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New spiro-heterocyclic coumarin derivatives as antibacterial agents: design, synthesis and molecular docking

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Abstract

Coumarin derivatives were synthesized herein from 3-acetyl coumarin and 4-(pyrimidin-2-yldiazenyl) antipyrine, leading to the azo chalcone intermediate compound. The final spiro-heterocyclic coumarins were produced through the cyclization of the azo-chalcone with thiourea, guanidine hydrochloride, benzene-1,2-diamine, 2-aminophenol, and hydroxylamine hydrochloride, respectively. The obtained target compounds were purified by column chromatography and characterized by FT-IR, 1H NMR, 13C-NMR and elemental analysis. The antibacterial activity of the synthesized compounds was evaluated *in vitro* against Gram-negative and Gram-positive bacteria. One of the compounds showed significant antibacterial activity. Furthermore, the docking study of this compound with DNA gyrase for *E. coli* and *S. aureus* bacterial strains was investigated, which revealed vital interactions and binding.

Keywords

spiro cyclic
coumarin derivatives
antibacterial agent
molecular docking

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Key findings

- Five spirocyclic coumarin derivatives were successfully synthesized and characterized by spectroscopic techniques.
- Compound (3a) among others exhibited the best activity against two types of Gram-negative and Gram-positive bacteria.
- The molecular docking study of compound (3a) and binding conformations with DNA gyrase for *E. coli* and *S. aureus* revealed that it was the most affected target.

Supplementary materials

1. Introduction

Coumarins (α-benzopyrones, 2*H*-chromen-2-ones) are a large family of naturally occurring compounds that exhibit numerous biological and medicinal activities [1, 2], such as antioxidant, anticancer, anticoagulant, and anti-inflammatory ones [3]. Also, they are used as additives in food, cosmetics, and dyes [4]. Their pharmacological properties depend on the substitution pattern [5].

Bacterial infections are widespread [6]. According to statistics, it is expected that 10 million individuals will die annually around the world unless the medical interventions are improved [7]. The reason behind this is the increase in multidrug resistance of pathogenic microorganisms [8]. Coumarins and their derivatives are extensively used as precursors to produce effective antibacterial agents. Many such agents have been developed by adding new groups to improve the interaction with DNA gyrase as a drug target. Several studies have proven that antibiotics that contain coumarin in their structure, such as novobiocin and clorobiocin, are antimicrobials via DNA gyrase inhibition. DNA gyrase (topoisomerases II) is an enzyme that is mainly responsible for bacterial chromosome replication. It can introduce a double-stranded break in DNA and is believed to have a central role in reducing topological strain in an ATP-dependent manner [9]. Bacterial growth can be prevented by DNA gyrase inhibitors through two particular mechanisms: directly inhibiting DNA gyrase, called “gyrase poisoning”, or indirectly, by inhibiting gyrase ATPase activity [10]. As a result, DNA gyrase has become a common target for various medicinal products, particularly for bacterial strains such as *Escherichia coli* [11]. Spiro compounds are

essential substances due to their balance between conformational restriction and flexibility. Recent progress in the isolation and characterization of new spiro building blocks from synthetic or natural products has facilitated their involvement in more molecules with pharmaceutical applications, such as anti-Alzheimer's, anticancer, antimicrobial agents [12]. They are free from permeability and absorption issues compared to aromatic heterocycles, making them attractive and probable targets for new drug discovery [13]. Antibacterial **I** and **II** [14, 15], and antifungal **III** [16], containing a spirocyclic ring, are prominent examples (Figure 1).

Therefore, in this study, some spiro-heterocyclic coumarin derivatives were synthesized (Scheme 1). Spectroscopic techniques and elemental analysis were used to characterized all the newly prepared compounds. In addition, the in *vitro* assessment of their antibacterial activities was evaluated. The molecular docking simulation of compound (**3a**) with DNA gyrase enzyme in *E. coli* and *S. aureus* was also studied.

2. Materials and methods

2.1. Materials

All reagents and solvents used were purchased from Sigma-Aldrich and BDH. The purity of the synthesized compounds was regularly checked on TLC plates coated with silica gel 60F254 aluminum sheets (TLC, Darmstadt, Germany) and purified using column chromatography in an ethyl acetate-hexane and dichloromethane system, ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Bio Spin Gmb (400 MHz for ^{1}H and 100 MHz for ^{13}C) in DMSO-d_6 with Me_4Si as the internal standard. Infrared spectra were recorded on Bruker Alpha (Platinum ATR, Germany). Elemental analyses were performed with Elementar Analysensysteme GmbH, CHNS Model, S. No. 11086109. Melting points were determined using a Stuart SMP3 apparatus (Staffordshire, UK). The NMR, FT-IR spectra and elemental analysis data of all the compounds are shown in the supporting information (Figures S3–S3e).

2.2. Preparation of intermediate azo-chalcone

2.2.1. 3-(2-(1,5-dimethyl-2-phenyl-4-(pyrimidin-2-yldiazenyl) -1,2-dihydro-3H-pyrazol-3-ylidene) acetyl)-2H-chromen -2-one (3)

A solution of 3-acetyl-2H-chromen-2-one **1** (6 g, 3.2 mmol) and 4-(pyrimidin-2-yldiazenyl)-antipyrine **2** (0.94 g, 3.2 mmol) in 15 mL absolute ethanol was stirred. Then, 0.5 mL of piperidine was added with rapid stirring. The mixture was refluxed for 8 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, it was cooled, and the precipitate was filtered off and recrystallized from ethanol. Orange powder, m.p: 258–260 °C, *Rf* = 0.78, IR spectrum, ν, cm^{-1}: 1731 (C=O$_{lactone}$), 1672 (C=O$_{carbonyl}$), 1595 (N=N). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm; 8.57 (2H, *d*, *J* = 12.0 Hz, pyrimidine), 8.39 (*s*, 1H, coumarin), 8.08–8.01 (*m*, 1H, pyrimidine), 7.84–7.76 (*m*, 1H, Ar H), 7.68–7.61 (*m*, 1H, Ar H), 7.47–7.37 (*m*, 3H, Ar H), 7.36–7.19 (*m*, 1H, Ar H), 6.67 (*s*, 1H, CH=), 2.78 (*s*, 3H, CH_3), 2.13 (*s*, 3H, CH_3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm; 184.0 (C=O$_{carbonyl}$); 163.6 (C$_{beta}$); 161.0 (C=O$_{lactone}$); 152.3 (C$_{Pyrimidine}$); 147.0 (C$_{olefinic}$); 143.4 (C$_{Pyrimidine}$); 143.1 (C$_{olefinic}$);140.2 (C$_{Ar}$); 136.0 (C$_{Ar}$); 134.0 (C$_{olefinic}$); 131.2 (C$_{Ar}$); 130.2 (C$_{Ar}$); 129.5 (C$_{Ar}$); 127.7 (C$_{Ar}$); 127.3 (C$_{Ar}$); 126.1 (C$_{Ar}$); 123.5 (C$_{Ar}$); 120.6 (C$_{Ar}$); 117.5 (C$_{Pyrimidine}$); 114.4 (C$_{olefinic}$); 114.3 (C$_{alpha}$); 32.7 (C$_{methyl}$); 10.6 (C$_{methyl}$). Found, %: C 67.32; H 4.38; N 8.26. $C_{26}H_{20}N_6O_3$. Calculated, %: C 67.23; H 4.34; N 8.23.

2.3. Preparation of target spirocyclic pyrimidines (3a, 3b)

General method. To a solution of azo-chalcone **3** (1.5 mmol), thiourea, and/or guanidine (1.5 mmol) in absolute ethanol (15 mL), a solution of potassium hydroxide (0.2 g) in water (1 mL) was added; the reaction mixture was refluxed for 7 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the alcohol was evaporated under vacuum till dryness, and the residue was acidified with diluted. HCl. The solid formed was filtered, washed with water, dried under vacuum, and purified through column chromatography using hexane, ethyl acetate, and dichloromethane (3.5:1:0.5 v/v/v) as eluent to obtain the pure target compounds.

2.3.1. 3-(2,3-dimethyl-1-phenyl-4-(pyrimidin-2-yldiazenyl)-7-thioxo-1,2,6,8-tetraazaspiro [4.5] deca-3,9-dien-9-yl)-2H-chromen-2-one (3a)

Light brawn powder, m.p: 290-292 °C, *Rf*=0.68, IR spectrum, ν, cm^{-1}: 1704 (C=O), 1662(C=S$_{thione}$), 1624 (N=N$_{azo}$). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm; 10.01 (*s*, 1H, NH), 8.64–8.61 (2H, *d*, *J* = 10.0 Hz, pyrimidine), 8.35 (*s*, 1H, coumarin), 8.11–8.08 (*m*, 1H, pyrimidine), 7.82–7.77 (*m*, 2H, Ar H), 7.70–7.65 (*m*, 3H, Ar H), 7.37 (2H, *d*, *J* = 2.5 Hz, Ar H), 5.01 (*s*, 1H, CH=), 3.09 (*s*, 3H, CH_3), 1.80 (*s*, 3H, CH_3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm; 183.2 (C=S$_{thione}$); 162.4 (C=O$_{lactone}$); 160.5 (C$_{Pyrimidine}$); 156.8 (C$_{Ar}$); 152.2 (C$_{Ar}$); 151.8 (C$_{Ar}$); 150.4 (C$_{olefinic}$); 144.9 (C$_{Pyrimidine}$); 138.0 (C$_{olefinic}$); 134.5 (C$_{Ar}$); 132.1 (C$_{Ar}$); 131.2 (C$_{olefinic}$); 128.7 (C$_{Ar}$); 127.4 (C$_{olefinic}$); 124.4(C$_{Ar}$); 121.0 (C$_{olefinic}$); 120.1 (C$_{Ar}$); 118.3 (C$_{Ar}$); 117.4 (C$_{Pyrimidine}$); 114.6 (C$_{Ar}$); 111.2 (C$_{spiro}$); 108.6 (C$_{olefinic}$); 46.0 (C$_{methyl}$); 12.8 (C$_{methyl}$). Found, %: C 62.12; H 4.28; N 21.47; S, 6.19. $C_{27}H_{22}N_8O_2S$. Calculated, %: C 62.06; H 4.24; N 21.44; S, 6.13.

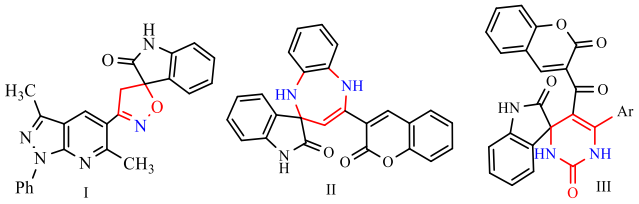

Figure 1 Examples of some active spirocyclic compounds.

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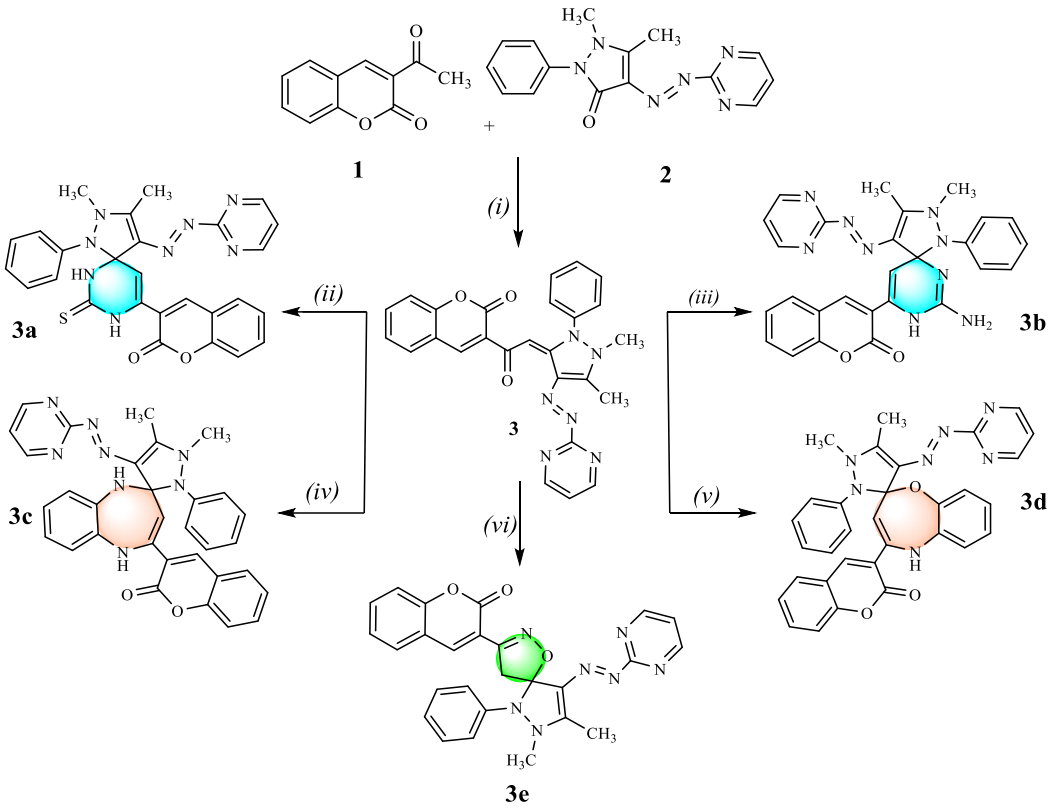

Scheme 1 Synthesis of spiro heterocycles (**3 and 3a-e**) *Reagents and conditions*: *(i)* Abs. EtOH, / pipridine, stirring, reflux 8 h, yield; 78%. *(ii)* Abs. EtOH /thiourea (0.2 g) of KOH, reflux 7 h, yield; 71%. *(iii)* Abs. EtOH /guanidine hydrochloride, (0.2 g) of KOH, reflux 7 h, yield :74%. *(iv)* Abs. EtOH / benzene-1,2-diamine, Gla.CH_3CO_2H, reflux 10 h, yield; 74%. *(v)* Abs. EtOH / 2-aminophenol, Gla.CH_3CO_2H, reflux 10 h, yield; 70%. *(vi)* Abs. EtOH /hydroxylamine hydrochloride, (0.2 g) of KOH, reflux 7 h, yield ;72%.

2.3.2. 3-(7-amino-2,3-dimethyl-1-phenyl-4-(pyrimidin-2-yldiazenyl)-1,2,6,8-tetraazaspiro [4.5] deca-3,6,9-trien-9-yl)-2H-chromen-2-one(3b)

Brownish powder, m.p: 272-274 °C, *Rf* = 0.82, IR spect rum, ν, cm^{-1}: 3551, 3483 (NH_2), 3190 (NH), 1697 (C=$O_{lactone}$), 1653 (C=N_{imine}), 1606 (N=N_{azo}). ^{1}H NMR spectrum (400 MHz, DMSO- d_6), δ, ppm; 10.12 (s, 1H, NH_2), 8.68 (2H, *d*, *J* = 12.6 Hz, pyrimidine), 8.49 (*s*, 1H, coumarin), 8.19–8.07 (*m*, 2H, Ar H), 7.68–7.57 (*m*, 2H, Ar H), 7.39–7.15 (*m*, 1H, pyrimidine), 6.98–6.74 (*m*, 2H, Ar H), 6.63 (*s*, 1H, C=CH), 5.61 (*s*, 2H, NH_2), 3.14 (*s*, 3H, CH_3), 2.20 (*s*, 3H, CH_3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm; 161.7 (C=$O_{lactone}$); 160.5 (C_{amine});160.4 ($C_{Pyrimidine}$); 158.0 (C=N_{imine}); 154.69(C_{Ar}); 152.1 (C_{Ar}); 150.3 ($C_{olefinic}$); 142.9 ($C_{olefinic}$); 136.1 (C_{Ar}); 135.1 (C_{Ar}); 133.0 ($C_{Pyrimidine}$); 129.5 ($C_{olefinic}$); 129.2 (C_{Ar}); 128.4 (C_{Ar}); 127.0 (C_{Ar}); 124.2 ($C_{olefinic}$); 121.5 ($C_{olefinic}$); 119.3 (C_{Ar}); 116.8 (C_{Ar}); 116.8 ($C_{Pyrimidine}$);113.2 ($C_{olefinic}$); 112.1 (C_{spiro}); 31.2 (C_{methyl}); 10.2 (C_{methyl}). Found, %: C 64.21; H 4.62; N 24.97. $C_{27}H_{23}N_9O_2$. Calculated, %: C 64.15; H 4.59; N 24.94.

2.4. Preparation of target spirocyclic diazepine (3c) and oxazepine (3d)

General method. Azo-chalcone **3** (1.5 mmol) was added to a solution of benzene-1,2-diamine and/or 2-aminophenol (1.5 mmol) in absolute ethanol (15 mL), and glacial acetic acid (0.5 mL) was added. The reaction mixture was refluxed with stirring for 10 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured on crushed ice. The solid formed was filtered, washed with water, dried under vacuum, and purified through column chromatography using hexane, ethyl acetate, and dichloromethane (3:1:1 v/v/v) as eluent to obtain the pure target compounds.

2.4.1. 3-(1',5'-dimethyl-2'-phenyl-4'-(pyrimidin-2-yldiazenyl)-1,1',2',5-tetrahydro spiro [benzo [b] [1,4] diazepine-2,3'-pyrazol]-4-yl)-2H-chro men-2-one (3c)

Light orange powder, m.p: 282–284 °C, *Rf* = 0.77, IR spectrum, ν, cm^{-1}:3204(NH), 1706 (C=O lactone), 1652 (N=N azo). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm; 9.69 (*s*, 2H, NH), 8.74–8.63 (2H, *d*, *J* = 11.8 Hz, pyrimidine), 8.39 (*s*, 1H, coumarin), 8.19–8.12 (*m*, 1H, Pyrimidine), 8.08–8.02 (*m*, 1H, Ar H), 7.93 (2H, *d*, *J* = 8.2 Hz, Ar H), 7.78–7.72 (*m*, 1H, Ar H), 7.65–7.62 (*m*, 1H, Ar H), 7.60–7.53 (*m*, 1H, Ar H), 7.49–7.39 (*m*, 2H, Ar H), 7.34–7.24 (*m*, 2H, Ar H), 6.47 (*s*, 1H, CH=), 3.21 (*s*, 3H,CH_3), 2.38 (*s*, 3H, CH_3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm; 160.5 (C=O lactone) ;154.9 (C Pyrimidine); 150.4 (C Ar); 150.0 (C_{Ar}); 148.6 (C_{Ar}); 147.8 (C_{Ar}); 143.2 ($C_{Pyrimidine}$); 140.7 (C_{Ar}); 136.4 (C_{Ar}); 134.6 (C_{Ar}); 132.9 (C_{Ar}); 130.5 ($C_{olefinic}$); 129.6 ($C_{olefinic}$); 129.4 ($C_{olefinic}$); 127.2 (C_{Ar}); 125.8 (C_{Ar}); 124.3 (C_{Ar}); 123.1 (C_{Ar}); 122.9 (C_{Ar}); 122.3 ($C_{olefinic}$); 119.6 (C_{Ar}); 118.7 (C_{Ar}); 116.9 ($C_{Pyrimidine}$); 116.0 (C_{Ar}); 113.2 ($C_{olefinic}$); 109.9 ($C_{olefinic}$); 106.5 (C_{spiro}); 48.1 (C_{methyl}); 12.2

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(C_{methyl}). Found, %: C 69.36; H 4.72; N 20.26. $C_{32}H_{26}N_8O_2$. Calculated, %: C 69.30; H 4.73; N 20.20.

2.4.2. 3-(1',5'-dimethyl-2'-phenyl-4'-(pyrimidin-2-yldiazenyl)-1',2'-dihydro-5H-spiro [benzo[b] [1,4] oxazepine-2,3'-pyrazol]-4-yl)-2H-chromen-2-one(3d)

Light orange powder, m.p: 286–288 °C, *Rf* = 0.71, IR spectrum, ν, cm^{-1}: 3116 (NH), 1720 ($C=O_{lactone}$), 1664 (N=N azo). ^1H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm; 10.03 (*s*, 1H, NH), 8.65 (2H, *d*, *J* = 8.5 Hz, pyrimidine), 8.39 (*s*, 1H, coumarin), 8.19–8.12 (*m*, 1H, Pyrimidine), 8.08–8.02 (*m*, 1H, Ar H), 7.95–7.91 (*m*, 1H, Ar H), 7.74 (2H, *d*, *J* = 12.1 Hz, Ar H), 7.65–7.62 (*m*, 2H, Ar H), 7.49–7.39 (*m*, 2H, Ar H), 7.34–7.24 (*m*, 2H, Ar H), 4.52 (*s*, 1H, CH=), 3.12 (*s*, 3H,CH_3), 2.20 (*s*, 3H,CH_3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm; 160.6 ($C=O_{lactone}$); 155.0 ($C_{Pyrimidine}$) 150.5 (C_{Ar}); 150.1 (C_{Ar}); 148.7 ($C_{Pyrimidine}$); 147.9 ($C_{olefinic}$); 143.3 ($C_{olefinic}$); 140.8 (C_{Ar}); 136.5 (C_{Ar}); 134.7 (C_{Ar}); 133.0 (C_{Ar}); 130.6 ($C_{olefinic}$); 129.7 ($C_{olefinic}$); 129.6 (C_{Ar}); 127.3 (C_{Ar}); 125.9 (C_{Ar}); 124.4 ($C_{olefinic}$); 123.2 (C_{Ar}); 123.0 (C_{Ar}); 122.4 (C_{Ar}); 119.7 (C_{Ar}); 118.8 (C_{Ar}); 117.0 (C_{Ar}); 116.1 ($C_{Pyrimidine}$); 113.3 (C_{Ar}), 110.0 (C_{spiro}); 106.6 ($C_{olefinic}$); 48.1 (C_{methyl}); 12.3 (C_{methyl}). Found, %: C 69.25; H 4.56; N 17.56. $C_{32}H_{25}N_7O_3$. Calculated, %: C 69.18; H 4.54; N 17.59.

2.5. Preparation of target spirocyclic isoxazole 3-(7,8-dimethyl-6-phenyl-9-(pyrimidin-2-yldiaze nyl)-1-oxa-2,6,7-triaza spiro [4.4] nona-2,8-dien-3-yl)-2H-chromen-2-one(3e)

General method. A mixture of azo-chalcone **3** (0.7 g, 1.5 mmol) and hydroxylamine hydrochloride (0.1 g, 1.5 mmol) in 15 mL of ethanol and a solution of potassium hydroxide (0.2 g) in water (2 ml) were added to the reaction mixture and refluxed for 7 h. The progress of the reaction was monitored using TLC. After completion of the reaction, the reaction mixture was poured into crushed ice and neutralized with dilute HCl. Finally, the solid formed was filtered, washed with water, dried under vacuum, and purified through column chromatography using hexane, ethyl acetate, and dichloromethane (3.5:1:0.5 v/v/v) as eluent to obtain the pure target compounds.

Light orange powder, m.p: 266–268 °C, *Rf*=0.79, IR spectrum, ν, cm^{-1}: 3204(NH), 1733 ($C=O_{lactone}$), 1662(N=N azo). ^1H NMR (400 MHz, DMSO-d_6), δ, ppm; 8.19 (2H, *d*, *J* = 12.3 Hz, Pyrimidine), 7.98 (*s*, 1H, coumarin), 7.80–7.72 (*m*, 1H, Pyrimidine), 7.55–7.47 (*m*, 2H, Ar H), 7.41–7.30 (*m*, 2H, Ar H), 7.05–6.97 (*m*, 2H, Ar H), 3.04 (*d*, 2H, *J* = 7.3 Hz, CH_2), 2.74 (*s*, 3H, CH_3), 2.15 (*s*, 3H, CH_3). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm; 160.5 ($C=O_{lactone}$); 160.4 ($C=N_{imine}$) ; 158.0 ($C_{Pyrimidine}$); 154.6 ($C_{Pyrimidine}$); 150.3 (C_{Ar}); 142.9 ($C_{olefinic}$); 136.1 ($C_{olefinic}$); 135.1 (C_{Ar}); 133.0 (C_{Ar}); 130.4 (C_{Ar}); 129.5 (C_{Ar}); 128.4 (C_{Ar}); 127.0 ($C_{olefinic}$); 121.5 ($C_{olefinic}$); 119.3 (C_{Ar}); 116.8 ($C_{Pyrimidine}$); 113.2 (C_{Ar}); 113.2 (C_{Ar}); 112.1 (C_{Ar}); 108.8 (C_{spiro}); 44.9 ($C_{isoxazole}$); 24.9 (C_{methyl}); 13.1 (C_{methyl}). Found, %: C 65.19; H 4.45; N 20.48. $C_{26}H_{21}N_7O_3$. Calculated, %: C 65.13; H 4.41; N 20.45.

2.6. Study of antibacterial activity

All five synthesized spirocyclic coumarin derivatives were biologically evaluated against *S. aureus* (ATCC5923) and *E. coli* (ATCC25922), which were chosen for extended evaluation of antibacterial properties of the studied compounds, with dimethyl sulfoxide (DMSO) and ceftriaxone (30μg/mL) as negative and positive controls. The agar-disc diffusion approach was used to assess the antibacterial activities according to the Özer H. et al. method [17]. In summary, we dissolved 25 and 50 g/mL of each compound in DMSO. The diameter of bacterial colonies was measured, and the percentage of bacterial growth inhibition was determined by the formula [18]:

$$I\ (\%) = (C - T)\cdot 100/C, \quad (1)$$

where *I* (%) is the degree of inhibition of bacterial growth. *C* is the diameter of the bacterial growth in the standard; *T* is the diameter of the bacterial growth in the test.

2.7. Molecular docking studies

Molecular docking investigations were carried out using the AutoDock Vina 1.1.2 software for molecular docking simulation studies. The 3D structure of DNA gyrase proteins was obtained from the RCSB Protein Data Bank: *E. coli* (PDB ID: 1KZN) and *S. aureus* (PDB ID: 3g75). All water molecules and the ligand were eliminated prior to the docking calculations for compound 3a, and the 3D structures of the ligand and conformations were generated using the ChemAxon Marvin Sketch 5.3.735 program and saved in the mol2 format. Gaussian 09 software was used to optimize and minimize the energy of compound **3a**. Auto Dock Tools (ADT) 1.5.6 was used to prepare the ligand and protein. The binding affinities of compound 3a to the DNA gyrase protein of *E. coli* (PDB ID: 1KZN) and *S. aureus* (PDB ID: 3g75) were simulated by mimicking the interactions between the protein and compound. Discovery Studio Visualizer (BIOVIA, Discovery Studio, v4.0.100.13345) was employed to analyze the interactions between the ligand and the targeted proteins [19].

3. Results and Discussions

Various compounds were used as nucleophilic reagents, such as thiourea, guanidine, benzene-1,2-diamine, 2-amino phenol and hydroxyl amine hydrochloride, and reacted with azo-chalcone [20] to form the heterocyclic compounds such as pyrimidine [21], diazepine and oxazepine [22], and isoxazole [23] **(3a–e),** respectively (Scheme 1). All the synthesized compounds were studied by FT-IR, ^1H NMR, ^{13}C-NMR and elemental analysis. Some of the compounds exhibited significant antibacterial activity, however, compound **3a** revealed the highest activity against both *S. aureus* and *E. coli* bacteria as shown in Figure 2.

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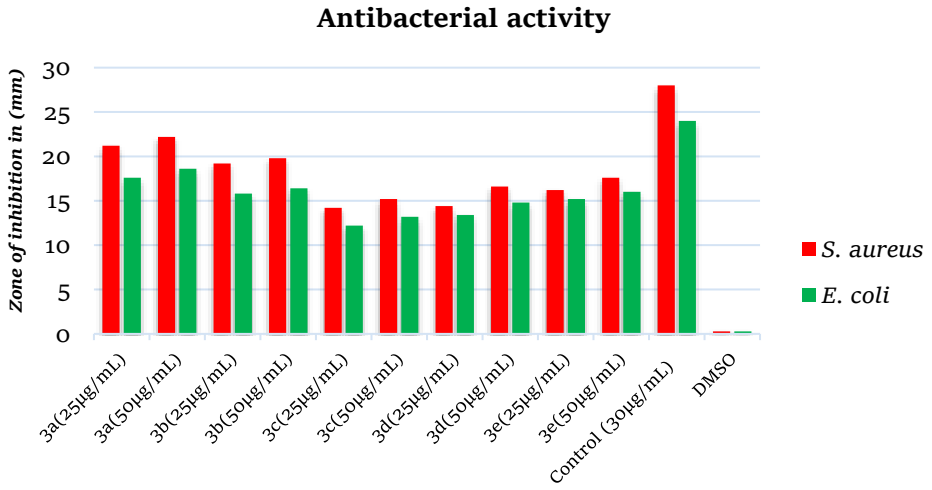

Figure 2 Antibacterial activity of synthesized heterocyclic coumarin derivatives.

Molecular docking results revealed that compound **3a** shows binding modes with DNA gyrase that closely resemble clorobiocin binding mode. Figure 3 (1A-1C) illustrates the formation of **3a** ligand-protein complex in the active site of *E. coli* DNA gyrase (ID: 1KZN) with $\Delta G_{\text{binding energy}}$ value of –9.9 kcal/mol. The formed complex was stabilized by hydrogen bonds (shown by green, dotted lines) with two amino acids ASN32 and GIY63. Different types of bonding interactions (like pi-alkyl, pi-Anion, and pi-Sigma, etc.) are shown by different colors of the dotted lines in Figure 3. As compared with the known antibiotic clorobiocin, the ligand-protein complex inside the active site of the *E. coli* DNA gyrase has $\Delta G_{\text{binding energy}}$ value of -7.2 kcal/mol, see Figure 3 (2A–2C).

On the other hand, Figure 4 (3A-3C) showed the formation of compound **3a** ligand-protein complex in the active site of *S. aureus* DNA gyrase (ID: 3g75) with $\Delta G_{\text{binding energy}}$ value of -8.2 kcal/mol.

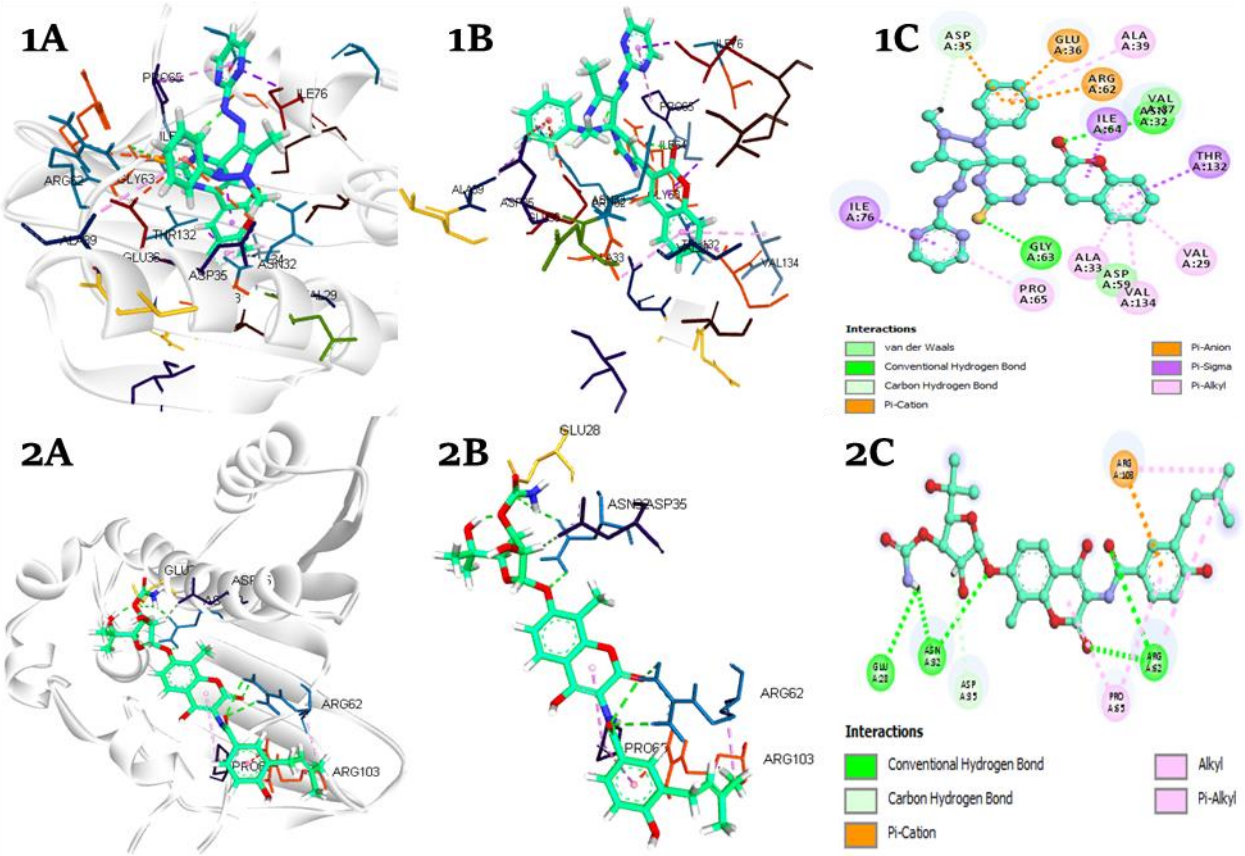

Figure 3 The formed interactions between compound 3a **(1A)**, and the clorobiocin **(2A)**, with *E. coli* DNA gyrase (ID: 1KZN) in a 3D ribbon. Stick model for compound 3a **(1B)**, and the clorobiocin **(2B)** show 3D interactions with specific amino acids residues of *E. coli* DNA gyrase (ID: 1KZN). Compound 3a **(1C)**, and with clorobiocin **(2C)**: shows 2D interactions with specific amino acid residues of *E. coli* DNA gyrase (ID: 1KZN).

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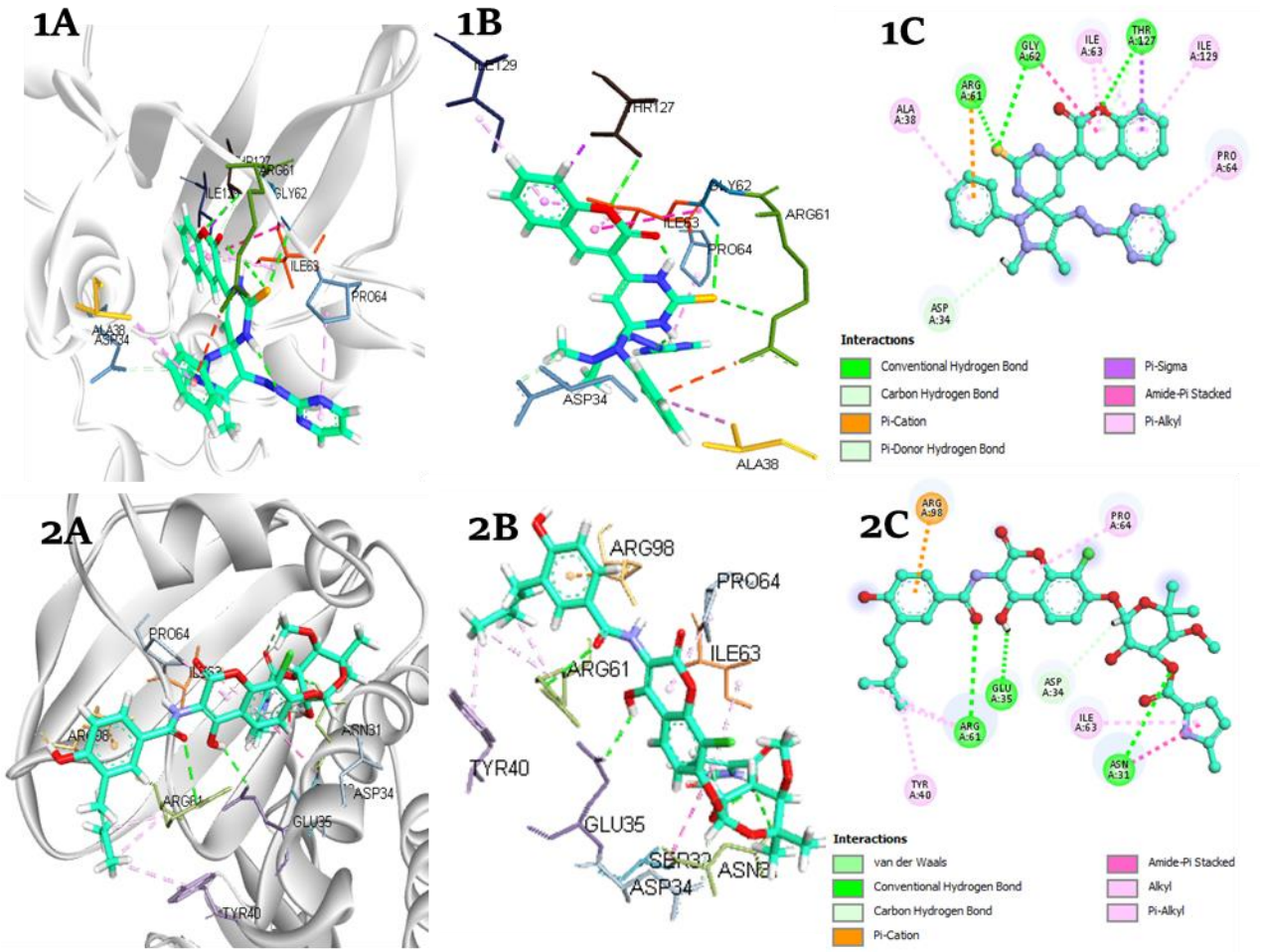

Figure 4 The formed interactions between the compound **3a (3A)**, and the clorobiocin **(4A)**, with the *S. aureus* DNA gyrase (ID: 3g75) in a 3D ribbon. Stick model for compound **3a (3B)**, and the clorobiocin **(4B)** show 3D interactions with specific amino acids residues of *S. aureus* DNA gyrase (ID: 3g75). Compound **3a (3C)**, with clorobiocin **(4C)**: show 2D interactions with specific amino acids residues of *S. aureus* DNA gyrase (ID: 3g75).

The ligand-protein complex was stabilized by hydrogen bonds (shown by green dotted lines) with three amino acids THR A:127, GLY A:62 and ARG A:61, interactions and different types of bonding interactions (like pi-cation, amide-pi stacked, and pi-Sigma etc.). As compared with the known antibiotic clorobiocin, the ligand-protein complex inside the active site of the *S. aureus* DNA gyrase has $\Delta G_{\text{binding energy}}$ of –6.8 kcal/mol (4A–4C). Compound **3a** showed both main hydrogen bond and interactions with different groups such as pyrimidine, thiol group, and lactone of coumarin. Also, possible antibacterial effects may be attributed to further interactions with amino acid residues. According to these findings, the selected compound **3a** has a better docking score of $\Delta G_{\text{binding energy}}$ with bacterial strains compared to clorobiocin. The results provide a sufficient explanation and a convenient correlation between *in vitro* antibacterial assay and the results of docking study.

4. Limitations

The synthesis of spiro- heterocyclic coumarin derivatives, which are important in various fields such as medicine, food industry, and organic chemistry, can present several limitations. The key challenges may be represented by harsh reaction conditions (acids and bases), low yield (long reaction time with low-moderate yield), catalyst requirements (large amount of catalyst and high cost) and environmental concerns (difficulty in disposal of organic reactants and solvents). The aforementioned issues can be a limitation for the synthesis of diverse heterocyclic coumarin derivatives.

5. Conclusions

In this research, spiro-heterocyclic coumarin derivatives were synthesized, characterized and evaluated for their biological activity against *S. aureus* (ATCC 5923) and *E. coli* (ATCC 25922). Compound **3a** showed better antibacterial activity of 22.2 and 18.6 mm zone of inhibition at 50 μg/mL concentration against the bacterial strains, respectively. Furthermore, these results have been supported by molecular docking study, which revealed that the selected compound can bind with bacterial DNA gyrase more efficiently, as with the antibiotic clorobiocin, through its obtained binding energy parameter.

● Supplementary materials

This article contains supplementary materials, which are available on the corresponding online page.

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• Conflict of interest

The authors declare no conflict of interest.

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