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RESEARCH ARTICLE

Design, synthesis, antimicrobial evaluation and molecular docking studies of some new 2,3-dihydrothiazoles and 4-thiazolidinones containing sulfisoxazole

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Abstract

Microbial resistance to the available drugs poses a serious threat in modern medicine. We report the design, synthesis and *in vitro* antimicrobial evaluation of new functionalized 2,3-dihydrothiazoles and 4-thiazolidinones tagged with sulfisoxazole moiety. Compound **8d** was most active against *Bacillis subtilis* (MIC, 0.007 μ g/mL). Moreover, compounds **7c-d** and **8c** displayed significant activities against *B. subtilis* and *Streptococcus pneumoniae* (MIC, 0.03–0.06 μ g/mL and 0.06–0.12 μ g/mL versus ampicillin 0.24 μ g/mL and 0.12 μ g/mL; respectively). Compounds **7a** and **7c-d** were highly potent against *Escherichia coli* (MIC, 0.49–0.98 μ g/mL versus gentamycin 1.95 μ g/mL). On the other hand, compounds **7e** and **9c** were fourfolds more active than amphotericin B against *Syncephalastrum racemosum*. Molecular docking studies showed that the synthesized compounds could act as inhibitors for the dihydropteroate synthase enzyme (DHPS). This study is a platform for the future design of more potent antimicrobial agents.

Introduction

Recently, many drug-resistant human pathogenic microbes have been reported¹. This has been attributed to the widespread use of antibacterial and antifungal agents as well as the inaccurate diagnosis². Methicillin-resistant Staphylococcus aureus³, vancomycin-resistant Enterococci⁴ and azole-resistant Candida species⁵ are well-known examples of drug-resistant microbes. It is particularly challenging to treat infections caused by these microbes especially in the case of immunocompromised patients⁶. Therefore, it is crucial to search for new potent antimicrobial agents⁷. There are two main strategies for the discovery of new drugs; either to search for a novel lead compound or to modify the structure of a known drug⁸. In the second strategy, two or more pharmacophores are often combined in one molecule to obtain the synergistic effect⁹. Sulfonamide derivatives have numerous biological activities including antibacterial¹⁰, carbonic anhydrase inhibitor¹¹, insulin release inducer¹², antiviral¹³, antifungal¹⁴, anticancer¹⁵ and anti-inflammatory activities¹⁶. It is known that sulfonamides reduce the biosynthesis of dihydrofolic acid through the competitive inhibition of the dihydropteroate synthase enzyme (DHPS)¹⁷; and sulfisoxazole is an example of sulfa drugs that inhibits the binding of p-amino benzoic acid (PABA) to DHPS binding pocket. To the best of our knowledge, only two crystal structures of a sulfa-related drug bound to the active site of DHPS

Keywords

Antimicrobial agents, molecular docking, sulfonamide, thiazole

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were solved and reported in the Protein Data Bank (PDB). The crystal structure of *Bacillus anthracis* dihydropteroate synthase (BaDHPS) bound to sulfathiazole-6-hydroxymethyl-7,8-dihydropterin-pyrophosphate (STZ-DHPP) adduct was reported by Yun et al¹⁸. In this structure, the STZ-DHPP adduct occupies both the PABA and pterin-binding pockets of DHPS (Figure I, Supplementary data)¹⁸. Trimethoprim, the dihydrofolate reductase inhibitor, is used in combination with sulfa drugs to increase the therapeutic efficacy¹⁹. On the other hand, 2,3-dihydrothiazoles and 4-thiazolidinones have occupied a unique position in the design and synthesis of biologically active antimicrobial agents^{20–22}.

In this study, we used the reported STZ-DHPP adduct¹⁸ as a lead compound to design potent antimicrobial agents targeting both the PABA and pterin-binding pockets of the DHPS enzyme, thereby inhibiting the biosynthesis of the dihydrofolic acid. The thiazole, methylene and pteridine moieties were replaced by the 3,4-dimethyl-isoxazole, carbonyl and substituted thiazole rings; respectively (Figure 1). Sulfisoxazole was used as a starting material and the target compounds were tested *in vitro* for their antibacterial and antifungal activities against human pathogenic microbes. The observed biological results were rationalized by molecular docking and lipophilicity studies.

Methods

Chemistry

Melting points were determined on digital Gallen-Kamp MFB-595 instrument (UK) using open capillary tubes and were uncorrected. IR spectra were recorded on Schimadzu FTIR 440

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Figure 1. Design of thaizole derivatives bearing sulfisoxazole moiety as DHPS inhibitors.



spectrometer (Kyoto, Japan) using KBr pellets. Mass spectra were performed on Shimadzu Qp-2010 plus mass spectrometer at 70 eV (Kyoto, Japan). ¹H NMR and ¹³C NMR spectra were recorded on a Bruker model (500 MHz or 400 MHz) Ultra Shield NMR spectrometer (Billerica, MA) in DMSO-d₆ using tetramethylsilane (TMS) as an internal standard; chemical shifts are reported as δ_{ppm} units. The elemental analyses were done at the Microanalytical Center, Cairo University, Cairo, Egypt. Sulfisoxazole **1** was purchased from Sigma-Aldrich (St. Louis, MO). 1-Cyanoacetyl-3,5-dimethylpyrazole **2**²³ and 2-cyano-*N*-(4-{[(3,4-dimethylisoxazole-5-yl)amino]sulfonyl}phenyl)acetamide **3** (24) were synthesized according to the reported procedures.

4-Amino-2,3-dihydro-3-phenyl-2-thioxothiazole-5-*N*-(4-{[(3,4-dimethylisoxazole-5-yl)amino]sulfonyl}phenyl) carboxamide (4)

Phenylisothiocyanate (1.35 g, 0.01 mol), sulfur (0.32 g, 0.01 mol)and triethylamine (0.3 mL) were added to a hot solution of compound **3** (3.34 g, 0.01 mol) in a mixture of absolute ethanol (20 mL) and dimethylformamide (DMF) (5 mL). The reaction mixture was heated at $65 \degree \text{C}$ for 8 h. It was allowed to cool, and then poured on ice water (200 mL) and the medium was neutralized by dilute HCl. The obtained solid was filtered off, washed with water (50 mL), air dried and recrystallized from ethanol/DMF to afford the target compound.

Pale yellow powder, yield (83%), mp 195–196 °C; IR (KBr) v_{max}/cm^{-1} : 3409–3351 (NH₂), 3279 (NH), 3206 (NH), 3045 (CH– Ar), 2986 (CH-sp³), 1659 (CO), 1175 (C=S); ¹H NMR (400 MHz, DMSO-d₆): $\delta_{ppm} = 1.64$ (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 7.10 (s, 2H, NH₂), 7.34 (d, J = 9.0 Hz, 2H, phenyl-H₂', phenyl-H₆'), 7.42 (d, J = 8.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.63 (d, J = 8.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 7.88 (m, 3H, phenyl-H₃', Phenyl-H₄', phenyl-H₅'), 10.74 (s, 1H, NHSO₂), 11.14 (s, 1H, NHCO); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 6.3$ (CH₃), 10.8 (CH₃), 105.3 (thiazole-C₅), 119.6, 120.6, 128.1, 128.5, 129.3, 130.6, 133.0, 134.9, 142.0, 143.0 (12C, C₆H₄, C₆H₅), 154.0 (isoxazole-C₄), 156.2, 161.0, 161.8, 162.0 (4C, isoxazole-C₃, isoxazole-C₅, thiazole-C₄, CONH), 187.0 (C=S); MS *m/z* (%): 501 (M⁺, 0.1), 293 (0.4), 266 (0.1), 251 (0.1), 235 (0.2), 175 (1.9), 136 (15.9), 111 (0.5), 96 (12.3), 77 (45.1), 52 (100); Anal. Calcd. for $C_{21}H_{19}N_5O_4S_3$ (501.60): C, 50.28; H, 3.82; N, 13.96%, Found: C, 50.31; H, 3.85; N, 13.99%.

General procedure for the synthesis of ketene N,S-acetal potassium salt derivatives 6a-e

Potassium hydroxide (0.56 g, 0.01 mol) was added to a solution of compound **3** (3.34 g, 0.01 mol) in DMF (30 mL). The reaction mixture was stirred for 1 h at room temperature. Substituted isothiocyanate derivatives 5a-e (0.01 mol) were then added dropwise and the mixture was stirred for 24 h at room temperature. Without separation, the obtained products were used for further reactions.

General procedure for the synthesis of functionalized 4-methylthiazole derivatives 7a-e

Chloroacetone (0.79 mL, 0.01 mol) was added dropwise to a wellstirred solution of the intermediate compounds **6a–e** (0.01 mol) in DMF (30 mL) at 0 °C. After complete addition, the reaction mixture was stirred at room temperature for 18 h then poured on ice water (200 mL). The medium was neutralized by dilute HCl and the obtained solid was filtered off, washed with water (50 mL), air dried and recrystallized from ethanol to afford the 4-methyl-thiaole derivatives **7a–e**.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3,4-dimethyl-thiazol-2(3H)-ylidene)-acetamide (7a)

Yellowish brown powder, yield (78%), mp 153–154 °C; IR (KBr) v_{max}/cm^{-1} : 3238 (NH), 3101 (NH), 3046 (CH–Ar), 2931 (CH-sp³), 2182 (CN), 1656 (CO); ¹H NMR (500 MHz, DMSOd₆): $\delta_{ppm} = 1.71$ (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 3.25 (s, 3H, NCH₃), 7.61 (s, 1H, thiazol-H₅), 7.71 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.81 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.89 (s, 1H, NHSO₂), 10.91 (s, 1H, CONH); MS m/z (%): 445 (M⁺, 0.2), 294 (0.2), 266 (0.4), 194 (0.6), 179 (1.00), 175 (1.5), 151 (1.5), 113 (2.1), 111 (6.1), 96 (5.8), 64 (100); Anal. Calcd. For C₁₉H₁₉N₅O₄S₂ (445.52): C, 51.22; H, 4.30; N, 15.72%, Found: C, 51.20; H, 4.28; N, 15.76%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-ethyl-4-methyl-thiazol-2(3H)-ylidene)-acetamide (7b)

Yellow powder, yield (74%), mp 162-163 °C; IR (KBr) v_{max}/cm⁻¹: 3362 (NH), 3283 (NH), 3049 (CH-Ar), 2977 (CHsp³), 2178 (CN), 1646 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{\text{ppm}} = 1.23$ (t, J = 7.0 Hz, 3H, CH₃), 1.71 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 3.25 (q, J = 7.0 Hz, 2H, CH₂), 7.63 (s, 1H, thiazol-H₅), 7.71 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.81 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.89 (s, 1H, NHSO₂), 10.93 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.9$ (CH₃), 10.2 (CH₃), 13.8 (CH₃), 27.9 (CH₃), 56.0 (CH₂), 94.0 (acetamide-C₂), 99.5 (CN), 104.9 (thiazole-C₅), 119.9 (2C, phenyl-C₂, phenyl-C₆), 127.4 (2C, phenyl-C₃, phenyl-C₅), 133.4, 143.6, 155.4, 155.5, 161.3, 163.3, 164.2 (7C, phenyl-C₁, phenyl-C₄, isoxazole-C₃, isoxazole-C₄, isoxazole-C₅, thiazole-C₂, thiazole-C₄), 165.9 (CONH); MS m/z (%): 460 ([M+H]⁺, 1.3), 459 (M⁺, 3.6), 208 (2.2), 193 (1.4), 175 (0.1), 127 (2.5), 111 (5.1), 96 (1.9), 92 (100); Anal. Calcd. for C₂₀H₂₁N₅O₄S₂ (459.54): C, 52.27; H, 4.61; N, 15.24%, Found: C, 52.32; H, 4.65; N, 15.27%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-allyl-4-methyl-thiazol-2(3H)-ylidene)-acetamide (7c)

Yellowish green powder, yield (63%), mp 118-119°C; IR (KBr) v_{max}/cm⁻¹: 3335 (NH), 3275 (NH), 3051 (CH-Ar), 2928 (CHsp³), 2189 (CN), 1649 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{\text{ppm}} = 1.64$ (s, 3H, CH₃), 1.70 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 3.87 (m, 2H, CH_2N), 5.22 (dd, J = 1.5 Hz, 17.5 Hz, 1H, $CH_2 =$), 5.30 (dd, J = 1.5 Hz, 17.5 Hz, 1H, CH₂=), 5.89 (m, 1H, CH=), 7.65 (s, 1H, thiazol-H₅), 7.71 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.81 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.93 (s, 1H, NHSO₂), 10.94 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.9$ (CH₃), 10.2 (CH₃), 27.9 (CH₃), 48.7 (CH₂N), 74.0 (acetamide-C₂), 100.1 (CN), 104.8 (thiazole-C₅), 117.0 (CH₂=), 119.9 (2C, phenyl-C₂, phenyl-C₆), 127.4 (2C, phenyl-C₃, phenyl-C₅), 128.1, 133.3, 133.5, 143.6, 155.6, 161.3, 163.3, 164.3 (8C, CH=, phenyl- C_1 , phenyl- C_4 , isoxazole- C_3 , isoxazole-C₄, isoxazole-C₅, thiazole-C₂, thiazole-C₄), 165.6 (CONH); MS m/z (%): 472 ([M+H]⁺, 9.4), 471 (M⁺, 5.8), 444 (5.5), 375 (3.5), 266 (2.5), 251 (2.9), 177 (9.5), 175 (7.1), 138 (100), 111 (19.4), 96 (9.9); Anal. Calcd. for C₂₁H₂₁Nz₅O₄S₂ (471.55): C, 53.49; H, 4.49; N, 14.85%, Found: C, 53.53; H, 4.53; N, 14.87%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-adamantyl-4-methyl-thiazol-2(3H)-ylidene)-acetamide (7d)

Yellow powder, yield (87%), mp 261-262 °C; IR (KBr) v_{max}/cm⁻¹: 3334 (NH), 3267 (NH), 3058 (CH-Ar), 2912 (CHsp³), 2263 (CN), 1652 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.65$ (m, 9H, 9 × adamantyl-H), 1.70 (s, 3H, CH₃), 2.01 (m, 3H, $3 \times$ adamantyl-H), 2.08–2.09 (m, 6H, CH₃, $3 \times$ adamantyl-H), 2.14 (s, 3H, CH₃), 7.65 (s, 1H, thiazol-H₅), 7.71 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.78 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.80 (s, 1H, NHSO₂), 10.90 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.7$ (CH₃), 10.3 (CH₃), 18.5, 28.1, 28.8, 29.0, 35.4, 35.5, 39.7, 39.8, 39.9, 40.1, 40.4 (11C, CH₃, 10 × adamantyl-C), 53.3 (acetamide-C₂), 100.8 (CN), 104.8 (thiazole-C₅), 120.0 (2C, phenyl-C₂, phenyl-C₆), 127.4 (2C, phenyl-C₃, phenyl-C₅), 128.1, 137.5, 143.1, 153.9, 160.7, 161.2, 164.1 (7C, phenyl- C_1 , phenyl- C_4 , isoxazole- C_3 , isoxazole-C₄, isoxazole-C₅, thiazole-C₂, thiazole-C₄), 165.8 (CONH); MS m/z (%): 566.71 ([M+H]⁺, 13.4), 565 (M⁺, 4.3), 175 (16.9), 135 (11.1), 111 (18.7), 96 (21.9), 93 (100);

Anal. Calcd. for $C_{28}H_{31}N_5O_4S_2$ (565.71): C, 59.45; H, 5.52; N, 12.38%, Found: C, 59.43; H, 5.53; N, 12.41%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(4-methyl-3-phenyl-thiazol-2(3H)-ylidene)-acetamide (7e)

Yellow powder, yield (79%), mp 130-131°C; IR (KBr) v_{max}/cm⁻¹: 3323 (NH), 3238 (NH), 3048 (CH-Ar), 2961 (CHsp³), 2239 (CN), 1649 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{\text{ppm}} = 1.70$ (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.15 (s, 3H, CH₃), 7.49-7.79 (m, 10H, C₆H₅, C₆H₄, thiazol-H₅), 9.21 (s, 1H, NHSO₂), 10.92 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSOd₆): $\delta_{ppm} = 6.3$ (CH₃), 10.7 (CH₃), 14.7 (CH₃), 67.3 (acetamide-C₂), 96.6 (CN), 105.4 (thiazole-C₅), 107.5, 121.0, 124.1, 127.9, 128.7, 129.4, 137.0, 138.9, 144.5, 156.0, 161.8, 162.2, 165.7 (17C, C₆H₅, C₆H₄, isoxazole-C₃, isoxazole-C₄, isoxazole-C₅, thiazole-C₂, thiazole-C₄), 167.0 (CONH); MS m/z (%): 508 ([M+H]⁺, 18.5), 507 (M⁺, 21.9), 481 (0.8), 411 (14.6), 396 (18.5), 332 (4.5), 294 (1.7), 266 (5.6), 256 (12.3), 175 (15.2), 130 (100), 96 (14.3), 93 (26.2), 77 (2.8); Anal. Calcd. for C₂₄H₂₁N₅O₄S₂ (507.58): C, 56.79; H, 4.17; N, 13.80%, Found: C, 56.83; H, 4.21; N, 13.84%.

General procedure for the synthesis of *N*-phenyl-thiazole derivatives 9a-e

A variety of α -halo carbonyl reagents **8a–e** (0.01 mol) were added dropwise to a well-stirred solution of the non-isolable potassium salt **6e** (0.01 mol) in DMF (30 mL) at 0 °C. The reaction mixture was stirred at room temperature for 18 h then poured on ice water (200 mL) and the medium was neutralized by dilute HCl. The obtained solid was filtered off, washed with water (50 mL), air dried and recrystallized from ethanol to afford the *N*-phenylthiazole derivatives **9a–e**.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(2,3-dihydro-5-acetyl-4-methyl-3-phenyl-2-ylidene-thiazol)-acetamide (**9a**)

Brown powder, yield (73%), mp 196-197 °C; IR (KBr) v_{max}/cm⁻¹: 3347 (NH), 3232 (NH), 3047 (CH-Ar), 2943 (CHsp³), 2201 (CN), 1705 (CO), 1663 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{\text{ppm}} = 1.69$ (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.11 (s, 3H, CH₃), 2.50 (s, 3H, CO<u>CH₃</u>), 7.13 (m, 1H, phenyl-H_{4'}), 7.35–7.45 (m, 2H, phenyl- $H_{2'}$ and phenyl- $H_{6'}$), 7.49–7.55 (m, 2H, phenyl-H_{3'} and phenyl-H_{5'}), 7.71 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.84 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.81 (s, 1H, NHSO₂), 10.95 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.9$ (CH₃), 10.2 (CH₃), 18.5 (CH₃), 56.0 (CH₃CO), 77.5 (acetamide-C₂), 104.9 (CN), 106.0, 111.1, 119.8, 120.5, 124.1, 127.4, 129.3, 130.0, 133.7, 141.0 (14C, C₆H₄, C₆H₅, thiazole- C_4 and thiazole- C_5), 143.4, 155.5, 161.3 (3C, isoxazole-C₃, isoxazole-C₄, isoxazole-C₅), 162.8 (CONH), 171.9 (thiazole-C₂), 195.0 (CH₃<u>CO</u>); MS *m*/*z* (%): 549 (M⁺, 0.9), 534 (12.0), 506 (27.4), 472 (18.0), 266 (39.1), 175 (3.8), 96 (77.4), 77 (51.2), 63 (100); Anal. Calcd. for C₂₆H₂₃N₅O₅S₂ (549.62): C, 56.82; H, 4.22; N, 12.74%, Found: C, 56.86; H, 4.27; N, 12.77%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(2,3-dihydro-5-ethylcarboxylate 4-methyl-3-phenyl-2-ylidene-thiazol)-acetamide (**9b**)

Yellow powder, yield (66%), mp 117–118 °C; IR (KBr) v_{max}/cm^{-1} : 3357 (NH), 3231 (NH), 3061 (CH–Ar), 2984 (CHsp³), 2191 (CN), 1701 (CO), 1652 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.32$ (s, 3H, CH₃), 1.69 (s, 3H, CH₃), 1.93 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 4.1 (s, 2H, O<u>CH₂</u>), 7.10–7.35 (m, 5H, C₆H₅), 7.73 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.86 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.88 (s, 1H, NHSO₂), 10.93 (s, 1H, CONH); MS m/z (%): 581 ([M+2]⁺, 65.7), 579 (M⁺, 61.1), 505 (48.1), 483 (57.4), 405 (61.1), 335 (100), 329 (48.1), 294 (62.9), 252 (29.6), 73 (44.4); Anal. Calcd. for C₂₇H₂₅N₅O₆S₂ (579.65): C, 55.95; H, 4.35; N, 12.08%, Found: C, 55.92; H, 4.37; N, 12.14%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(2,3-dihydro-4-ethylacetate-3-phenyl-2-ylidene-thiazol)acetamide (**9c**)

Brown powder, yield (53%), mp 119-120°C; IR (KBr) v_{max}/cm⁻¹: 3390 (NH), 3254 (NH), 3064 (CH-Ar), 2985 (CHsp³), 2194 (CN), 1725 (CO), 1653 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.63 - 1.70$ (m, 6H, CH₃ and <u>CH₃CH₂O)</u>, 2.08 (s, 3H, CH₃), 2.51 (s, 2H, CH₂COO), 4.09 (q, J = 7.5 Hz, 2H, CH₂OOC), 7.28 (s, 1H, thiazole-H₅), 7.38–7.50 (m, 5H, phenyl- $H_{2'}$, phenyl- $H_{3'}$, phenyl- $H_{4'}$, phenyl- $H_{5'}$ and phenyl- $H_{6'}$), 7.72 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.84 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.89 (s, 1H, NHSO₂), 10.95 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.8$ (CH₃), 10.2 (CH₃), 14.0 (CH₃), 39.0 (CH₂COO), 60.4 (CH₂OOC), 84.0 (acetamide-C₂), 105.0, 112.6 (2C, thiazole-C₅ and CN), 119.9, 120.4, 120.6, 120.9, 124.0, 127.4, 128.6, 129.3, 129.8 (13C, C₆H₄, C₆H₅ and thiazole-C₄), 143.4, 155.5, 161.3 (3C, isoxazole-C₃, isoxazole-C₄, isoxazole-C₅), 162.6 (CONH), 167.5 (COO), 171.0 (thiazole-C₂); MS m/z (%): 580 ([M+1]⁺, 5.2), 579 ($\overline{M^+}$, 7.5), 550 (7.4), 506 (6.6), 328 (1.9), 294 (1.9), 285 (1.4), 266 (4.1), 162 (100), 111 (19.7), 96 (19.6), 87 (4.1), 77 (11.6); Anal. Calcd. for C₂₇H₂₅N₅O₆S₂ (579.65): C, 55.95; H, 4.35; N, 12.08%, Found: C, 55.97; H, 4.31; N, 12.05%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(2,3-dihydro-3,4-diphenyl-2-ylidene-thiazol)-acetamide (**9d**)

Yellow powder, yield (87%), mp 256–257 °C; IR (KBr) v_{max}/cm^{-1} : 3413 (NH), 3288 (NH), 3060 (CH–Ar), 2981 (CH-sp³), 2196 (CN), 1634 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.70$ (s, 3H, CH₃), 2.08 (s, 3H, CH₃), 7.0–7.50 (m, 11H, C₆'H₅', C₆"H₅" and thiazole-H₅), 7.71 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.89 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 9.90 (s, 1H, NHSO₂), 10.91 (s, 1H, CONH); MS m/z (%): 570 ([M+1]⁺, 2.1), 569 (M⁺, 3.1), 394 (0.7), 303 (3.3), 294 (37.1), 275 (0.3), 251 (0.4), 175 (3.3), 111 (13.9), 96 (7.2), 77 (100); Anal. Calcd. for C₂₉H₂₃N₅O₄S₂ (569.65): C, 61.14; H, 4.07; N, 12.29%, Found: C, 61.11; H, 4.09; N, 12.33%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(2,3-dihydro-4-(4-chloro-phenyl)-3-phenyl-2-ylidene-thiazol)-acetamide (**9e**)

Yellow powder, yield (79%), mp 209–210 °C; IR (KBr) v_{max}/cm^{-1} : 3436 (NH), 3282 (NH), 3061 (CH–Ar), 2927 (CHsp³), 2199 (CN), 1640 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.70$ (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 7.11–7.16 (m, 1H, phenyl-H_{4'}), 7.33–7.38 (m, 4H, phenyl-H_{2'}, phenyl-H_{3'}, phenyl-H_{5'}, phenyl-H_{6'}), 7.53 (d, J = 8.5 Hz, 2H, phenyl-H_{2''}, phenyl-H_{6''}), 7.62 (d, J = 8.5 Hz, 2H, phenyl-H_{3''}, phenyl-H_{5''}), 7.72 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.86 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 7.96 (s, 1H, thiazole-H₅), 9.91 (s, 1H, NHSO₂), 10.94 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.9$ (CH₃), 10.3 (CH₃), 81.0 (acetamide-C₂), 104.9, 116.0 (2C, thiazole-C₅ and CN), 119.9, 121.0, 123.0, 124.5, 127.5, 128.4, 128.8, 129.4, 131.5, 132.4, 134.9, 135.3, 141.6 (19C, $2 \times C_6H_4$, C_6H_5 and thiazole-C₄), 143.4, 155.6, 161.3 (3C, isoxazole-C₃, isoxazole-C₄, isoxazole-C₅), 168.0 (CONH), 172.1 (thiazole-C₂); MS m/z (%): 604 (M⁺, 14.3), 492 (2.4), 352 (13.6), 337 (1.9), 309 (3.2), 294 (8.4), 111 (100), 96 (11.8), 77 (49.5); Anal. Calcd. for C₂₉H₂₂ClN₅O₄S₂ (604.10): C, 57.66; H, 3.67; N, 11.59%, Found: C, 57.70; H, 3.71; N, 11.63%.

General procedure for the synthesis of compounds 10a-e

Method (A)

Chloroacetylchloride (0.79 mL, 0.01 mol) was added slowly to the non-isolated potassium salts **6a–e** (0.01 mol) at 0 °C. Then the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was poured portion wise to ice water (200 mL) and neutralized by dilute HCl. The obtained solid was separated by filtration, washed with water (50 mL), air dried and recrystallized from ethanol to afford the 4-thiazolidinone derivatives **10a–e**.

Method (B)

Chloroacetonitrile (0.63 mL, 0.01 mol) was added to the nonisolated potassium salts **6a–e** (0.01 mol) at 0 °C. Then the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was poured portion wise to ice water (200 mL) and the medium was neutralized by HCl. The obtained solid was separated by filtration, washed with water (50 mL), air dried and recrystallized from ethanol. Compound **11** could not be obtained and instead the product was identical in all respects (mp., m.mp., IR spectra) with **10a** isolated from reaction of **6a** with chloroacetyl chloride.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-methyl-4-oxothiazolidin-2-ylidene)-acetamide (**10a**)

Yellow powder, yield (73%), mp 206-207 °C; IR (KBr) v_{max}/cm⁻¹: 3343 (NH), 3112 (NH), 3062 (CH-Ar), 2930 (CHsp³), 2201 (CN), 1747 (CO), 1662 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.65$ (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 3.55 (s, 3H, NCH₃), 3.91 (s, 2H, CH₂), 7.72 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.84 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 10.09 (s, 1H, NHSO₂), 10.96 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.8$ (CH₃), 10.2 (CH₃), 31.2 (CH₃), 32.7 (CH₂), 77.0 (acetamide-C₂), 105.0 (CN), 116.2 (phenyl-C₄), 120.5 $(2C, phenyl-C_2, phenyl-C_6), 127.5 (2C, phenyl-C_3, phenyl-C_5),$ 134.3 (phenyl- C_1), 142.8, 155.4, 161.3 (3C, isoxazole- C_3 , isoxazole-C₄, isoxazole-C₅), 163.4, 172.1, 173.7 (3C, CONH, COCH₂, thiazole-C₂); MS m/z (%): 447 (M⁺, 13.7), 432 (1.8), 421 (3.6), 351 (4.9), 272 (21.1), 266 (15.7), 181 (47.2), 175 (5.6), 111 (29.1), 96 (1.9), 93 (100); Anal. Calcd. for $C_{18}H_{17}N_5O_5S_2$ (447.49): C, 48.31; H, 3.83; N, 15.65%, Found: C, 48.36; H, 3.87; N, 15.67%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-ethyl-4-oxothiazolidin-2-ylidene)-acetamide (**10b**)

Brown powder, yield (71%), mp 139–140 °C; IR (KBr) v_{max}/cm^{-1} : 3369 (NH), 3251 (NH), 3052 (CH–Ar), 2928 (CH-sp³), 2194 (CN), 1732 (CO), 1663 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.27$ (t, J = 7.0 Hz, 3H, CH₃), 1.65 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 3.92 (s, 2H, CH₂), 4.15 (q, J = 7.0 Hz, 2H, NCH₂), 7.72 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.82 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 10.13 (s, 1H, NHSO₂), 10.96 (s, 1H, CONH); ¹³C NMR (125 MHz, DMSO-d₆): $\delta_{ppm} = 5.8$ (CH₃), 10.2 (CH₃), 13.7 (CH₃), 31.3 (CH₂), 40.1 (CH₂), 76.3 (acetamide-C₂), 105.0 (CN), 116.0 (phenyl-C₄), 120.5 (2C, phenyl-C₂, phenyl-C₆), 127.5 (2C, phenyl-C₃, phenyl-C₅), 134.3 (phenyl-C₁), 142.8, 155.4, 161.3 (3C, isoxazole-C₃, isoxazole-C₄, isoxazole-C₅), 163.4, 170.9, 173.7 (3C, CONH, COCH₂), New 2,3-dihydrothiazoles and 4-thiazolidinones containing sulfisoxazole 5

thiazole-C₂); MS m/z (%): 461 (M⁺, 9.3), 435 (10.0), 432 (1.7), 350 (42.1), 266 (11.1), 210 (29.0), 92 (100); Anal. Calcd. for C₁₉H₁₉N₅O₅S₂ (461.51): C, 49.45; H, 4.15; N, 15.17%, Found: C, 49.50; H, 4.17; N, 15.19%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-allyl-4-oxothiazolidin-2-ylidene)-acetamide (**10c**)

Greenish brown powder, yield (64%), mp 129–130 °C; IR (KBr) ν_{max}/cm^{-1} : 3371 (NH), 3230 (NH), 3061 (CH–Ar), 2929 (CH-sp³), 2193 (CN), 1716 (CO), 1653 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.65$ (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 3.86 (m, 2H, CH₂N), 3.95 (s, 2H, CH₂S), 5.29 (dd, J = 1.5 Hz, 17.5 Hz, 1H, CH_{2a}=), 5.36 (dd, J = 1.5 Hz, 17.5 Hz, 1H, CH_{2b}=), 5.86 (m, 1H, CH=), 7.71 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.83 (d, J = 9.0 Hz, 2H, phenyl-H₃, phenyl-H₅), 10.11 (s, 1H, NHSO₂), 10.98 (s, 1H, CONH); MS m/z (%): 473 (M⁺, 0.3), 362 (1.7), 294 (1.5), 207 (3.8), 179 (12.6), 175 (2.2), 111 (21.4), 96 (10.0), 92(100); Anal. Calcd. for C₂₀H₁₉N₅O₅S₂ (473.53): C, 50.73; H, 4.04; N, 14.79%, Found: C, 50.77; H, 4.08; N, 14.82%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(3-adamantyl-4-oxothiazolidin-2-ylidene)-acetamide (10d)

Greenish brown powder, yield (88%), mp 132–133 °C; IR (KBr) v_{max}/cm^{-1} : 3380 (NH), 3233 (NH), 3036 (CH–Ar), 2911 (CH-sp³), 2191 (CN), 1717 (CO), 1653 (CO); ¹H NMR (500 MHz, DMSO-d₆): $\delta_{ppm} = 1.65$ (m, 9H, 9 × adamantyl-H), 1.69 (s, 3H, CH₃), 2.02 (m, 3H, 3 × adamantyl-H), 2.08–2.10 (m, 6H, CH₃, 3 × adamantyl-H), 3.99 (s, 2H, CH₂S), 7.72 (d, J = 9.0 Hz, 2H, phenyl-H₂, phenyl-H₆), 7.84 (d, J = 9.0 Hz, 2H, phenyl-H₃), 10.13 (s, 1H, NHSO₂), 11.01 (s, 1H, CONH); MS m/z (%): 568 ([M+H]⁺, 21.0), 567 (M⁺, 8.4), 471 (2.7), 393 (19.6), 273 (19.3), 252 (26.7), 175 (2.7), 112 (100), 111 (3.7), 96 (27.4); Anal. Calcd. for C₂₇H₂₉N₅O₅S₂ (567.68): C, 57.13; H, 5.15; N, 12.34%, Found: C, 57.17; H, 5.18; N, 12.39%.

2-Cyano-N-(4-{[(3,4-dimethylisoxazol-5-yl)amino]sulfonyl}phenyl)-2-(4-oxothiazolidin-3-phenyl-2-ylidene)-acetamide (**10e**)

Yellow powder, yield (79%), mp 145–146 °C; IR (KBr) v_{max}/cm^{-1} : 3354 (NH), 3262 (NH), 3056 (CH–Ar), 2939 (CHsp³), 2197 (CN), 1729 (CO), 1667 (CO); ¹H NMR (400 MHz, DMSO-d₆): $\delta_{ppm} = 1.64$ (s, 3H, CH₃), 2.08 (s, 3H, CH₃), 4.04 (s, 2H, CH₂), 7.46–7.79 (m, 9H, C₆H₄ and C₆H₅), 9.88 (s, 1H, NHSO₂), 10.95 (s, 1H, CONH); ¹³C NMR (100 MHz, DMSO-d₆): $\delta_{ppm} = 5.7$ (CH₃), 10.1 (CH₃), 32.6 (CH₂), 77.1 (acetamide-C₂), 105.0 (CN), 116.1, 120.5, 124.6, 128.4, 127.5, 134.3, 138.4, 141.2 (12C, C₆H₄ and C₆H₅), 143.9, 156.8, 161.9 (3C, isoxazole-C₃, isoxazole-C₄, isoxazole-C₅), 164.5, 169.3, 172.1 (3C, CONH, <u>CO</u>CH₂, thiazole-C₂); MS *m*/*z* (%): 509 (M⁺, 0.9), 414 (6.3), 243 (6.7), 175 (3.9), 96 (12.3), 92 (10.9), 77 (75.3), 63 (100); Anal. Calcd. for C₂₃H₁₉N₅O₅S₂ (509.56): C, 54.21; H, 3.76; N, 13.74%, Found: C, 54.25; H, 3.81; N, 13.77%.

Antimicrobial evaluation

Antimicrobial screening

The antimicrobial screening and measurement of minimal inhibitory concentrations (MICs) of the studied compounds were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. The newly synthesized compounds were evaluated for their *in vitro* antimicrobial activities against the human pathogens *Streptococcus pneumoniae* (RCMB 010010), *Bacillis subtilis* (RCMB 010067) and *Staphylococcus epidermidis* (RCMB 010024) as examples of

Gram-positive bacteria and Escherichia coli (RCMB 010052), Proteous vulgaris (RCMB 010085) and Klebsiella pneumonia (RCMB 010093) as examples of Gram-negative bacteria. The antifungal activities of the target compounds were evaluated against Aspergillus fumigatus (RCMB 02568), Syncephalastrum racemosum (RCMB 05922) and Geotricum candidum (RCMB 05097). The preliminary screening of the antibacterial and antifungal activities was carried out by the agar-diffusion method. The results were recorded for each tested compound as the average diameter of inhibition zones (IZs) of microbial growth around the disks in millimeters and were attributed to the original tested concentration (5 mg/mL) as a preliminary test. Whatman filter paper disks were prepared with standard size (6.0 mm diameter) and reserved into 1.0 Oz screw capped wide mouthed containers for sterilization. These containers were kept at a temperature of 150 °C. Then, the standard sterilized filter paper disks soaked with a solution of the test compound in DMF $(100 \,\mu\text{L}, 5 \,\text{mg/mL})$ were placed on nutrient agar plate seeded with the proper test organism in triplicates. Standard concentrations of 10⁶ CFU/mL (Colony Forming Units/mL) and 10⁴ CFU/mL were used for antimicrobial assays. Pyrex glass Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. Ampicillin and gentamycin were used as standard antibacterial agents; while amphotericin B was used as standard antifungal agent. Ampicillin, gentamycin, amphotericin B and sulfisoxazole were used as reference drugs. DMF alone was used as control at the same aforementioned concentration and, therefore, showed no visible change in bacterial growth. The plates were incubated at 37 °C for 24 h for bacteria and for 48 h at 25°C for fungi. The mean zone of inhibition is measured in $mm \pm standard$ deviation beyond well diameter (6 mm). Compounds that showed growth IZs (>12 mm) were further evaluated for their MICs using the twofold serial dilution technique²⁵.

Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Subouraud Liquid Medium (Oxoid) was used to assess the antibacterial and antifungal activities, respectively. Stock solutions of the tested compounds, ampicillin, gentamycin, amphotericin B and sulfisoxazole were prepared in DMF at a concentration of 1000 µg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500-0.007 µg/mL. Then, 10 mL of the broth containing about 10⁶ CFU/mL of test bacteria or 10⁴ CFU/mL of the test fungus was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 h for antibacterial activity and at 25 °C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the MICs values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and un-inoculated media were run in parallel to the test compounds under the same conditions.

Molecular modeling

For the purpose of this study, the crystal structure of DHPS from *B. anthracis* (BaDHPS) bound to the STZ-DHPP adduct¹⁸ was retrieved from the Protein Data Bank (PDB code: 3TYE) and processed as described previously²⁴. The studied compounds were docked into BaDHPS using the flexible Molecular Operating Environment (MOE) Dock methodology (Montreal, QC, Canada)²⁶. The three-dimensional docking box was defined around the co-crystallized ligand in the solvent-exposed binding pocket of the enzyme. The Alpha Triangle Matcher was used to generate the initial placement poses, which were then rescored

and prioritized using the London dG Scoring to maximize the hydrophobic, ionic and hydrogen-bond contacts to the protein. In a subsequent refinement stage, the generated poses were energy minimized using the MMFF94x force field. Finally, the optimized poses were ranked using the GBVI/WSA Δ G free-energy estimates²⁷. The predicted docking poses were visually inspected and interactions with binding pocket residues were analyzed using the ligand-interactions diagrams²⁸.

Results and discussion

Chemistry

Scheme 1 outlines the synthetic pathway for the preparation of 4-amino-3-phenylthiazol-2-(3H)-thione **4**, 2,3-dihydrothiazoles **7a–e** and **9a–e** and thiazolidin-4-ones **10a–e**. The precursor *N*-(2-cyanoacetyl)sulfisoxazole **3** was prepared by cyanoacetylation of sulfisoxazole **1** with 1-cyanoacetyl-3,5-dimethylpyrazole **2** in boiling dioxane as previously described from our laboratory²⁴. The Gewald reaction²⁹ of compound **3**, as a nitrile containing active methylene moiety, with sulfur and phenyl isothiocyanate in warming ethanol containing a catalytic amount of triethylamine afforded 4-amino-3-phenylthiazol-2(3H)-thione **4** in good yield. The chemical structure of **4** was confirmed by ¹H NMR spectrum which lacked a signal assignable for the active methylene group and exhibited signals due to the aromatic ring protons at 7.34–7.88 ppm, and three exchangeable signals corresponding

to one NH₂ and two NH protons at 7.10, 10.74 and 11.14 ppm, respectively. The IR spectrum of compound **4** showed absorption bands at 3409–3351, 3279, 3206, 1659, 1175 cm⁻¹ characteristic for NH₂, two NH, C=O and C=S functions, respectively. The mass spectrum of **4** showed a molecular ion peak at m/z = 501, which agrees with its molecular formula (C₂₁H₁₉N₅O₄S₃).

Next, we described the behavior of N-(2-cyanoacetyl)sulfisoxazole 3 towards alkyl/phenyl isothiocyanates and α -halo carbonyls as a convenient route to attain some new functionalized thiazole derivatives. Thus, the base prompted nucleophilic addition of the active methylene group of 3 to a series of substituted isothiocyanates 5 in DMF gave the non-isolable adduct 6a-e that reacted in situ with chloroacetone to afford the functionalized 2,3dihydrothiazoles 7a-e. Structures 7a-e were elucidated on the basis of elemental analyses and spectral data. IR spectra of 7a-e revealed only two NH bands at 3362-3323 and 3283-3238 cm⁻¹ in addition to lower conjugated CN and C=O bands at 2189-2178 and 1656–1646 cm⁻¹ regions. ¹H NMR spectra of 7a-e exhibited the H-5 proton of the 2,3-dihydrothiazole ring as a sharp singlet signal at $\delta = 7.61 - 7.65$ ppm. The ¹³C NMR spectrum of **7b**, as a representative example of the synthesized analogues, showed five aliphatic carbons at δ 5.9, 10.2, 13.8, 27.9 and 56.0 ppm due to four CH₃ and CH₂N, in addition to the nitrile and carbonyl carbons of the amide function at δ 99.5 and 165.9 ppm, respectively.



Scheme 1. Synthetic route to functionalized thiazoles tagged with sulfisoxazole.

In a similar manner, heterocyclization of the non-isolable potassium salt **6e** with a variety of α -halo carbonyl reagents at room temperature afforded the diverse substituted 2,3-dihy-drothiazoles **9a–e**.

On the other hand, in situ heterocyclization of 6a-e with chloroacetyl chloride afforded the 4-thiazolidinone derivatives 10a-e. The structures of 10a-e were established through different spectroscopic techniques (IR, ¹H NMR, ¹³C NMR, MS) and elemental analyses data. IR spectra of 10a-e revealed two NH bands at 3380-3343 and 3280-3233 cm⁻¹ in addition to a nitrile and two C=O bands at 2201-2193, 1747-1716 and 1667-1653 cm⁻¹ regions, respectively. Their mass spectra showed, in addition to the molecular ion peak, in each case, a fragment ion peak at m/z 111 corresponding to 3,4-dimethylisoxazolylamino radical cation. ¹H NMR spectra of **10a-e** exhibited the methylene group of the 4-thiazolidinone ring as a sharp singlet signal at δ 4.04–3.91 ppm. The ¹³C NMR spectrum of **10a**, as a representative example of the synthesized analogues, showed the methylene carbon of the thiazolidinone ring at $\delta 32.7$ ppm in addition to the carbonyl carbons of the amide and 4-thiazolidinone ring functions at $\delta 163.4$ and 172.1 ppm, respectively. Moreover, the chemoselectivity of compounds 10a-e was chemically confirmed by the alternate synthesis of 10a as a representative example of the series. Thus, *in situ* reaction of the potassium salt **6a**, prepared from 3 and methyl isothiocyanate in DMF in the presence of potassium hydroxide, with chloroacetonitrile followed by acid hydrolysis gave a product that proved identical in all respects (mp., m.mp., IR spectra) with 10a isolated from reaction of 6a with chloroacetyl chloride (see Methods section). On the basis of this finding, the other isomeric structure, 5-thiazolidinone, was discarded.

To account for the formation of the products **7**, **9** and **10**, it is assumed, as depicted in Scheme 1, that the reaction of **3** with alkyl/aryl isothiocyanates starts with nucleophilic addition to give **6** as intermediate which then undergoes *in situ* cyclization with α -halo carbonyl reagents *via* elimination of HCl and water to give **7**, **9** and **10** as end products. This sequence is compatible with literature reports on reactions of thiocarbamoyl with α -halo carbonyl reagents to afford the respective thiazole derivatives^{30,31}.

Antimicrobial evaluation

Antibacterial activity

Preliminary antibacterial screening was carried out for the target compounds and the data are presented in Table 1. Interestingly, some of the synthesized *N*-substituted sulfisoxazole derivatives displayed better antibacterial activities compared to the reference standards ampicillin and gentamycin and were superior to sulfisoxazole, which has weaker antibacterial activities than ampicillin and gentamycin (Table 1).

Some of the synthesized 4-methylthiazoles 7a-e showed excellent activities against *B. subtilis*, *S. pneumoniae*, *E. coli* and *K. pneumonia*. Compounds 7c and 7d were fourfold more potent than ampicillin against *B. subtilis* (MIC, 0.06 µg/mL versus 0.24 µg/mL). Additionally, 7c had twofold the activity of ampicillin against *S. pneumonia* (MIC, 0.06 µg/mL versus 0.12 µg/mL) and 7d was equipotent to ampicillin against the same microbe. On the other hand, 7b showed half the potency of ampicillin against *B. subtilis* (MIC, 0.49 µg/mL versus 0.24 µg/mL). On the other hand, 7a-e showed excellent activities against *E. coli* (MIC, 0.49–1.95 µg/mL versus 1.95 µg/mL for gentamycin) while 7d displayed half the potency of gentamycin against *K. pneumonia* (MIC, 0.06 µg/mL versus 0.03 µg/mL).

Additionally, the *N*-phenyl-thiazoles 9a-e showed good to excellent antimicrobial activities and 9c was the most active among this series; it was equipotent to ampicillin against

S. pneumoniae (MIC, $0.12 \,\mu\text{g/mL}$) and equipotent to gentamycin against *E. coli* (MIC, $1.95 \,\mu\text{g/mL}$). Moreover, the derivatives **9c–e** were more potent than ampicillin against *B. subtilis* (MIC, $0.007-0.12 \,\mu\text{g/mL}$ versus $0.24 \,\mu\text{g/mL}$; respectively). Interestingly, the thiazoles **9c** and **9d** showed promising results against *K. pneumonia* (MIC, $1.95, 0.24 \,\mu\text{g/mL}$; respectively).

Regarding the 4-thiazolidinones 10a-e, compound 10c was the most active against the tested Gram-positive bacteria and compounds 10a and 10b had half the potency of gentamycin against *E. coli* (MIC, $3.9 \,\mu\text{g/mL}$ versus $1.95 \,\mu\text{g/mL}$). Moreover, 10a,b displayed good activities against *P. vulgaris* and *K. pneumonia*.

On the other hand, the 4-aminothiazole 4 had weak to moderate antibacterial activities against most of the tested bacterial strains and it was completely inactive against *S. pneumonia*.

Antifungal activity

The results displayed in Table 2 showed that most of the target compounds had more potent antifungal activities than sulfisoxazole and that the latter had weaker antifungal activities than amphotericin B against the tested microbes (Table 2).

The 4-methylthiazole **7c** showed excellent activity against *G. candidum* (MIC, 0.03 µg/mL) (Scheme 1) and was equipotent to amphotericin B against *A. fumigates and S. racemosum* (MIC, 0.12 µg/mL and 7.81 µg/mL; respectively). Moreover, 4-methylthiazole **7d** displayed double the potency of amphotericin B against *S. racemosum* (MIC, 3.9 µg/mL versus 7.81 µg/mL) and good activity against *G. candidum* (MIC, 0.98 µg/mL). Interestingly, 4-methylthiazole **7e** had four times the potency of amphotericin B against *S. racemosum* (MIC, 1.95 µg/mL versus 7.81 µg/mL) and excellent activity against *G. candidum* (MIC, 0.03 µg/mL). It was observed that the thiazoles **4, 7a** and **7b** had moderate antifungal activities against the tested fungal strains.

On the other hand, *N*-phenyl-thiazole **9a** showed half the potency of amphotericin B against *A. fumigates* (MIC, 0.24 µg/mL) versus 0.12 µg/mL) and good activity against *G. candidum* (MIC, 0.49 µg/mL). While, *N*-phenyl-thiazoles **9c**, **9d** and **9e** displayed half the potency of amphotericin B against *S. racemosum* (MIC, 15.63 µg/mL versus 7.81 µg/mL; respectively) and excellent activity against *G. candidum* (MIC, 0.06, 0.03 and 0.98 µg/mL; respectively).

Finally, 4-thiazolidinones **10a**, **10b** and **10d** showed moderate antifungal activities. While, 4-thiazolidinones **10c** displayed good activity against *A. fumigates* and *G. candidum* (MIC, 1.95 and 0.24 μ g/mL; respectively) and fourfold the potency of amphotericin B against *S. racemosum*.

Molecular modeling

MOE docking results

The docking results obtained from MOE-Dock showed that the studied compounds can be accommodated in the binding pocket of BaDHPS with a comparable orientation to the one observed in the STZ-DHPP covalent adduct in the reported crystal structure¹⁸. The top-ranked docking poses reproduce the key interactions observed in the STZ-DHPP–BaDHPS complex (Figure 2A).

Most notably, the benzene-sulfonamide moiety in all predicted binding modes interacts with Ser221 *via* H-bonding between its sulfonamide group and the backbone NH group, while the phenyl ring packs against the side chains of Lys220 and Pro69. Moreover, the central phenyl moiety in the studied compounds makes faceto-edge interaction with Phe189 (H-to-C distances ranging from 3.0 to 3.6 Å). As shown in Figure 2, this characteristic orientation of the benzene-sulfonamide is common among all studied

Table 1. Antibacterial inhibition zone in $mm \pm standard$ deviation and minimal inhibitory concentrations (MIC, $\mu g/mL$, between brackets) of some newly synthesized compounds.

		Gram-positive bacteria	L	Gram-negative bacteria			
Compounds	S. pneumoniae	B. subtilis	S. epidermidis	E. coli	P. vulgaris	K. pneumonia	
4	_a	$17.4 \pm 0.25 (15.63)$	15.2 ± 0.44 (62.5)	$11.2 \pm 0.33 \text{ (ND^b)}$	9.2 ± 0.53 (ND)	14.6 ± 0.58 (62.5)	
7a	$16.0 \pm 0.44 (31.25)$	18.3 ± 0.67 (7.81)	14.0 ± 0.58 (250)	$21.2 \pm 0.58 (0.98)$	13.0 ± 0.46 (500)	$23.4 \pm 0.63 (0.24)$	
7b	$21.2 \pm 0.43 (0.98)$	$22.3 \pm 0.53 (0.49)$	18.8 ± 0.38 (3.9)	$20.3 \pm 0.63 (1.95)$	17.2 ± 0.58 (15.63)	$22.8 \pm 0.19 (0.24)$	
7c	$24.6 \pm 0.34 (0.06)$	$24.6 \pm 0.25 (0.06)$	$20.6 \pm 0.34 (0.98)$	$20.1 \pm 0.58 (0.98)$	$19.8 \pm 0.42 (1.95)$	$22.3 \pm 0.44 (0.49)$	
7d	$24.2 \pm 0.44 \ (0.12)$	$25.1 \pm 0.25 (0.06)$	$21.2 \pm 0.63 (0.98)$	$22.3 \pm 0.33 (0.49)$	-	$24.8 \pm 0.63 (0.06)$	
7e	$22.4 \pm 0.29 (0.49)$	$21.3 \pm 0.41 (0.98)$	19.3 ± 0.56 (3.9)	$19.5 \pm 0.37 (1.95)$	$16.3 \pm 0.48 (31.25)$	17.9 ± 0.30 (7.81)	
9a	$16.7 \pm 0.38 (15.63)$	19.1 ± 0.41 (3.9)	$18.7 \pm 0.68 (3.9)$	$17.1 \pm 0.37 (15.63)$	18.2 ± 0.50 (7.81)	16.9 ± 0.31 (15.63)	
9b	-	19.3 ± 0.53 (3.9)	17.7 ± 0.43 (7.81)	13.7 ± 0.25 (15.63)	10.8 ± 0.44 (ND)	15.4 ± 0.44 (62.5)	
9c	$23.6 \pm 0.58 \ (0.12)$	$25.6 \pm 0.63 (0.03)$	$20.3 \pm 0.44 (1.95)$	19.8 ± 0.25 (1.95)	16.3 ± 0.25 (31.25)	20.3 ± 0.44 (1.95)	
9d	$19.5 \pm 0.44 \ (1.95)$	$29.8 \pm 0.58 \ (0.007)$	$17.4 \pm 0.53 (15.63)$	17.6 ± 0.19 (7.81)	15.2 ± 0.44 (62.5)	$22.6 \pm 0.63 \ (0.24)$	
9e	$19.6 \pm 0.44 \ (1.95)$	$23.7 \pm 0.63 \ (0.12)$	$16.1 \pm 0.33 (31.25)$	14.4 ± 0.25 (125)	_	15.6 ± 0.25 (31.25)	
10a	$16.9 \pm 0.38 (15.63)$	18.1 ± 0.44 (7.81)	$16.1 \pm 0.68 (31.25)$	18.9 ± 0.37 (3.9)	$21.4 \pm 0.31 \ (0.98)$	$19.6 \pm 0.50 \ (1.95)$	
10b	$16.2 \pm 0.15 (31.25)$	$19.8 \pm 0.42 \ (1.95)$	14.3 ± 0.58 (250)	19.2 ± 0.63 (3.9)	$17.4 \pm 0.53 (15.63)$	$20.3 \pm 0.58 (1.95)$	
10c	$20.9 \pm 0.44 \ (0.98)$	$20.3 \pm 0.58 (1.95)$	$19.6 \pm 0.27 (1.95)$	$17.2 \pm 0.58 (15.63)$	16.1 ± 0.53 (31.25)	18.3 ± 0.58 (7.81)	
10d	18.1 ± 0.63 (7.81)	$20.0 \pm 0.32 \ (1.95)$	$17.2 \pm 0.58 (15.63)$	13.9 ± 0.46 (250)	-	$21.2 \pm 0.45 \ (0.98)$	
10e	15.0 ± 0.43 (62.5)	17.4 ± 0.53 (15.63)	13.6 ± 0.44 (250)	11.2 ± 0.33 (ND)	-	14.3 ± 0.44 (250)	
Sulfisoxazole	$19.6 \pm 0.44 \ (1.95)$	$20.8 \pm 0.63 \ (0.98)$	17.5 ± 0.72 (7.81)	15.2 ± 0.25 (62.5)	-	18.2 ± 0.58 (7.81)	
Ampicillin	$23.8 \pm 0.20 \ (0.12)$	$32.4 \pm 0.30 \ (0.24)$	25.4 ± 0.18 (0.06)	NT ^c	NT	NT	
Gentamycin	NT	NT	NT	$19.9 \pm 0.30 \ (1.95)$	$23.4 \pm 0.30 \ (0.24)$	$26.3 \pm 0.15 \ (0.03)$	

^aNo activity.

^bND, not determined.

^cNT, not tested.

Table 2.	Antifungal inhibition	zone in mm \pm sta	andard deviation	and minimal	l inhibitory	concentrations	(MIC, μg/mL,	, between bra	ckets) (of some 1	newly
synthesiz	zed compounds.										

		Fungi			Fungi			
Compounds	A. fumigatus	S. racemosum	G. candidum	Compounds	A. fumigatus	S. racemosum	G. candidum	
4	16.3 ± 0.25 (31.25)	15.2 ± 0.58 (62.5)	$17.3 \pm 0.17 (15.63)$	9c	$21.3 \pm 0.39 \ (0.98)$	$17.2 \pm 0.16 (15.63)$	$24.6 \pm 0.58 \ (0.06)$	
7a	13.6 ± 0.25 (250)	11.7 ± 0.34 (ND*)	16.5 ± 0.58 (31.25)	9d	$22.3 \pm 0.25 (0.49)$	16.5 ± 0.25 (15.63.)	$25.8 \pm 0.58 (0.03)$	
7b	15.3 ± 0.55 (62.5)	13.4 ± 0.35 (500)	11.5 ± 0.58 (ND)	9e	20.4 ± 0.39 (1.95)	$16.5 \pm 0.16 (15.63)$	$21.4 \pm 0.58 (0.98)$	
7c	$24.3 \pm 0.63 \ (0.12)$	17.6 ± 0.27 (7.81)	$26.9 \pm 0.35 \ (0.03)$	10a	13.9 ± 0.29 (250)	12.1 ± 0.18 (500)	14.6 ± 0.35 (62.5)	
7d	$17.1 \pm 0.53 (15.63)$	18.8 ± 0.42 (3.9)	$20.9 \pm 0.31 \ (0.98)$	10b	15.7 ± 0.33 (31.25)	13.8 ± 0.25 (250)	16.8 ± 0.34 (15.63)	
7e	$20.3 \pm 0.39 (1.95)$	$20.3 \pm 0.16 (1.95)$	$26.6 \pm 0.58 (0.03)$	10c	$20.2 \pm 0.36 (1.95)$	$16.9 \pm 0.27 (1.95)$	$22.6 \pm 0.65 (0.24)$	
9a	$23.0 \pm 0.34 (0.24)$	14.6 ± 0.52 (125)	$21.9 \pm 0.53 (0.49)$	10d	$16.2 \pm 0.36 (31.25)$	15.0 ± 0.44 (62.5)	17.6 ± 0.58 (7.81)	
9b	17.6 ± 0.58 (7.81)	15.4 ± 0.25 (62.5)	12.6 ± 0.38 (500)	10e	11.3 ± 0.34 (ND)	12.1 ± 0.25 (500)	15.3 ± 0.38 (62.5)	
Amphotericin B	$23.7 \pm 0.1 \ (0.12)$	19.7±0.2 (7.81)	$28.7 \pm 0.2 \ (0.007)$	Sulfisoxazole	15.1 ± 0.39 (62.5)	13.2 ± 0.58 (125)	16.8 ± 0.58 (15.63)	

*ND, not determined.

compounds indicating that all of them can occupy the PABA pocket preventing the key substrate from binding, which is the basis of the inhibitory action of all sulfa drugs against DHPS^{32,33}. In addition to the distinctive interactions in the PABA-binding pockets, the studied compounds exhibit interactions with residues in the pterin-binding pocket of DHPS that vary slightly between chemical classes. For instance, the 4-methylthiazole derivative **7a** exhibits cation– π interaction between its thiazole ring and the charged side chain of Lys220, as well as a hydrogen bond between its central amide group and the side chain of Arg254, which could further stabilize its interaction with DHPS (Figure 2B).

The bulky phenyl substituent in the *N*-phenyl-thiazole series **9a–e** enforces a bound conformation where the thiazole ring points deeper into the pterin-binding pocket making interaction with Lys220 *via* cation– π contact. Compounds **9b** and **9c** bear an ester substitution on their terminal thiazole rings. This unique decoration allows for a water-mediated hydrogen bond to the side chain of Asp184 deeper in the pterin-binding pocket (Figure 2E), which utilizes the W286 water molecule observed in the employed crystal structure¹⁸. Conversely, the second phenyl

substituent in compounds **9d** and **9e** was accommodated in a hydrophobic pocket lined by Met145, Leu214 and Ile143 (Figure 2F), which is in agreement with the reported flexibility of the binding pocket of BaDHPS¹⁸.

The 4-thiazolidinone derivative 10a presented the same Hbond to Arg254 and formed an extra H-bond from the thiazolidinone oxygen to the side chain of Asp184 in the back of the pterin-binding pocket (Figure 2C). On the other hand, the thiazolidinone ring of compound 10e exhibited 180° rotation, thereby forming a hydrogen bond to the acidic side chain of Asp101 instead (Figure 2D).

Lipophilicity study

The lipophilic characters of sulfisoxazole and the target compounds were calculated by the online program OSIRIS Property Explorer. The results are summarized in Table 3 and revealed that most of the target compounds were more lipophilic than sulfisoxazole. Thus, they were expected to have an improved intracellular penetration and consequently better antimicrobial activities than sulfisoxazole.

New 2,3-dihydrothiazoles and 4-thiazolidinones containing sulfisoxazole 9



Figure 2. Predicted binding modes of the studied compounds generated by MOE-Dock. (A) Interactions with key residues in the crystal structure of the STZ-DHPP adduct (orange carbons) with BaDHPS (PDB code 3TYE, STZ: sulfathiazole, DHPP: 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate). (B–F) Docking poses for compounds **7a**, **9b**, **9e**, **10a** and **10e**, respectively (green: ligand's carbon, red: oxygen, blue: nitrogen, yellow: sulfur, white: hydrogen). Protein is shown as light gray cartoons, hydrogen bonds as dotted red lines, and crystal water W286 as magenta sphere. Colors are available in the online version of the paper.

Regarding the 4-methylthiazoles **7a–e** (Scheme 1), the larger the *N*-alkyl substitution the higher the lipophilic character (Table 3); and consequently the best antimicrobial activities were observed in the case of *N*-allyl and *N*-adamantyl substitutions. The high lipophilic characters of the *N*-phenyl-thiazoles **9a–e** (Scheme 1) were among the factors responsible for their observed good antimicrobial activities (Tables 1 and 2). Finally, 4-thiazolidinones 10a-e were more polar than 4methylthiazoles 7a-e and consequently had lower antimicrobial activities than compounds 7a-e (Table 1). It was obvious that the 4-thiazolidinone 10a was less polar but more active than sulfisoxazole against the examined Gram-negative bacteria; thus, there should be other factors than its lipophilicity responsible for the observed activity.

Table 3. Calculated lipophilic characters of the target compounds.

Compounds	Clog P*	Compounds	C log P	
Sulfisoxazole	1.02	9c	3.76	
4	1.86	9d	5.04	
7a	2.25	9e	5.65	
7b	2.66	10a	0.91	
7c	2.93	10b	1.31	
7d	4.33	10c	1.58	
7e	3.75	10d	2.98	
9a	3.58	10e	2.40	
9b	3.74			

*Calculated by online program OSIRIS Property Explorer.

Structure activity relationship

The results obtained from this study revealed that most of the target compounds were more active than sulfisoxazole (Tables 1 and 2). The high lipophilic characters of the target compounds, the synergistic antimicrobial effect of the thiazole and sulfisoxazole moieties as well as their ability to occupy both the PABA and pterin-binding pockets of DHPS enzyme (Figure 2) could explain the observed good antimicrobial activities of the target compounds. Regarding the 4-methylthiazoles 7a-e (Scheme 1), the larger the N-alkyl substitution the better is the antimicrobial activity. The type and size of the substitutions on the thiazole ring of the target compounds 9a-e (Scheme 1) as well as the lipophilic characters are among the factors responsible for the improved antimicrobial activities. The smaller the substitution at position 5 of the thiazole ring the higher is the antimicrobial activity; i.e. the effect of the substitutions was in the following order $(H>CH_3CO->CH_3CH_2OOC-)$. On the other hand, substitutions at position 4 of the thiazole ring affected significantly the antimicrobial activities; the effect was in the following descending order $(CH_3CH_2OOCCH_2 -> C_6H_5 -> 4 - Cl - C_6H_5 -> CH_3 -)$. The antimicrobial results summarized in Tables 1 and 2 indicate that the presence of ester moiety on the thiazole ring of the target compounds 9a-e resulted in improved antimicrobial activities; this result was consistent with the molecular docking results (Figure 2C). Additionally, the presence of phenyl ring at position 4 of the thiazole ring of the target compounds **9a-e** could occupy the specified hydrophobic pocket within the DHPS (Figure 2D) and was responsible for the observed superior biological activities. However, the activity was reduced by the introduction of chloro-substituent at the para position of the phenyl ring, which could be caused by steric hindrance.

Finally, the presence of thiazolidinone oxygen in compounds **10a–e** allows for the H-bond formation in the back of the pterinbinding pocket (Figure 2E and F). Notably, the *N*-allyl substitution gave good results against the Gram-positive bacteria; while the *N*-methyl substitution gave good results against the Gramnegative bacteria.

Conclusion

A new series of 4-aminothiazole, 4-methylthiazole, *N*-phenylthiazole, 4-thiazolidinone derivatives bearing sulfisoxazole moieties were designed, synthesized and evaluated for their *in vitro* antimicrobial activities. Most of newly synthesized compounds were more potent than sulfisoxazole and some of them exhibited better antimicrobial activities than the reference drugs ampicillin, gentamycin and amphotericin B. The relatively higher lipophilicity of the target compounds could account for the observed good antimicrobial activities due to the increased intracellular concentration. Moreover, docking studies indicated that the target compounds could occupy both the PABA and pterin-binding pockets of DHPS. Additionally, the synergistic effect of both the sulfonamide and the thiazole ring could explain the targets' superior antimicrobial profiles. Particularly, compounds **7a**, **7c–e**, **9c–d** and **10c** displayed promising antimicrobial activities.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary material available online Supplementary Figure I

