however, the imino tautomer is exclusively found in the solid phase. According to a recent study on the sulfathiazole polymorphism two intramolecular interactions are constantly observed; one between the thiazole sulfur atom ($S^{\delta+}$) and an oxygen ($O^{\delta-}$) in the sulfone group, while the other interaction is attributed to the thiazole ring NH proton with the sulfone oxygen atom in the imino tautomer [29].

The ^{1}H NMR spectrum of 3 showed two new peaks at 4.25 and 9.34 ppm which were assigned to NH_2 and NH protons, respectively. The HMBC spectrum of 2 showed three landmark cross peaks. The first peak appears at 4.53, 165.81 ppm due to the ^{2}J ^{1}H-^{13}C coupling interaction between the carbonyl carbon and the neighboring CH_2 of the spacer. The two other cross peaks appear at 6.78 and 165.81 ppm and 7.26 and 165.81 ppm were assigned to the ^{3}J and ^{4}J ^{1}H-^{13}C coupling interactions between the carbonyl carbon and the two protons of the thiazole ring. Examination of the HMBC spectrum of 3 revealed a pertinent peak at 7.26, 166.34 ppm due to the ^{4}J ^{1}H-^{13}C interaction between the thiazole ring proton and the carbonyl carbon. Other cross peaks appeared at 7.26, 48.11 ppm and 4.53, 127.91 ppm were attributed to the ^{3}J ^{1}H-^{13}C interaction between the thiazole ring and the CH_2 of the spacer. Additionally, a cross peak appeared at 5.82, 112.75 ppm referred to the ^{3}J ^{1}H-^{13}C coupling interaction between the amino group protons and the carbon atoms of the phenyl ring. The ^{1}H NMR spectra of 4a-h exhibited resonance peaks at δ 12.01–9.91 ppm characteristic for CO-NH proton, whereas proton –N=C–H appeared at δ 8.73–7.43 ppm. The observed two groups of



Scheme 1. Reagent and conditions: (a) ethyl bromoacetate, K_2CO_3, acetone, reflux, 6 h; (b) hydrazine hydrate, ethanol, reflux, 4 h; (c) acetyl chloride, pyridine, stirring, rt, 4 h; (d) KOH, MeOH, then acidify with HCl; (e) phenyl isothiocyanate, ethanol, reflux, 72 h.

resonance in the ^{1}H NMR spectra in DMSO-d_6 of the synthesized hydrazones are due to the hindered rotation in the CO–NH group. According to the ^{1}H NMR and based on the previous studies on the stereo isomers of hydrazones, the HC=N protons are predominantly present in solutions as E geometrical imine isomer which has low steric hindrance compared to the Z isomer [30].

Our goal also was to synthesize sulfonamide derivatives containing multiple thiazole moieties. The synthesis of new thiazole ring was achieved by exploiting Hantzsch method as depicted in Scheme 3. Firstly, N-phenylthiourea 5 was formed upon the treatment of 4 with ammonium thiocyanate in H_2O/HCl mixture with stirring at reflux for 3 h. Secondly, N-phenylthiourea 5 was allowed to react with 2-bromo-1-(4-chlorophenyl) ethan-1-one in refluxing ethanol to afford dithiazole 6. Similarly, 5 reacted with 2-bromo-1-phenylethan-1-one which afforded dithiazole 7 in high yield. In an attempt to synthesis a trithiazole-containing compound 6, 2-bromo-*N*-(thiazol-2-yl)acetamide was reacted with 5 in absolute ethanol but compound 8 was obtained instead. A plausible mechanism explaining the formation of 8 is presented below (Figure 1).

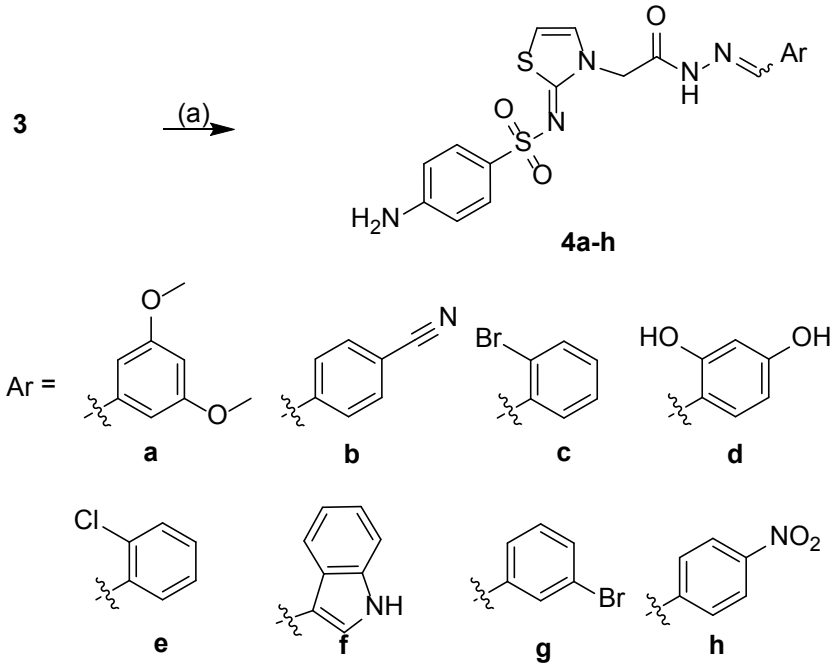
Antimicrobial Activity

The antimicrobial screening was achieved according to the Kirby Bauer disc diffusion method using 4.0 mM of the test compounds in DMSO, which was used as a solvent and a negative control [31]. The antimicrobial activity of all the newly synthesized compounds reported in this study was investigated in vitro against six microorganisms; two Gram-positive strains (Staphylococcus aureus and Bacillus cereus), two Gram-negative strains (Escherichia coli and Salmonella typhimurium), and two yeast species (Candida albicans and Saccharomyces cerevisiae). Only six compounds exhibited antimicrobial activity against the tested pathogens.

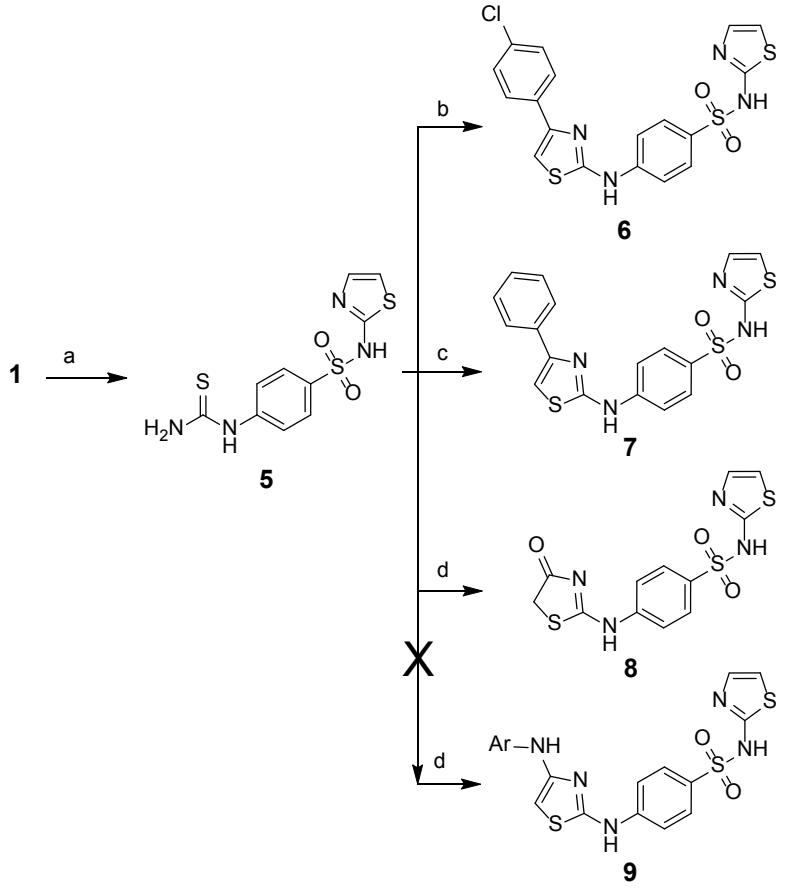
Among all the tested microorganisms *S. typhimurium* and *S. areus* showed susceptibility to most of tested antimicrobial compounds, while *E. coli* and *B. cereues* showed resistance to the tested compounds. On the other hand, the hydrazone 4g showed potent antimicrobial activity against all tested microorganisms (Table 1). The bacterial deactivation of 4g was 52, 79, 52, and 88% against *S. aureus, B. cereus, E. coli,* and *S. typhimurium,* respectively, compared to sulfamethoxazole as a reference antibacterial agent. The results showed that the substituted thiourea 5 showed the least activity in general. It was tolerant to *B. cereus* and *E. coli* as well as the fungal species under investigation on the other hand it showed moderate activity toward *S. aureus* and *S. typhimurium*. Compounds 6, 7, and 8 showed better activities towards the microorganisms compared to their precursor 5. This could be attributed to the contribution of the new formed thiazole ring (6 and 7) and thiazolone rings (8). Compound 6 that has two thiazole rings showed strong activity toward *B. cereus* (84%), *S. aureus,* (79%), and *E. coli* (76%) while it showed low activity toward *S. typhimurium* (55%), and *S. cerevisiea,* on the other hand it showed no activity toward *C. albicans*. S. aureus was susceptible to all compounds except for 8. *B. cereus* was susceptible to 4g and 6 and tolerant to the rest of the test compounds. *E. coli* showed resistance to compounds 4d, 5, and 8. *S. typhimurium* showed susceptibility to 4d, 4g, 5, 6, and 8 and resistance to 7. *C. albicans* showed susceptibility to 4d, 4g, 7, and 8 and resistance to 5 and 6. *S. cerevisiea* showed susceptibility to 4d, 4g, 6, and 8 and resistance to 5 and 7.

Docking studies with DHPS

The search for gaining information about the binding mode of the ligand and the DHPS active site motivated us for conducting the docking study. The docking study was essentially performed to predict and get closer and in depth visualization of the mode of interaction of the sulfathiazole derivatives with the active site of DHPS (5*J*Q9). The structural geometry optimizations of 5*J*Q9 and the sulfathiazole derivatives were obtained using quantum mechanics force field MMFF94x applying Gasteiger (PEOE) method for partial charges and the optimized structures were used as receptor and ligands, respectively, through in silico docking studies of sulfathiazole in the active site inside the 5*J*Q9 cavity. The most preferred binding modes of four molecules (4d, 4g, 6, and 7) with DHPS are illustrated below. The sulfathiazole derivatives interact with the active site of 5*J*Q9 via H-bonding, ionic, or hydrophobic interactions due to the elaboration of seventeen amino acid residues. The highest score poses obtained from the docking results showed that all the compound under investigation interacted with the active site residuals in a similar manner observed for pterin–sulfonamide conjugates [32]. Additionally, the acquired poses generally preserve most of the key interactions detected in the aforementioned conjugates. The benzene-



Scheme 2. Reagent and conditions: (a) absolute ethanol, reflux, 6-8 h.



Scheme 3. Reagent and conditions: (a) NH_4SCN, H_2O/HCl, 3 h; (b) 2-bromo-1-(4-chlorophenyl)ethan-1-one, ethanol, reflux,7 h; (c) 2-bromo-1-phenylethan-1-one, ethanol, reflux, 3 h; (d) 2-bromo-*N*-(thiazol-2-yl)acetamide, ethanol, reflux, 7 h.

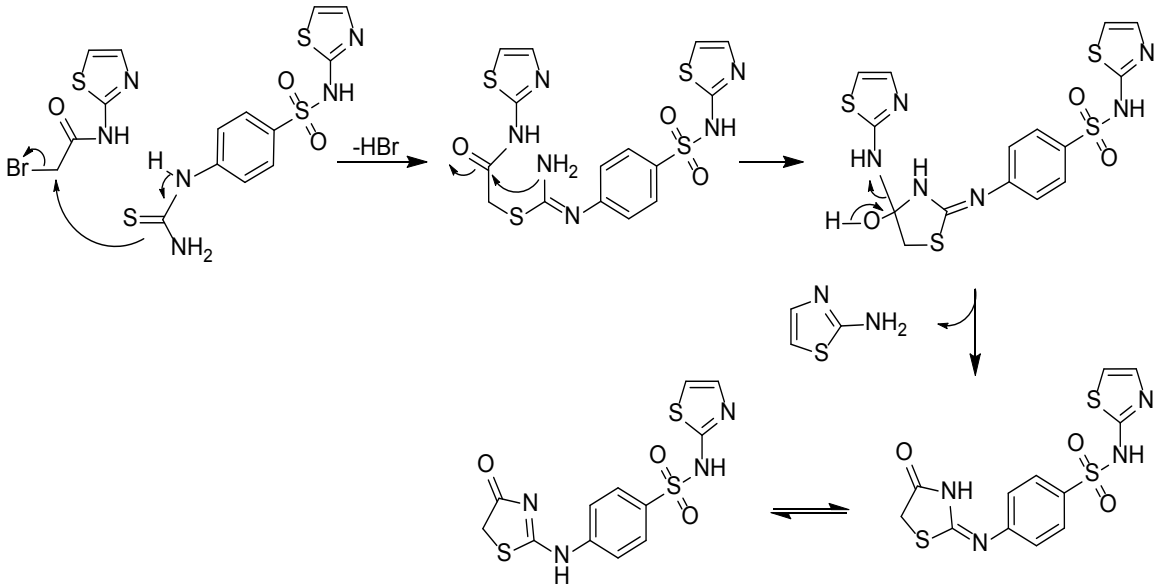


Figure 1. A plausible mechanism for the formation of 4-((4-oxothiazolidin-2-ylidene)amino)-*N*-(thiazol-2-yl) benzenesulfonamide (8).

TABLE 1. Antimicrobial activity of tested compound against different microbial strains. Inhibition zones diameter are indicated in mm.

Compounds		*S. aureus*	*B. cereus*	*E. coli*	*S. typhimurium*	*C. albicans*	*S. cerevisiea*
4d	9[a]	N/A	N/A	9.0	10	13	
4g	10	15	11	16	13	16	
5	10	N/A	N/A	11	N/A	N/A	
6	15	16	16	10	NA	11	
7	16	N/A	15	N/A	10	N/A	
8	N/A	N/A	N/A	11	12	10	
DMSO	N/A	N/A	N/A	N/A	N/A	N/A	
Sulfamethoxazole	19	19	21	18	-	-	
Fluconazole	-	-	-	-	22	21	

[a]Results are expressed as the mean of 4 replicates ± standard deviation.

*inhibition zone including diameter of the hole (6 mm) impregnated with 40 µL of different compounds.

N/A: not active (indicates that no inhibition zone determined).

sulfonamide moiety of hydrazide 4g is positioned suitably inside the active site of the enzyme thus creating the characteristic H-bond between the sulfonamide moiety and the amino acid residues of Asn22 (2.73 Å), Pro232 (2.84 Å), Thr62 (2.44Å) and His257 (2.74, 2.59Å) (Figure 2). Additionally, 3-bromophenyl residue acquires hydrophobic interactions. Whereas the compound 4d belongs to the hydrazones family in this study, it forms two H-bonds, one with Asp185 (2.82 Å, donor) and the other with Asp258 (2.80 Å, donor), and also there are different hydrophobic interactions with the amino acid residues of the cavity (Figure 2). Docking visualization of 6, which contains two thiazole rings showed four H-bonds contributed by the newly formed thiazole ring (Asn22, 2.57, acceptor) and the sulfonamide moiety; the NH group (Ser61, 2.28 Å, donor), the sulfoxide group (Arg255, 2.39 Å, acceptor), and the nitrogen atom of the thiazole ring (Arg 255, 2.89 Å, acceptor) as illustrated in Figure 2c. Compound 7 which is similar in structure to 6 but differs in the lack of 4-Cl group, interestingly, showed weak interaction with the receptor active site (Figure 2c-d). Thus, the sulfoxide group bound to Ser222 (255 Å, H-acceptor) and the NH interacts with Gly189 (2.11 Å) through H-bonds and there are other hydrophobic interactions. The types of the

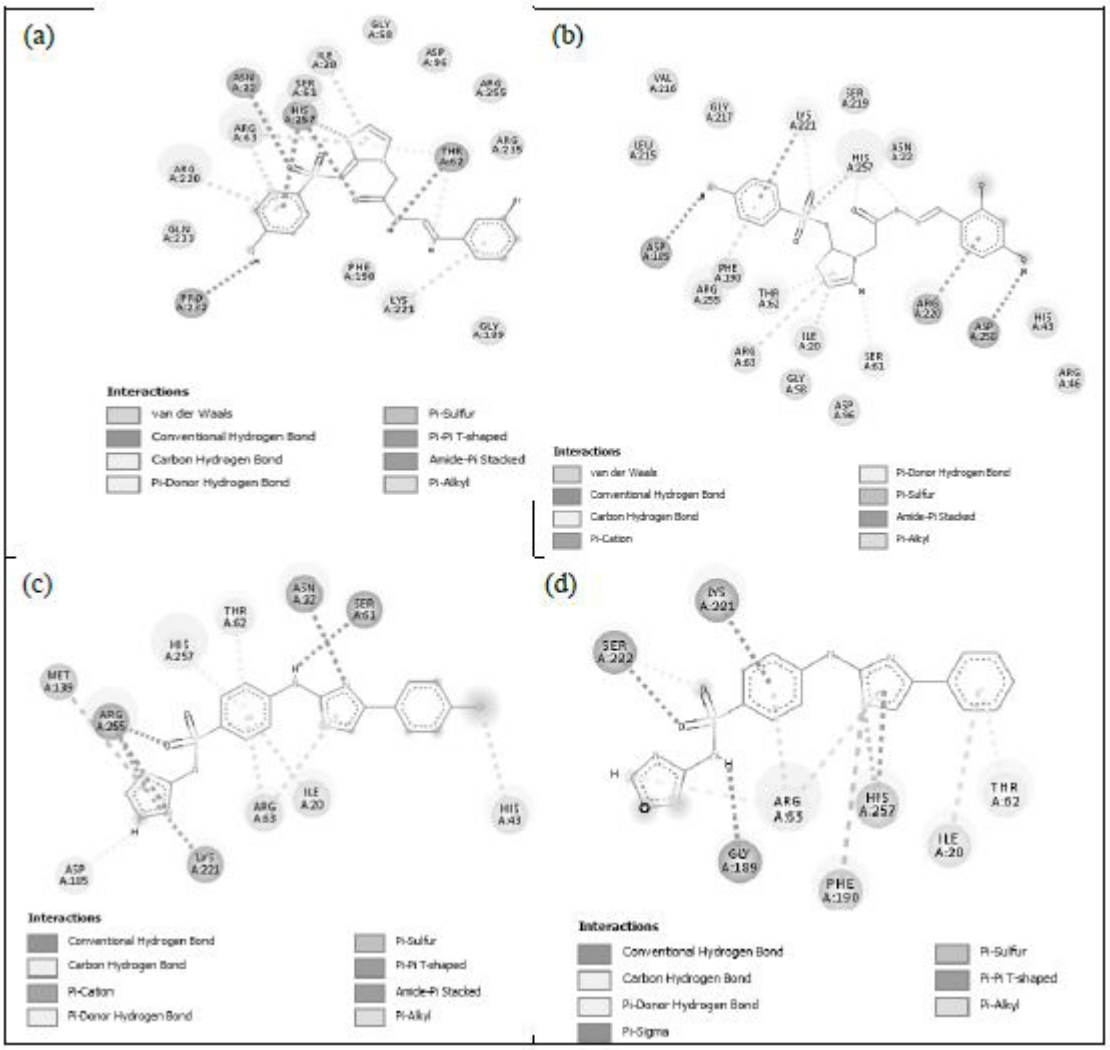


Figure 2. (a) 2D view of amino acid residues close to 4g in the best docked pose inside the binding pocket of 5JQ9. (b) 2D view of amino acid residues close to 4d in the best docked pose inside the binding pocket of 5JQ9. (c) 2D view of amino acid residues close to 6 in the best docked pose inside the binding pocket of 5JQ9. And (d) 2D view of amino acid residues close to 7 in the best docked pose inside the binding pocket of 5JQ9.

three H-bonds of the sulfanilamide (one donor and two acceptors) and the H-bond of the second thiazole ring in addition to the hydrophobic bonds could explain the higher antimicrobial activity of 6 compared to 7 even though they have similar structures. The biological activity assay supported by the molecular docking results, suggested that modification of the sulfonamide moiety by electron withdrawing group may be required for improved potential antibacterial activity of sulfonamides. The predicted binding modes of the hydrazides (4d and 4g), 6, and 7 residing in the pocket of DHPS is preventing the key substrate from binding which is common for all sulfa drugs and is the basis of their inhibitory action against DHPS.

Conclusion

In the present work, novel sulfathiazole derivatives were synthesized and their chemical structures were elucidated using several spectroscopic techniques such as 1D-NMR (^{1}H, ^{13}C), 2D-NMR (COSY, HMBC, and HSQC), FTIR, mass spectrometry, and microanalyses. Their antimicrobial activities were evaluated. Some of the synthesized compounds demonstrated potent inhibition against all the strains tested. It should be noted that compounds 4g, 6, and 7 showed good antimicrobial activities compared to the commercial antimicrobial agents. The docking study was achieved to give us a closer look on the binding mode of the ligand and the receptor (DHPS) active site. The results showed that 4g is positioned suitably inside the active site of the enzyme thus creating the characteristic H-bond between the sulfonamide moiety and the amino acid residues of Asn22 (2.73 Å), Pro232 (2.84 Å), Thr62 (2.44Å) and His257 (2.74, 2.59Å). These results could be a start point in the near future for seeking antimicrobial agents with reasonable economical cost for application in pharm.

Supplementary Information (SI)

Copies of the original spectra (^{1}H, ^{13}C, COSY, HMBC, and HSQC NMR, etc.) of the molecules reported in the experimental section are included in Supplementary Information.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Chen D., Milacic V., Frezza M., Dou Q.P., Metal complexes, their cellular targets and potential for cancer therapy. *Curr. Pharm. Des.,* **15** (7), 777-791 (2009).

2. Drews *J*., Drug discovery: A historical perspective. *Science*, **287** (5460), 1960-1964 (2000).

3. Homem V., Santos L., Degradation and removal methods of antibiotics from aqueous matrices – A review. *J. Environ. Econ. Manag.*, **92** (10), 2304-2347 (2011).

4. Tačić A., Nikolić V., Nikolić L., Savić I., Antimicrobial sulfonamide drugs. *Adv. Technol.,* **6** (1), 58-71 (2017).

5. Ballagi-Pordány G., Köszeghy A., Koltai M.-Z., Aranyi Z., Pogátsa G., Divergent cardiac effects of the first and second generation hypoglycemic sulfonylurea compounds. *Diabetes Res. Clin. Pr.,* **8** (2), 109-114 (1990).

6. Marble A., Glibenclamide, a new sulphonylurea: whether oral hypoglycaemic agents? *Drugs*, **1** (2), 109-115 (1971).

7. Malaisse W.*J*., Gliquidone contributes to improvement of Type 2 diabetes mellitus management. *Drug. R&D,* **7** (6), 331-337 (2006).

8. Rosa M.M., Dias T., Chapter 54 - Commonly used endocrine drugs, in: B. *J*osé, M.F. *J*osé (Eds.), *Handbook of Clinical Neurology, Elsevierpp*. 809-824 (2014).

9. Kremer E.K., Facchin G., Estevez E., Albores P., Baran E.*J*., Ellena *J*., Torre M.H., Copper complexes with heterocyclic sulfonamides: Synthesis, spectroscopic characterization, microbiologicaland SOD-like activities: Crystal structure of [Cu(sulfisoxazole) 2(H_2O)$_4$].2H_2O. *J. Inorg. Biochem.,* **100**, 1167-1175 (2006).

10. Cole D.C., Lennox W.*J*., Stock *J*.R., Ellingboe *J*.W., Mazandarani H., Smith D.L., Zhang G., Tawa G.*J*., Schechter L.E., Conformationally constrained N1-arylsulfonyltryptamine derivatives as 5-HT6 receptor antagonists. *Bioorg. Med. Chem. Lett.,* **15**, 4780-4785 (2005).

11. Friedberg C.K., Taymor R., Minor *J*.B., Halpern M., The use of diamox, a carbonic anhydrase inhibitor, as an oral diuretic in patients with

congestive heart failure. *N. Engl. J. Med.,* **248** (21), 883-889 (1953).

12. McCullough *J*.L., Maren T.H., Inhibition of dihydropteroate synthetase from Escherichia coli by sulfones and sulfonamides. *Antimicrob. Agents Chemother.*, **3** (6), 665-669 (1973).

13. Vinnicombe H.G., Derrick *J*.P., Dihydropteroate synthase from Streptococcus pneumoniae: Characterization of substrate binding order and sulfonamide inhibition. *Biochem. Biophys. Res. Commun.,* **258** (3), 752-757 (1999).

14. Levy C., Minnis D., Derrick *J*eremy P., Dihydropteroate synthase from Streptococcus pneumoniae: structure, ligand recognition and mechanism of sulfonamide resistance. *Biochem. J.,* **412** (2), 379-388 (2008).

15. Zani F., Vicini P., Antimicrobial activity of some 1,2-benzisothiazoles having a benzenesulfonamide moiety. *Arch. Pharm.,* **331** (6), 219-223 (1998).

16. Supuran C.T., Scozzafava A., *J*urca B.C., Ilies M.A., Carbonic anhydrase inhibitors - Part 49: Synthesis of substituted ureido and thioureido derivatives of aromatic/heterocyclic sulfonamides with increased affinities for isozyme I. *Euro. J. Med. Chem.,* **33** (2), 83-93 (1998).

17. Babaoglu K., Qi *J*., Lee R.E., White S.W., Crystal structure of 7,8-dihydropteroate synthase from bacillus anthracis. *Structure*, **12** (9), 1705-1717 (2004).

18. Hevener K.E., Yun M., Qi *J*., Kerr I.D., Babaoglu K., Hurdle *J*.G., Balakrishna K., White S.W., Lee R.E., Structural studies of pterin-based inhibitors of dihyropteroate synthase. *J. Med. Chem.,* **53** (1), 166-177 (2010).

19. Glicklich E.A., Sulfathiazole ointment in the treatment of pyogenic dermatoses. *N. Engl. J. Med.,* **226** (25), 981-983 (1942).

20. Gordin F.M., Simon G.L., Wofsy C.B., Mills *J*., Adverse reactions to trimethoprim-sulfamethoxazole in patients with the acquired immunodeficiency syndrome. *Ann. Intern. Med.,* **100** (4), 495-499 (1984).

21. Lin D., Tucker M.*J*., Rieder M.*J*., Increased adverse drug reactions to antimicrobials and anticonvulsants in patients with HIV infection. *Ann. Pharmacother.,* **40** (9), 1594-1601 (2006).

22. Summan M., Cribb A.E., Novel non-labile covalent binding of sulfamethoxazole reactive metabolites to cultured human lymphoid cells. *Chem.-Biol. Interact.,* **142** (1–2), 155-173 (2002).

23. Kamel E.M., Gaballah S.T., Mohamed Y.M.A., Mohamed N.R., Synthesis of New Pyrazolones and Fused Pyrazole Derivatives as Antimicrobial Agents. *Egy. J. Chem.,* **53** (5), 731-744 (2010).

24. Fosbinder R.*J*., Walter L.A., Sulfanilamido Derivatives of Heterocyclic Amines. *J. Am. Chem. Soc.,* **61** (8), 2032-2033 (1939).

25. Leitch L.C., Baker B.E., Brickman L., Synthesis of Sulphanilylthiourea and Related Compounds. *Can. J. Res. Sec. B: Chem. Sci.,* **23** (4), 139 (1945).

26. (MOE) M.O.E., Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A2R7, (2014).

27. Roberts R.M., Gilbert *J*.C., Rodewald L.B., Wingrove A.S., *An Introduction to Modern Experimental Organic Chemistry,* Holt, Rinehart and Winston, New York (1974).

28. Boyle *J*., Otty S., Sarojini V., A safer and convenient synthesis of sulfathiazole for undergraduate organic and medicinal chemistry classes. *J. Chem. Edu.,* **89** (1), 141-143 (2012).

29. Sovago I., Gutmann M.*J*., Hill *J*.G., Senn H.M., Thomas L.H., Wilson C.C., Farrugia L.*J*., Experimental electron density and neutron diffraction studies on the polymorphs of sulfathiazole. *Cryst. Growth Des.,* **14** (3), 1227-1239 (2014).

30. Palla G., Pelizzi C., Predieri G., Vignali C., Conformational study on N-acylhydrazones of aromatic aldehydes by NMR spectroscopy. *Gazz. Chim. Ital.,* **112**, 339–341 (1982).

31. Bauer A.M., Kirby W.M., Sherris C., Turck M., Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Path.*, **45**, 493-496 (1966).

32. Zhao Y., Shadrick W.R., Wallace M.*J*., Wu Y., Griffith E.C., Qi *J*., Yun M.-K., White S.W., Lee R.E., Pterin–sulfa conjugates as dihydropteroate synthase inhibitors and antibacterial agents. *Bioorg. Med. Chem. Lett.,* **26** (16), 3950-3954 (2016).