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ORIGINAL PAPER

Synthesis, in vitro evaluation and molecular docking of new pyrazole derivatives bearing 1,5,10,10a-tetrahydrobenzo[*g*] quinoline-3-carbonitrile moiety as potent antibacterial agents

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Abstract

In the current study, a new series of pyrazole derivatives **4a**–**d**, **5a**–**d** and **6a**–**d** possessing 1,5,10,10a-tetrahydrobenzo[*g*] quinoline-3-carbonitrile moiety were synthesized by treating chalcones (**3a**–**d**) with hydrazine monohydrate/acetic anhydride, hydrazine monohydrate/formic acid and 4-chlorophenylhydrazine in ethanol, respectively. These reactions proceeded smoothly with satisfactory yields, and the obtained compounds were characterized using FTIR, ^{1}H-NMR, ^{13}C-NMR and elemental analyses. All the synthesized compounds were subjected to antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacteria and molecular docking studies. The antibacterial studies showed that all compounds exhibited good to excellent antibacterial activity against the tested bacterial strains. The structure–activity relationship studies revealed that the compounds **3b**, **4b**, **5b** and **6b** bearing a chlorine atom at the para position of the phenyl group attached to pyrazole moiety in displayed the highest antibacterial activity against all the tested bacteria exceeding the standard drug ampicillin. Moreover, the molecular docking results showed that the highest scores docking have been obtained from the most active antibacterial compounds **3b**, **3d**, **4b**, **4d**, **4c**, **5b** and **6b** with good energy binding score within the active site of topoisomerase II DNA gyrase enzymes (PDB ID: 2XCS), suggesting that they can act by the inhibition of DNA replication. These compounds are auspicious candidates as antibacterial agents that would deserve further investigations.

Keywords Heterocycles · Pyrazole · Quinoline · Antibacterial activity · Molecular docking

Introduction

The transmission and infection by microbes, such as bacteria and fungi, are considered the main origin of diseases in humans in both developed and developing countries [1, 2]. The major reason for the increase in microbial infections was ascribed to the resistance developed by these microbial strains against currently available antimicrobial agents. The emergence of multidrug resistance (MDR) phenomena has forced medicinal chemists to develop more potent antimicrobial agents having attractive structural motifs and more effective biological activities [3]. In recent years, heterocyclic compounds bearing pyrazole nucleus have gained considerable attention owing to their broad spectrum of remarkable pharmacological activity [4].

Heterocyclic compounds possessing pyrazole moiety have occupied a privileged position in medicinal chemistry, and they have been found to denote versatile biological activities

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such as antifungal [5, 6], antibacterial [7, 8], antioxidant [9], analgesic [10], anticancer [11], anti-inflammatory [12], antidiabetic [13] and antitubercular [14].

Various synthetic strategies for the preparation of substituted pyrazoles have been reported including the cyclocondensation of hydrazine and comparable derivatives with carbonyl systems, dipolar cycloadditions and multicomponent reactions [15]. Furthermore, chalcones were used as intermediates in the synthesis of numerous pyrazole derivatives [16, 17].

In synthetic medicinal chemistry, the quinoline scaffold has been known as a trendy core structure for the synthesis of many compounds with potent pharmacological activity [18]. Quinoline derivatives are marked by pharmacokinetics that favors their use in all localized infections. Heterocycles containing a quinoline nucleus have been reported to exhibit a broad spectrum of interesting biological activity including antimalarial [19], anti-inflammatory and analgesic [20], antifungal [21], antidepressant and anticonvulsant [22], antibacterial [23], antioxidant [24], antineoplastic [25]. Since most of the pyrazole and quinoline derivatives display interesting antimicrobial activity, the combination of these synthesized compounds is also expected to present antimicrobial activity.

In 2018, El Shehry et al. [26] described a method to synthesize three series of quinoline derivatives bearing a pyrazole moiety with promising antimicrobial activity. In the first series, 4-(quinolin-2-yloxy)benzaldehyde and 4-(quinolin-2-yloxy)acetophenone were first reacted with ketone or aldehyde derivatives to yield the corresponding chalcones that were further cyclized with hydrazine to give new pyrazoline derivatives. The second series was synthesized through the reaction of 2-hydrazinylquinoline with formylpyrazoles, and the third series was obtained via the treatment of 2-hydrazinylquinoline with ethoxyethylidene, dithioacetal and arylidene derivatives.

As an extension to our work aiming to synthesize novel heterocyclic ring systems having potent pharmacological activity [27–29], we report herein a modification of a substituted quinoline into a variety of novel condensed pyrazole derivatives incorporating the tetrahydrobenzo[*g*]quinoline-3-carbonitrile moiety. In order to explore the biological potential of the novel synthesized pyrazole derivatives, in vitro antibacterial evaluation of the synthesized compounds against bacterial strains was carried out. Besides, molecular docking studies have been conducted to obtain accurate predictions on the optimized conformations for both the pyrazole derivatives (as ligands) and protein targets to form a stable complex.

Results and discussion

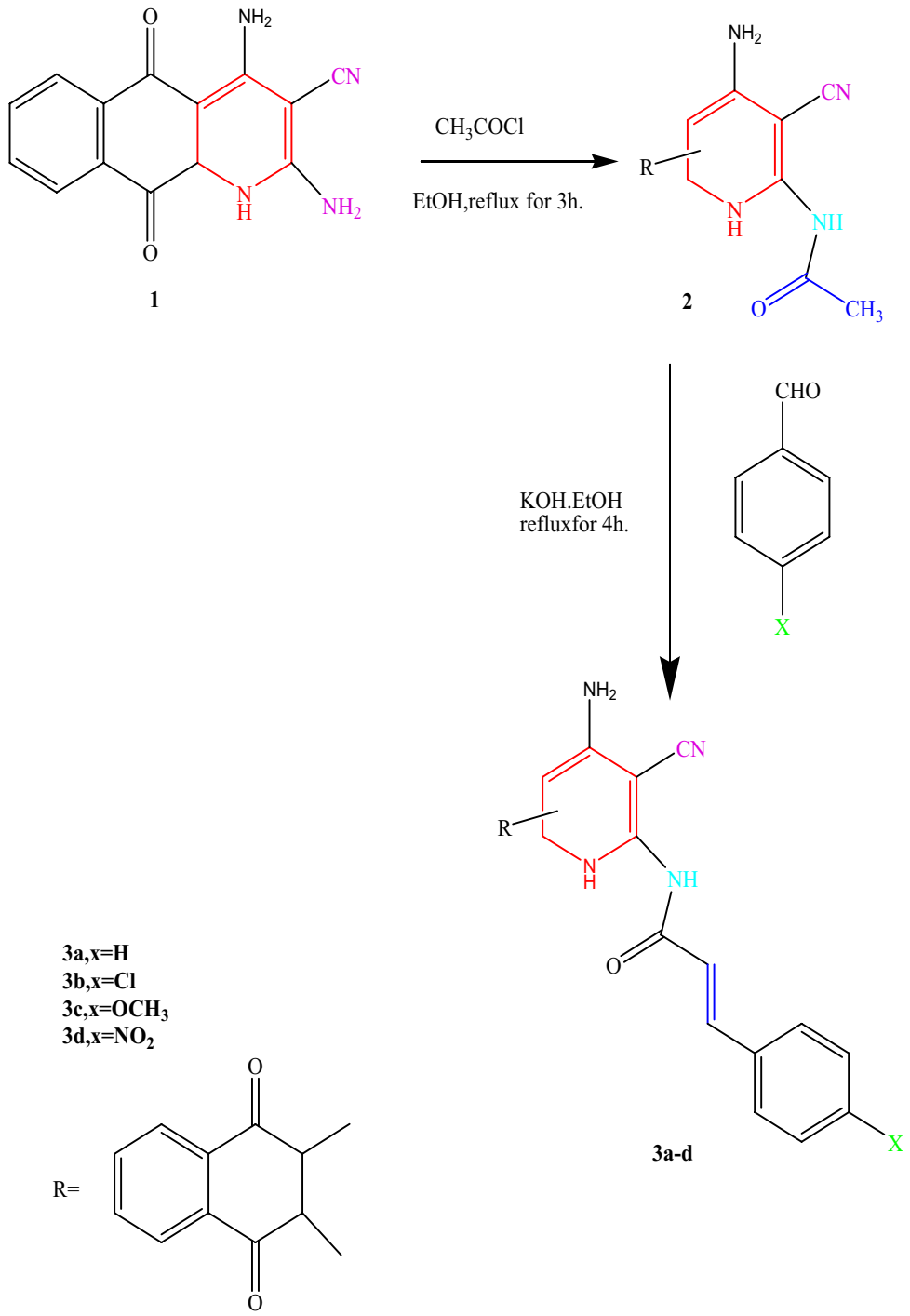
Chemistry

The required starting compound, namely 2,4-diamino-5,10-dioxo-1,5,10,10a-tetrahydrobenz[*g*]quinoline-3-carbonitrile (**1**), was prepared according to a previous reported method [30]. The chemical synthesis of compounds **2** and **3a–d** is illustrated in Scheme 1. The treatment of compound **1** and acetyl chloride in ethanol solvent afforded the corresponding compound *N*-(4-amino-3-cyano-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinolin-2-yl)acetamide (**2**). The structure identification of compound (2) was carried out by spectroscopic analyses (FTIR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry). On the other hand, compound **2** reacted with a different aromatic aldehyde (benzaldehyde, *p*-chloro benzaldehyde, *p*-methoxy benzaldehyde, *p*-nitro benzaldehyde) in the presence of ethanol as solvent and potassium hydroxide to yield chalcones **3a–d** (Scheme 1). The chemical structures of compounds **3a–d** were elucidated based on spectroscopic data (mass spectrometry, ^{13}C-NMR, ^{1}H-NMR and FTIR). The subsequent treatment of chalcones **3a–d** with hydrazine monohydrate, acetic anhydride and ethanol gave the corresponding 2-((1-acetyl-5-(4-substituted phenyl)-4,5-dihydro-1H-pyrazole-3-yl) amino)-4-amino-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**4a–d**) as illustrated in Scheme 2. The chemical structures of the obtained compounds were confirmed based upon their elemental and spectral data.

Due to the medicinal importance of pyrazole and quinoline derivatives, many attempts have been made in this work to further design potent bioactive pyrazole derivatives bearing 1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile moiety. For this reason, different functional groups were attached to the para position of the pyrazole moiety of the synthesized compounds to manipulate their physicochemical and biological properties. Accordingly, chalcones **3a–d** were reacted with hydrazine hydrate and formic acid to produce the corresponding 4-amino-2-((1-formyl-5-substituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**5a–d**) (Scheme 3). All these reactions proceeded smoothly and provided the corresponding compounds **5a–d** with satisfactory yields varying between 71 and 81%. All these new compounds were characterized and identified by spectral data.

Chalcones **3a–d** were treated with 4-chlorophenylhydrazine in ethanol under heating for 4 h affording 4-amino-2-((1-(4-substituted phenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**6a–d**) in good yields (66–76%) (Scheme 4). The structural assignment of compounds **6a–d**

Scheme 1 Synthesis of chalcone derivatives **3a–d**

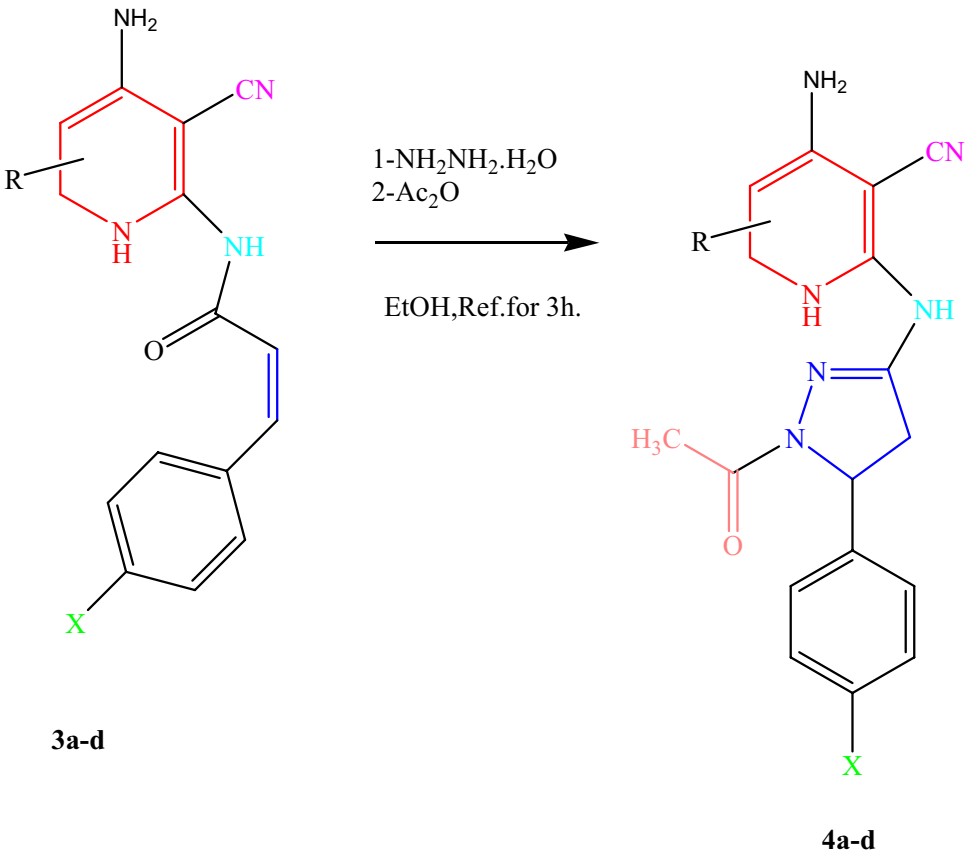


was performed based on elemental and spectral FTIR, ^{1}HNMR, ^{13}C NMR and mass spectrometry analyses as detailed in the experimental section.

Biological activity and SAR studies

After the successful design and elucidation of the chemical structures of all the newly synthesized compounds, we assessed their antibacterial activity in vitro against four species of bacteria, including gram-positive, *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative, *Escherichia coli*, *Pseudomonas aeruginosa* bacteria. Ampicillin was used as a reference standard antibacterial agent, and the mean inhibition diameter values ($n = 3$) are presented in Table 1 in mm/mg sample. Mainly, all the new synthesized compounds exerted good in vitro antibacterial activity against all the tested strains. Compound **4b** exhibited the highest antibacterial activity against *E. coli* bacteria with an

Scheme 2 Synthesis of pyrazole derivatives (**4a–d**)

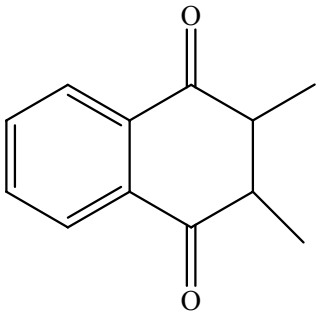


3a-d

4a-d

3,4a,x=H
3,4b,x=Cl
3,4c,x=OCH$_3$
3,4d,x=NO$_2$

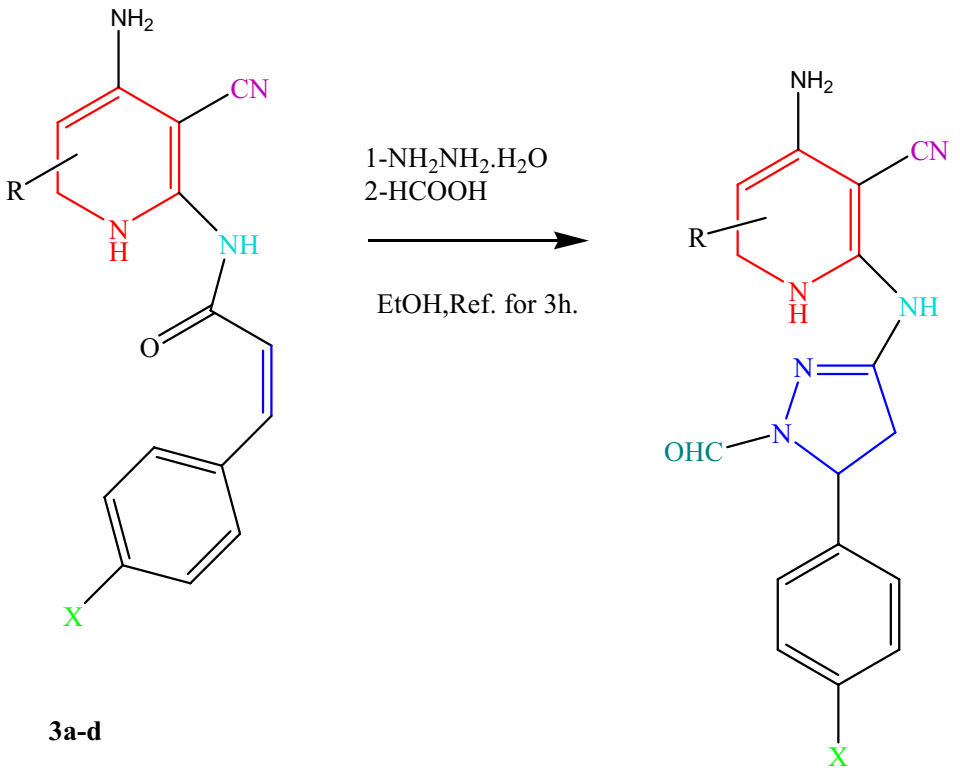
R=



inhibition zone diameter ($d = 35$ mm/mg sample) exceeding that of the reference Ampicillin ($d = 24$ mm/mg sample). In fact, the replacement of the hydrogen atom, present at the para position of the phenyl group attached to pyrazole moiety in compounds (**4a**, **5a** and **6a**), by a withdrawing chlorine atom (**4b**, **5b** and **6b**) showed significant improvement in the antibacterial activity against *Bacillus subtilis, Staphylococcus aureus* and *gram-negative, Escherichia coli, Pseudomonas aeruginosa* bacteria. However, the substitution of the chlorine atom present at the para position of the phenyl group attached to pyrazole moiety in compounds (**3b**, **4b**, **5b**, and **6b**) by an electron-donating methoxy group (**3c**, **4c**, **5c**, and **6c**) and electron-withdrawing nitro group (**3d**, **4d**, **5d** and **6d**) decreased slightly their biological activity against all tested gram-positive and gram-negative bacteria. It is worth mentioning that compounds **1**, **2**, **3a**, **5c** and **6c** displayed the least antibacterial activity against the tested bacterial strains. These results are in agreement with those obtained by the group of Reddy [31] who studied the antimicrobial activity of thiazolidinones–pyrazoles derivatives against various bacterial strains.

A look at the results given in Table 1 and the SAR studies suggests that the presence of chlorine atom at the para position of the pyrazole moiety in compounds (**3b**, **4b**, **5b**, and **6b**) enhances meaningfully their antibacterial activity

Scheme 3 Synthesis of pyrazole derivatives **5a–d**

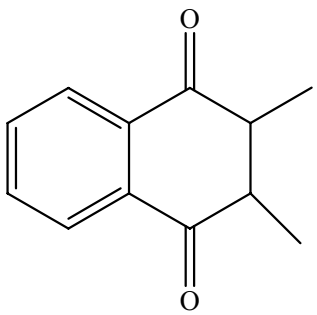


3a-d

5a-d

3,5a,x=H
3,5b,x=Cl
3,5c,x=OCH_3
3,5d,x=NO_2

R=



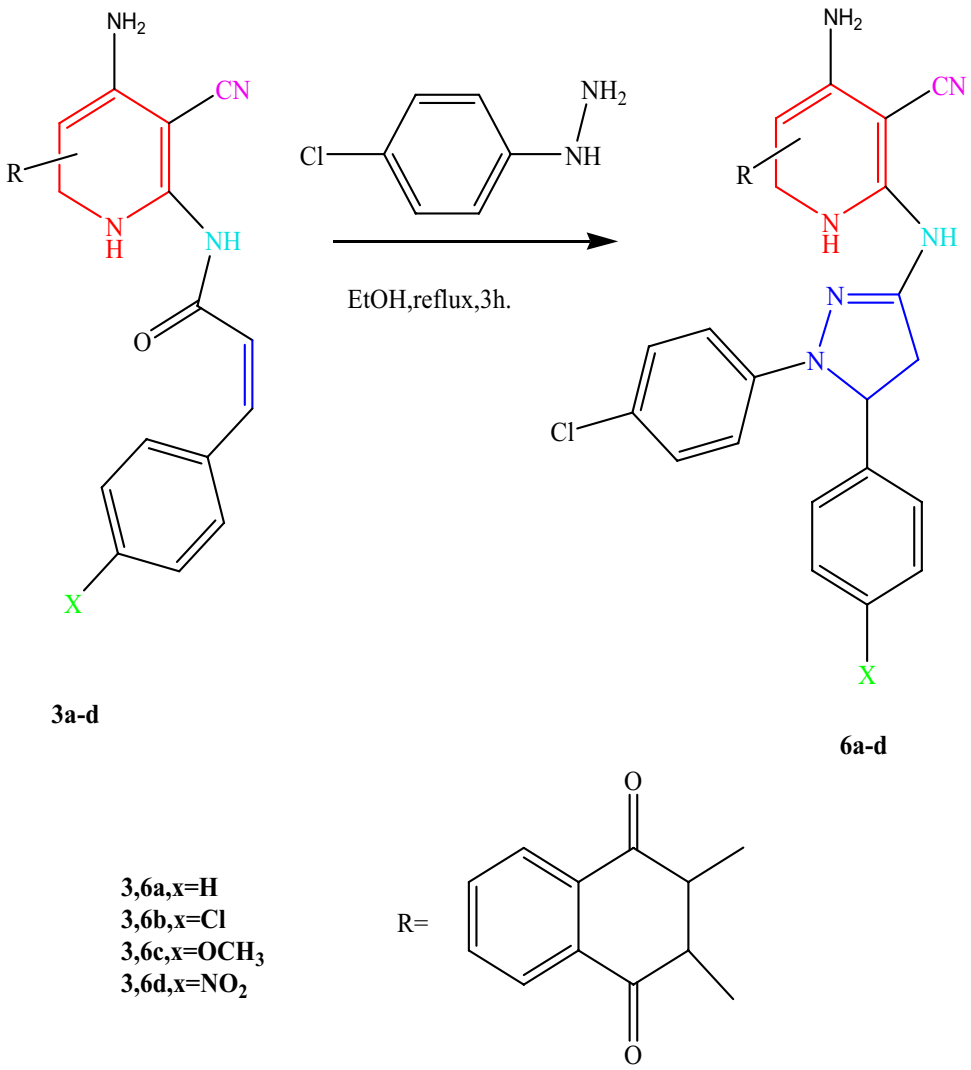
against the gram-positive and gram-negative bacteria compared to the methoxy and nitro-derivatives.

Molecular docking study

In the present work, docking studies were realized to obtain precise predictions on the optimized conformations for both the new synthesized pyrazole derivatives (as ligands) and protein targets to form a stable complex. For this aim, the most active antibacterial prepared compounds **3b**, **3d**, **4c**, **4b**, **4d**, **5a**, **5b**, and **6b** were subjected to molecular simulation study within the active site of topoisomerase II by the use of MOE, 2010, Chemical Computing Group Inc., to predict the action mechanism of these novel candidates. The crystal structure of topoisomerase II was downloaded from PDB (code: 2XCS). GSK299423 was redocked into topoisomerase II with a root mean standard deviation (RMSD) = 1.1524, the main binding of GSK299423 with topoisomerase II resulted in two main residues Asp A1083 and Asp B1083 producing two hydrogen bondings with NH group with binding energy = −17.45 kcal/mol (Fig. 1).

The binding energy scores and hydrogen bonds formed with the amino acids from group interaction atoms obtained from this study are presented in Table 2. From these data, compound benzo[*g*]quinolin (**3b**) recorded high binding energy (− 19.31 kcal/mol) with five hydrogen bonds as

Scheme 4 Synthesis of pyrazole derivatives **6a–d**



follows: (1) C=O with Asp B1083 through water molecule, (2) CN group with Asp B1083 through water molecule, (3) Arg A1122 with CN, (4) NH_2 group with Ser B1084 via water molecule, and (5) C=O moiety with DC D11 (Fig. 2).

Regarding compound **3d**, it demonstrated seven hydrogen bonding interactions with Asp b1083, Asp A1083 and Arg B1122 (Fig. 3).

On the other hand, 2-((1-acetyl-5-(4-methoxyphenyl)-4, 5-dihydro-1H-pyrazol-3-yl) amino)-4-amino-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**4c**) exhibited good fitting with topoisomerase binding site forming five hydrogen bonds as follows: (1) Dc D12 with OCH_3, (2) Ala 1118 with C=O, (3) Met A1075 with CN, (4) Asp B1083 with CN and (5) Asp B1083 with CN (Fig. 4).

The target compound **4d** recorded three binding with DG D10, Arg 1122 and DC D11 through hydrogen bondings with NH2, CN and C=O moieties, sequentially (Fig. 5).

Furthermore, the target compound **5a** exhibited good fitting with topoisomerase II. It showed arene–arene interaction with phenyl group, in addition to five interactions through H bondings as below (Fig. 6):

(a) Arg A1122 with C=O
(b) Ser B1084 with CN
(c) Ser B1084 with CN via water molecule
(d) Ser B1084 with NH_2 via water molecule
(e) Asp A1083 with CN

In addition, the candidate **5b** demonstrated three H bondings with DG D10 with the amino group, DG D11 with carbonyl group and Arg A1122 with cyano moiety (Fig. 7).

Finally, the most active antibacterial compound **4b** revealed the highest energy binding score − 20.44 kcal/mol recording six hydrogen bonding within the active site of topoisomerase II with Dc D12, DG C9, Ser A1084, DG C8, DA C7 and Glu A1088 amino acids (Fig. 8).

Table 1 In vitro antibacterial activity of the synthesized compounds against *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* bacteria

Sample	Bacterial species			
	Gram-positive		Gram-negative	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Inhibition zone diameter (mm/mg sample)[a]				
Control (DMSO)	0	0	0	0
Ampicillin	28	21	26	24
1	15	13	11	12
2	18	16	14	17
3a	20	17	15	22
3b	29	23	25	26
3c	24	21	19	17
3d	25	22	23	24
4a	22	18	18	19
4b	30	22	35	26
4c	26	20	22	21
4d	28	21	23	25
5a	23	19	21	18
5b	27	21	29	25
5c	21	19	20	22
5d	26	20	22	24
6a	22	18	23	19
6b	25	20	27	26
6c	22	17	25	21
6d	23	18	18	23

[a]The mean values of three experiments are given

Furthermore, the candidate **6b** showed three hydrogen bonds within the receptor with Ser B438, Ala A1118 and DG D10 through binding with CN and NH2 functional groups (Fig. 9).

Experimental

Chemistry

Unless otherwise mentioned, all reagents and solvents were purchased from Sigma-Aldrich and used without any further purification. Reaction progress was monitored using thin-layer chromatography (TLC) on silica gel pre-coated F254 Merck plates (Darmstadt, Germany), and spots were visualized by ultraviolet irradiation. The melting points of all synthesized compounds were determined with an electrothermal device Gallenkamp electrothermal (Weiss-Gallenkamp, Loughborough, UK) and were used without correction. Infrared spectra data were recorded on a PerkinElmer Lambda 40 spectrometer using KBr pellets. Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) was run on a Varian VXR300/5 FT NMR spectrometer at 300 MHz in DMSO-d6 using TMS as an internal standard, and chemical shift (δ) values were recorded in ppm. Electron ionization mass spectrometry (EIMS) was carried out with a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elemental analysis data were performed by PerkinElmer elemental analyzer, and it was carried out at the Micro analytical Center of Cairo University.

Synthesis of compounds (1)

The synthesis of compound (**1**) was carried out as per the protocol previously mentioned in a previous Ref. [30].

Synthesis of *N*-(4-amino-3-cyano-5,10-dioxo-1,5,10,10a tetrahydrobenzo[g]quinolin-2-yl) acetamide (2)

A mixture of compound (**1**) (2.66 g, 0.01 mol), ethanol (20 mL) and acetyl chloride (0.78 g, 0.01 mol) [32, 33] was put in 250-mL round flask and was stirred under reflux for 3–4 h until complete reaction. The obtained product was treated with cold water, filtered and recrystallized from ethanol to afford compound (**2**). 75% yield; Mp: 210–212 °C; IR (V_{max}, cm^{-1}), 1715–1615 (acyclic C=O), 1650 (2 CO

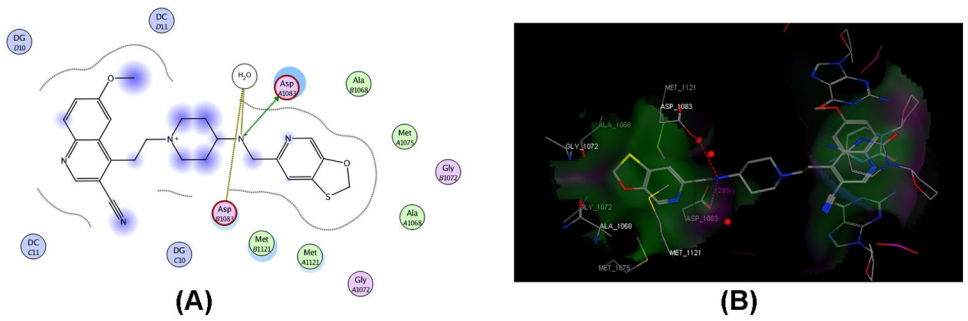


Fig. 1 **a** Compound GSK299423 within topoisomerase II (3D structure), **b** 2D interaction between topoisomerase II with GSK299423

Table 2 Binding energy results of compounds **3b**, **3d**, **4c**, **4b**, **4d**, **5a**, and **5b**

Compound	Affinity kcal/mol	No. of hydrogen bonds	Distance (Å) from the main residue		Functional group
3b	− 19.31	5	Asp B1083	2.88	C=O
			Asp B1083	2.17	CN
			Arg A1122	2.99	C=O
			Ser B1084	2.87	NH2
			DC D11	3.08	C=O
3d	− 19.11	7	Arg B1122	2.78	NH_2
			Asp A1083	2.65	NH_2
			Asp B1083	2.76	C=O
			Asp B1083	3.16	C=O
			Asp B1083	2.98	C=O
			Asp B1083	2.54	NH
			Asp B1083	2.32	NH
4b	− 20.44	6	DG C9	2.51	NH_2
			Dc D12	2.87	CN
			Ser A1084	3.24	C=O
			DG C8	2.98	CN
			DA C7	2.45	CN
			Glu A1088	2.87	CN
4c	− 18.65	5	Dc D12	2.88	OCH_3
			Ala 1118	2.62	C=O
			Met A1075	3.21	CN
			Asp B1083	3.67	CN
			Asp B1083	3.34	CN
4d	− 18.98	3	DG D10	3.87	NH2
			Arg 1122	3.11	CN
			DC D11	3.43	C=O
5a	− 17.98	5	Arg A1122	2.88	C=O
			Ser B1084	3.15	CN
			Ser B1084	2.56	CN
			Ser B1084	3.53	NH_2
			Asp A1083	3.66	CN
5b	− 18.45	3	DG D10	2.76	NH
			DG D11	3.12	C=O
			Arg A1122	3.54	CN
6b	− 19.98	3	Ser B438	2.56	CN
			Ala A1118	2.73	CN
			DG D10	3.02	NH_2
GSK299423	− 17.45	2	Asp A1083	3.76	NH
			Asp B1083	3.42	NH

quinone ring), 2214 (CN), 3390–3375 (2 NH), 3185 (NH_2); ^{1}H-NMR δ_H: 2.57 (s, 3H, CH_3 group), 6.55 (s, 2H, NH_2), 4.67 (s, 1H, CH), 7.01–8.02 (m, 4H, $J = 7.42$ Hz, Ar–H), 10.24, 10.36 (s, 2H, 2NH) ppm; ^{13}C NMR δ_C: 23.58 (CH_3), 59.46 (CH), 74.78, 110.36, 145.37, 156.54, 130.73, 136.38,126.25, 133.64, 126.47, 134.46 (C=C), 115.83 (CN), 170.31, 183.32, 196.54 (3CO) ppm; MS (El, *m*/*z*): 308; analysis calculated for: $C_{16}H_{12}N_4O_3$ (308.29); C, 62.33; H, 3.92; N, 18.17, Found; C, 62.36; H, 3.95; N, 18.22.

General procedure for the synthesis of compounds (3a–d)

Compound (**2**) (3.08 g, 0.01 mmol) was treated with substituted benzaldehydes (benzaldehyde, *p*-chloro benzaldehyde, *p*-methoxy benzaldehyde, *p*-nitro benzaldehyde) (0.01 mol) and KOH (0.1 g) in ethanol (7 mL), and the mixture was refluxed for 4 h before cooling to room temperature. The reaction undergoes condensation process according to

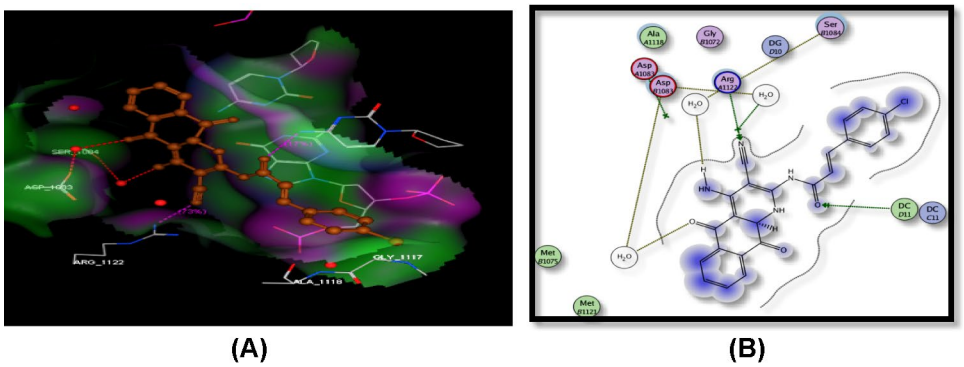


Fig. 2 **a** Compound **3b** within topoisomerase II (3D structure), **b** 2D interaction between topoisomerase II with **3b**

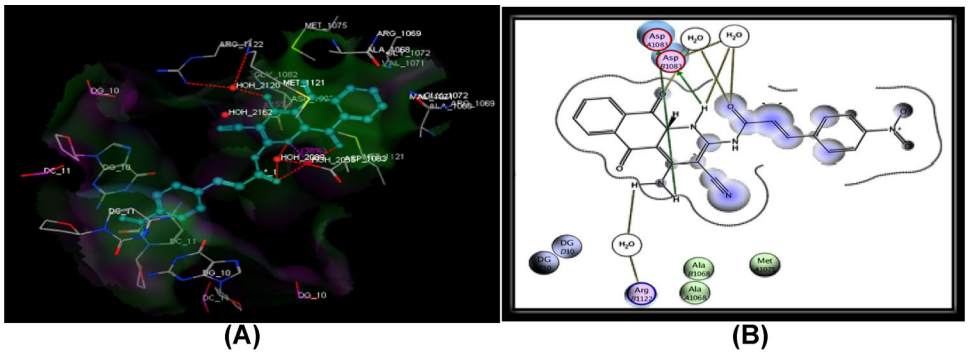


Fig. 3 **a** Compound **3d** within topoisomerase II (3D structure), **b** 2D interaction between topoisomerase II with **3d**

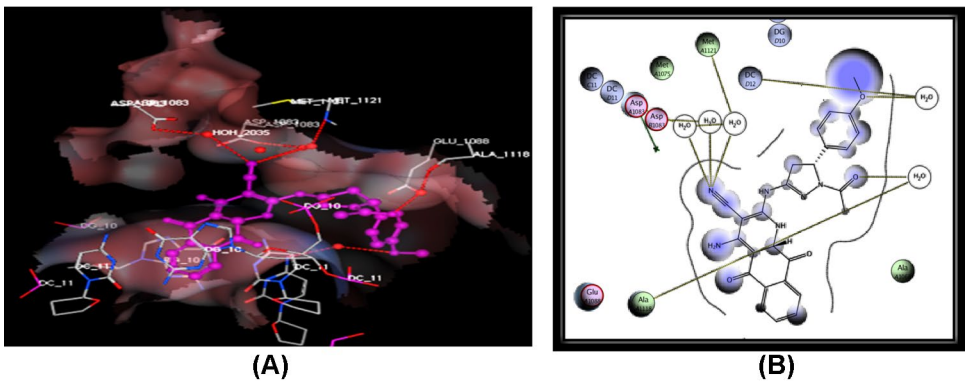


Fig. 4 **a** Compound **4c** within topoisomerase II (3D structure), **b** 2D interaction between topoisomerase II with **4c**

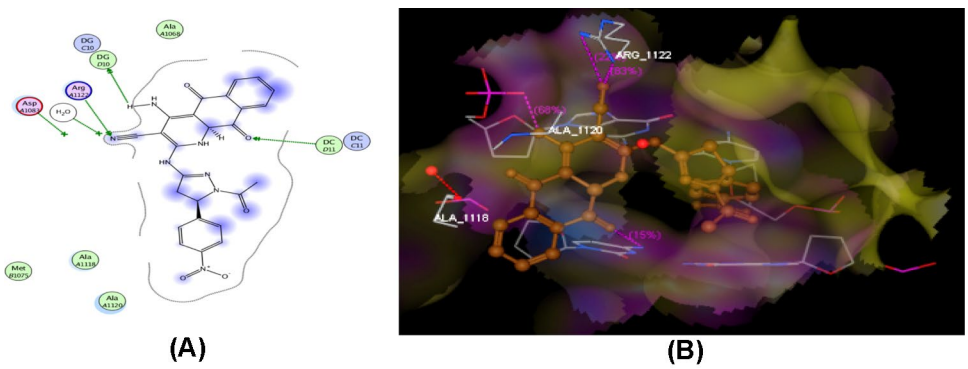


Fig. 5 **a** Compound **4d** within topoisomerase II (2D structure), **b** 3D interaction between topoisomerase II with **4d**

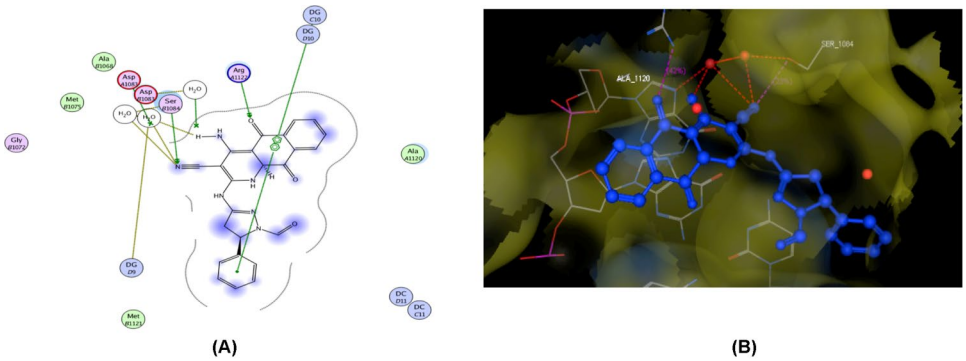


Fig. 6 **a** Compound **5a** within topoisomerase II (2D structure), **b** 3D interaction between topoisomerase II with **5a**

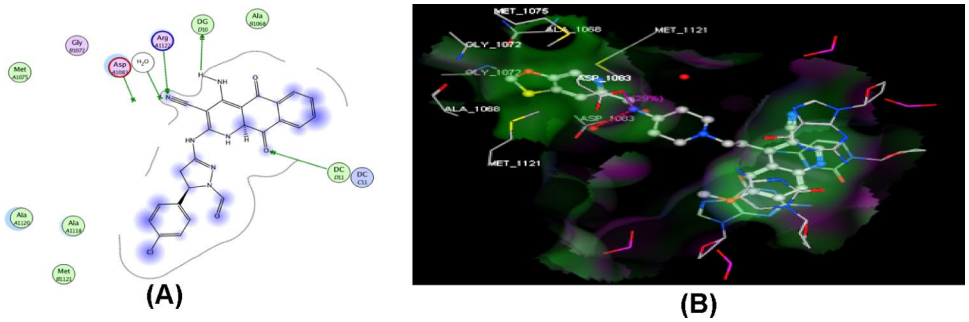


Fig. 7 **a** Compound **5b** within topoisomerase II (2D structure), **b** 3D interaction between topoisomerase II with **5b**

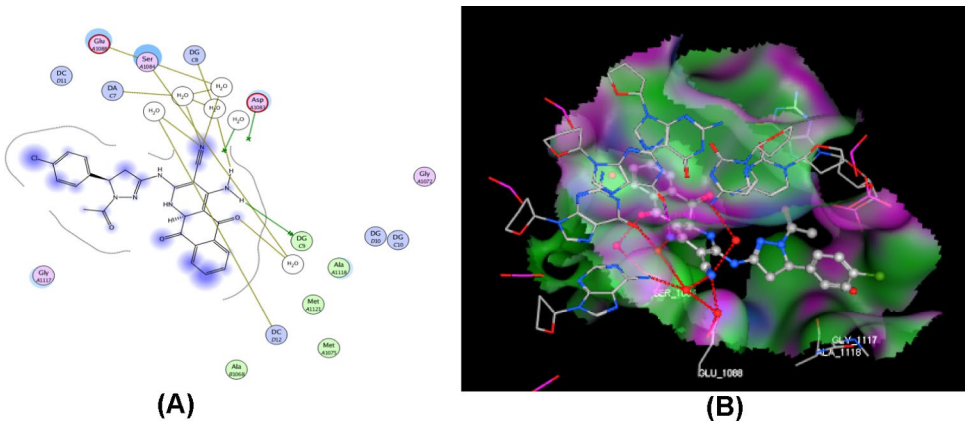


Fig. 8 **a** Compound **4b** within topoisomerase II (2D structure), **b** 3D interaction between topoisomerase II with **4b**

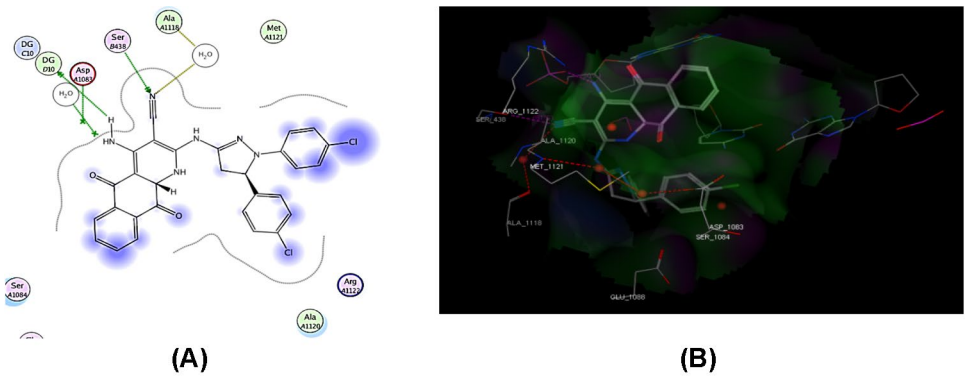


Fig. 9 **a** Compound **6b** within topoisomerase II (2D structure), **b** 3D interaction between topoisomerase II with **6b**

Claisen–Schmidt method. The solvent was removed under reduced pressure, and the residue was filtered and recrystallized with ethanol and dried.

Synthesis of 4-amino-3-cyano-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinolin-2-yl)cinnamamide (**3a**)

Yield 67%; Mp: 185–187 °C; IR (V_{max}, cm^{-1}), 3400–3100 (NH_2, 2NH), 2216 (CN), 1711–1615 (acyclic C=O), 1663 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 6.98 (d, 1H, $J=7.11$ Hz, CH=), 6.78 (d, 1H, $J=7.15$ Hz, O=C–CH), 6.57 (s, 2H, NH_2), 4.69 (s, 1H, CH), 7.02–8.52 (m, 9H, $J=7.22$ Hz, Ar–H), 10.25, 10.36 (s, 2H, 2NH); ^{13}C NMR δ_C: 59.46 (CH), 74.78, 110.36, 126.25, 126.47, 130.73, 133.64, 134.46, 136.38, 145.37, 156.54 (C=C) 127.96, 128.58, 128.67, 135.31 (C=C), 109.62, 141.83 (C=C chalcone),115.83 (CN), 165.52, 183.32, 196.54 (3 CO) ppm; MS (El, *m*/*z*): 396; analysis calculated for: $C_{23}H_{16}N_4O_3$ (396.4); C, 69.69; H, 4.07; N, 14.13, Found; C, 69.72; H, 4.13; N, 14.17.

Synthesis of *N*-(4-amino-3-cyano-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinolin-2-yl)-3-(4-chlorophenyl) acrylamide (**3b**)

Yield 64%; Mp: 243–245 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, 2NH), 2223 (CN), 1717–1615 (acyclic C=O), 1667 (2 CO) cm^{-1}; ^{1}H-NMR δ (ppm): 6.99 (d, 1H, $J=7.14$ Hz, CH=CH),6.82 (d, 1H, $J=7.18$ Hz, O=C–CH=CH), 6.61 (s, 2H, NH_2), 4.73 (s, 1H, CH), 7.02–8.52 (m, 8H, $J=7.58$ Hz, Ar–H), 10.26, 10.39 (s, 2H, 2 NH); ^{13}C NMR δ_C: 59.46 (CH), 74.78, 110.36, 126.25, 126.47, 130.73, 133.64, 134.46, 136.38,145.37,156.54 (C=C) 128.88, 129.07, 133.39,133.57 (C=C phenyl), 109.62, 141.83 (C=C chalcone),115.83 (CN), 165.52, 183.32, 196.54 (3CO) ppm; MS (El, *m*/*z*): 430; analysis calculated for: $C_{23}H_{15}ClN_4O_3$ (430.8); C, 64.12; H, 3.51; Cl, 8.23; N, 13.00, Found; C, 64.15; H, 3.56; Cl, 8.23; N, 13.05.

Synthesis of *N*-(4-amino-3-cyano-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinolin-2-yl)-3-(4-methoxyphenyl)acrylamide (**3c**)

Yield 68%; Mp: 255–257 °C; IR (V_{max}, cm^{-1}), 3400–3100 (NH_2, 2NH), 2225 (CN), 1720–1615 (acyclic C=O), 1668 (2 CO) cm^{-1}; ^{1}H-NMR δ (ppm): 3.63 (s, 3H, CH_3),6.94 (d, 1H, $J=7.12$ Hz, CH=CH), 6.80 (d, 1H, $J=7.16$ Hz, O=C–CH=CH), 6.59 (s, 2H, NH_2), 4.70 (s, 1H, CH), 7.02–8. 52 (m, 8H, $J=7.26$ Hz, Ar–H), 10.25, 10.37 (s, 2H, 2NH); ^{13}C NMR δ_C: 55.86 (CH_3), 59.46 (CH), 74.78, 110.36, 126.25, 126.47, 130.73, 133.64, 134.46, 136.38, 145.37, 156.54 (C=C), 114.28, 127.57, 130.14, 159.88 (C=C phenyl), 109.62, 141.83 (C=C chalcone), 115.83 (CN), 165.52, 183.32, 196.54 (3CO) ppm; MS (El, *m*/*z*): 426: analysis calculated for: $C_{24}H_{18}N_4O_4$ (426.4); C, 67.60; H, 4.25; N, 13.14, Found; C, 67.64; H, 4.28; N, 13.17.

Synthesis of *N*-(4-amino-3-cyano-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[g]quinolin-2-yl)-3-(4-nitrophenyl)acrylamide (**3d**)

Yield 72%; Mp: 267–269 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, 2NH), 2226 (CN), 1723–1615 (acyclic C=O), 1668 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 4.78 (s, 1H, CH), 6.95 (d, 1H, $J=7.13$ Hz, CH=CH),6.81 (d, 1H, $J=7.20$ Hz, O=C–CH=CH), 6.61 (s, 2H, NH_2), 7.02–8.52 (m, 8H, $J=7.38$ Hz, Ar–H), 10.24,10.36 (s, 2H, 2NH); ^{13}C NMR δ_C: 59.46 (CH), 74.78, 110.36, 126.25, 126.47, 130.73, 133.64, 134.46, 136.38, 145.37,156.54 (C=C),123. 84,129.08, 141.41, 147.23 (C=C phenyl), 109.62, 141.83 (C=C chalcone), 115.83 (CN), 165.52, 183.32, 196.54 (3CO) ppm; MS (El, *m*/*z*): 441: analysis calculated for: $C_{23}H_{15}N_5O_5$ (441.4); C, 62.58; H, 3.43; N, 15.87, Found; C, 62.61; H, 3.47; N, 15.92.

General procedure for the synthesis of compounds (**4a–d**)

A mixture of chalcone (**3a–d**) (0.01 mol), hydrazine monohydrate (0.5 g, 0.01 mol) and EtOH (10 mL) was refluxed under vigorous stirring for 1 h. Subsequently, the acetic anhydride (1.5 mL) was added to the mixture, and the solution was refluxed for 3 h. The precipitate formed was filtered and purified via crystallization with EtOH/CH_2Cl_2 (1:3) as eluent to obtain compounds (**4a–d**).

Synthesis of 2-((1-acetyl-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)amino)-4-amino-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**4a**)

Yield 66%; Mp: 223–225 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2221 (CN), 1720–1615 (acyclic C=N), 1660 (2 CO) cm^{-1}; ^{1}H-NMR δ (ppm): 2.15 (s, 3H, CH_3), 3.47 (d, 2H, $J=7.14$ Hz, CH_2), 4.67 (s, 1H, CH), 4.94 (t, 1H, $J=7.25$ Hz, CH), 6.58 (s, 2H, NH_2), 7.02–8.02 (m, 9H, $J=7.31$ Hz, Ar–H), 10.24 (s, 2H, 2 NH); ^{13}C NMR δ_C: 23.51 (CH_3), 39.85 (CH_2), 59.34, 63.84 (2CH), 72.57, 110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 158.37 (C=C), 126.75, 126.97, 128.59, 141.78 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 168.54, 183.55, 196.56 (3CO) ppm; MS (El, *m*/*z*): 452: analysis calculated for: $C_{25}H_{20}N_6O_3$ (452.46); C, 66.36; H, 4.46; N, 18.57, Found; C, 66.39; H, 4.51; N, 18.63.

Synthesis of 2-((1-acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)amino)-4-amino-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**4b**)

Yield 63%; Mp: 270–272 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2225 (CN), 1722–1617 (acyclic C=N), 1663 (2 CO) cm^{-1}, ^{1}H-NMR δ (ppm): 2.18 (s, 3H, CH_3), 3.49 (d, 2H, $J=7.16$ Hz, CH_2), 4.74 (s, 1H, CH), 4.97 (t, 1H, $J=7.29$ Hz, CH), 6.62 (s, 2H, NH_2), 7.02–8.02 (m, 8H, $J=7.55$ Hz, Ar–H), 10. 27 (s, 2H, 2 NH); ^{13}C NMR δ_C: 23.51 (CH_3), 39.85 (CH_2), 59.34, 63.84 (2CH), 72.57, 110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 158.37 (C=C), 127.31, 128.64, 132.37, 139.85 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 168.54, 183.55, 196.56 (3CO) ppm; MS (El, *m*/*z*): 486: analysis calculated for: $C_{25}H_{19}ClN_6O_3$ (486.91); C, 61.67; H, 3.93; Cl, 7.28; N, 17.26, Found; C, 61.71; H, 3.98; Cl, 7.33; N, 17.30.

Synthesis of 2-((1-acetyl-5-(4-methoxyphenyl)-4, 5-dihydro-1H-pyrazol-3-yl) amino)-4-amino-5, 10-dioxo-1, 5, 10, 10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**4c**)

Yield 70%; Mp: 220–223 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2221 (CN), 1722–1617 (acyclic C=N), 1663 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 2.16 (s, 3H, CH_3), 3.46 (d, 2H, $J=7.13$ Hz, CH_2), 3.69 (s, 3H, CH_3), 4.72 (s, 1H, CH), 4.96 (t, 1H, $J=7.23$ Hz, CH), 6.62 (s, 2H, NH_2), 7.02–8.02 (m, 8H, $J=7.21$ Hz, Ar–H), 10.25 (s, 2H, 2 NH); ^{13}C-NMR (DMSO–d_6 $\delta=23.51$ (CH_3), 39.85 (CH_2), 55.91 (CH_3), 59.34, 63.84 (2CH), 72.57, 110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 158.37 (C=C), 114.32, 126.74, 134.21, 158.71 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 168.54, 183.55, 196.56 (3CO) ppm; MS (El, *m*/*z*): 482: analysis calculated for: $C_{26}H_{22}N_6O_4$ (482.17); C, 64.72; H, 4.60; N, 17.42, Found; C, 64.75; H, 4.64; N, 17.47.

Synthesis of 2-((1-acetyl-5-(4-nitrophenyl)-4, 5-dihydro-1H-pyrazol-3-yl) amino)-4-amino-5, 10-dioxo-1, 5, 10, 10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**4d**)

Yield 65%; Mp: 229–231 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2225 (CN), 1722–1617 (acyclic C=N), 1664 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 2.20 (s, 3H, CH_3), 3.51 (d, 2H, $J=7.15$ Hz, CH_2), 4.80 (s, 1H, CH), 4.99 (t, 1H, $J=7.26$ Hz, CH), 6.65 (s, 2H, NH_2), 7.02–8.02 (m, 8H, $J=7.49$ Hz, Ar–H), 10.31 (s, 2H, 2 NH); ^{13}C NMR δ_C: 23.51 (CH_3), 39.85 (CH_2), 59.34, 63.84 (2CH), 72.57,110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 158.37 (C=C), 123.49, 123.81, 146.07, 147.86 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 168.54, 183.55, 196.56 (3CO) ppm; MS (El, *m*/*z*): 497: analysis calculated for: $C_{25}H_{19}N_7O_5$ (497.46); C, 60.36; H, 3.85; N, 19.71, Found; C, 60.40; H, 3.91; N, 19.75.

General procedure for the synthesis of compounds (**5a–d**)

A mixture of chalcones (**3a**–**d**) (0.01 mmol) and hydrazine monohydrate (0.5 g, 0.01 mol) in ethanol (10 mL) was stirred under reflux for 1 h. Subsequently, formic acid (1.5 mL) was added to the mixture, and the solution was refluxed for 3 h. The solid product was collected by filtration, washed with water, and recrystallized from ethanol.

Synthesis of 4-amino-2-((1-formyl-5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) amino)-5, 10-dioxo-1, 5, 10, 10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**5a**)

Yield 71%; Mp: 261–263 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2220 (CN), 1725–1615 (acyclic C=N), 1670 (C=O), 1660 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 3.46 (d, 2H, $J=7.12$ Hz, CH_2), 4.68 (s, 1H, CH),4.91 (t, 1H, $J=7.22$ Hz, CH), 6.56 (s, 2H, NH_2), 7.02–8.02 (m, 9H, $J=7.13$ Hz, Ar–H), 10. 22 (s, 2H, 2NH), 10.41 (s, 1H, aldehyde-H); ^{13}C-NMR δ_C 39.85 (CH_2), 59.34, 63.84 (2CH), 72.57, 110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 158.37 (C=C), 126.75, 126.97, 128.59,141.78 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 159.86, 183.55, 196.56 (3CO) ppm; MS (El, *m*/*z*): 438: analysis calculated for: $C_{24}H_{18}N_6O_3$ (438.44); C, 65.75; H, 4.14; N, 19.17; Found: C, 65.79; H, 4.17; N, 19.22.

Synthesis of 4-amino-2-((5-(4-chlorophenyl)-1-formyl-4,5-dihydro-1H-pyrazol-3-yl)amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (**5b**)

Yield 81%; Mp: 292–294 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2225 (CN), 1725–1620 (acyclic C=N),1670 (C=O), 1660 (2CO) cm^{-1}; 1H-NMR δ (ppm): 3.51 (d, 2H, $J=7.14$ Hz, CH_2), 4.71 (s, 1H, CH),4.99 (t, 1H, $J=7.25$ Hz, CH), 6.65 (s, 2H, NH_2), 7.02–8.02 (m, 8H, $J=7.47$ Hz, Ar–H), 10.30 (s, 2H, 2NH), 10.44 (s, 1H, aldehyde-H); ^{13}C NMR δ_C: 39.85 (CH_2), 59.34, 63.84 (2CH), 110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 72.57, 158.37 (C=C), 127.31, 128.64, 132.37, 139.85 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 159.86, 183.55, 196.56 (3CO) ppm; MS (El, *m*/*z*): 472: analysis calculated for: $C_{24}H_{17}ClN_6O_3$ (472.88): C, 60.96; H, 3.62; Cl, 7.50; N, 17.77; Found, 60.98; H, 3.66; Cl, 7.54; N, 17.82.

Synthesis of 4-amino-2-((1-formyl-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl) amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (5c)

Yield 75%; Mp: 232–234 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2221 (CN), 1720–1610 (acyclic C=N),1670 (C=O), 1663 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 3.45 (d, 2H, $J=7.11$ Hz, CH_2), 3.67 (s, 3H, CH_3), 4.72 (s, 1H, CH), 4.96 (t, 1H, $J=7.21$ Hz, CH), 6.63 (s, 2H, NH_2), 7.02–8.02 (m, 8H, $J=7.35$ Hz, Ar–H), 10. 24 (s, 2H, 2 NH), 10.42 (s, 1H, aldehyde –H); ^{13}C NMR δ_C: 39.85 (CH_2), 55.91 (CH_3), 59.34, 63.84 (2CH), 72.57, 110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 158.37 (C=C), 114.32, 126.74, 134.21, 158.71 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 159.86, 183.55, 196.56 (3 CO) ppm; MS (El, *m*/*z*): 468: analysis calculated for: $C_{25}H_{20}N_6O_4$ (468.46): C, 64.10; H, 4.30; N, 17.94; Found; C, 64.14; H, 4.34; N, 17.97.

Synthesis of 4-amino-2-((1-formyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (5d)

Yield 78%; Mp: 297–299 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2225 (CN), 1723–1615 (acyclic C=N), 1670 (C=O), 1664 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 3.53 (d, 2H, $J=7.13$ Hz, CH_2), 4.77 (s, 1H, CH), 5.12 (t, 1H, $J=7.26$ Hz, CH), 6.69 (s, 2H, NH_2), 7.02–8.02 (m, 8H, $J=7.53$ Hz, Ar–H), 10. 35 (s, 2H, 2 NH), 10.45 (s, 1H, aldehyde-H); ^{13}C-NMR δ_C: 39.85 (CH_2), 59.34, 63.84 (2CH), 110.41, 126.28, 126.45, 130.74, 133.66, 134.48, 136.35, 156.53, 72.57, 158.37 (C=C), 123.49, 123.81, 146.07, 147.86 (C=C phenyl), 115.85 (CN), 153.31 (C=N), 159.87, 183.55, 196.56 (3CO) ppm; MS (El, *m*/*z*): 483: analysis calculated for: $C_{24}H_{17}N_7O_5$ (483.44): C, 59.63; H, 3.54; N, 20.28; Found: C, 59.66; H, 3.57; N, 20.31.

General procedure for the synthesis of compounds (6a–d)

A mixture of chalcones (**3a**–**d**) (0.01 mol) and 4-chlorophenylhydrazine (1.42 g, 0.01 mol) in ethanol (10 mL) was refluxed for 3 h under stirring. The suspension was cooled down to room temperature to give a precipitate, which was collected by filtration and recrystallized from ethanol.

Synthesis of 4-amino-2-((1-(4-chlorophenyl)-5-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) amino)-5, 10-dioxo-1, 5, 10, 10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (6a)

Yield 76%; Mp: 226–228 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2223 (CN), 1720–1615 (acyclic C=N), 1663 (2CO) cm^{-1}. ^{1}H-NMR δ (ppm): 3.48 (d, 2H, $J=7.12$ Hz CH_2), 4.70 (s, 1H, CH), 4.95 (t, 1H, $J=7.22$ Hz, CH), 6.59 (s, 2H, NH_2), 7.02–8.02 (m, 13H, $J=7.31$ Hz, Ar–H), 10.23 (s, 2H, 2NH); ^{13}C NMR δ_C: 39.94 (CH_2), 58.41, 59.37 (2CH), 72.56, 110.39, 126.51, 130.75, 133.65, 134.43, 156.36, 158.34 (C=C), 153.21(C=N), 115.87 (CN), 114.95, 126.34, 126.75, 126.98, 128.56, 129.67, 141.94, 143.57 (C=C), 183.35, 196.46 (2CO) ppm; MS (El, *m*/*z*): 520: analysis calculated for: $C_{29}H_{21}ClN_6O_2$ (520.97): C, 66.86; H, 4.06; Cl, 6.81; N, 16.13; Found: C, 66.89; H, 4.11; Cl, 6.84; N, 16.18.

Synthesis of 4-amino-2-((1,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (6b)

Yield 73%; Mp: 273–275 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2226 (CN), 1723–1620 (acyclic C=N), 1665 (2CO) cm^{-1}. ^{1}H-NMR δ (ppm): 3.51 (d, 2H, $J=7.32$ Hz, CH_2), 4.72 (s, 1H, CH), 4.98 (t, 1H, $J=7.27$ Hz, CH), 6.62(s, 2H, NH_2), 7.02–8.02 (m, 12H, $J=7.61$ Hz, Ar–H), 10.25 (s, 2H, 2NH); ^{13}C NMR δ_C: 39.94 (CH_2), 58.41, 59.37 (2CH), 72.56, 110.39, 126.51, 130.75, 133.65, 134.43, 156.36, 158.34 (C=C), 153.21 (C=N), 115.87 (CN), 114.95, 126.34, 127.32, 128.67, 129.67, 132.35, 141.66, 141.94 (C=C), 183.35, 196.46 (2CO) ppm; MS (El, *m*/*z*): 555: analysis calculated for: $C_{29}H_{20}Cl_2N_6O_2$ (555.41); C, 62.71; H, 3.63; Cl, 12.77; N, 15.13; Found; C, 62.75; H, 3.66; Cl, 12.81; N, 15.16.

Synthesis of 4-amino-2-((1-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (6c)

Yield 69%; Mp: 224–226 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, NH), 2224 (CN), 1720–1613 (acyclic C=N),1665 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 3.44 (d, 2H, $J=7.29$ Hz CH_2), 3.68 (s, 3H, CH_3), 4.74 (s, 1H, CH), 4.96 (t, 1H, $J=7.23$ Hz, CH), 6.61 (s, 2H, NH_2), 7.02–8. 02 (m, 12H, $J=7.37$ Hz, Ar–H), 10.22 (s, 2H, 2 NH); ^{13}C NMR δ_C: 39.94 (CH_2), 55.86 (CH_3), 58.41, 59.37 (2CH), 72.56, 110.39, 126.51, 130.75, 133.65, 134.43, 156.36, 158.34 (C=C), 153.21 (C=N), 115.87 (CN), 114.18, 126.66, 135.85, 158.68, 114.95, 126.34, 129.67, 141.94 (C=C), 183.35, 196.46 (2 CO) ppm; MS (El, *m*/*z*): 551: analysis calculated for: $C_{30}H_{23}ClN_6O_3$ (551.00): analysis calculated for: C, 65.39; H, 4.21; Cl, 6.43; N, 15.25; Found; C, 65.39; H, 4.21; Cl, 6.43; N, 15.25.

Synthesis of 4-amino-2-((1-(4-chlorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)amino)-5,10-dioxo-1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile (6d)

Yield 66%; Mp: 233–235 °C; IR (V_{max}, cm^{-1}) 3400–3100 (NH_2, 2NH), 2225 (CN), 1724–1615 (acyclic C=N), 1664 (2CO) cm^{-1}; ^{1}H-NMR δ (ppm): 3.55 (d, 2H, J = 7.31 Hz, CH_2), 4.77 (s, 1H, CH), 5.15 (t, 1H, J = 7.25 Hz, CH), 6.71 (s, 2H, NH_2), 7.02–8. 02 (m, 12H, J = 8.21 Hz, Ar–H), 10.35 (s, 2H, 2 NH); ^{13}C NMR δ_C: 39.94 (CH_2), 55.86 (CH_3), 58.41, 59.37 (2CH), 72.56, 110.39, 126.51, 130.75, 133.65, 134.43, 156.36, 158.34 (C=C), 153.21 (C=N), 115.87 (CN), 114.95, 123.47, 123.78, 126.34, 129.67, 141.94, 145.87, 149.69 (C=C), 183.35, 196.46 (2CO) ppm; MS (El, *m/z*): 565: analysis calculated for: $C_{29}H_{20}ClN_7O_4$ (565.97): C, 61.54; H, 3.56; Cl, 6.26; N, 17.32; Found: C, 61.56; H, 3.61; Cl, 6.32; N, 17.37.

Antibacterial evaluation

All eighteen synthesized compounds were in vitro screened against gram-positive bacteria: *B. subtilis, S. aureus*, and gram-negative bacteria: *E. coli, P. aeruginosa*. The antibacterial activity of the synthesized compounds was assessed adopting the disk diffusion method as outlined in a previous study [34]. In brief, one microliter of the each gram-positive and gram-negative bacteria was grown in 10 mL of fresh media until reaching a count of 108 cells mL^{-1}. Subsequently, 100 µL of the bacterial suspension was spread onto the Mueller–Hinton agar medium. Plates impregnated with gram-positive bacteria including *S. aureus* and *B. subtilis*; gram-negative bacteria as *Escherichia coli* were incubated at 35–37 °C for 24–48 h. Filter disks impregnated with 10 µL of solvent were used as a negative control. The standard disk of ampicillin (an antibacterial agent), served as a positive control for antibacterial activity. All experiments were repeated and carried out in triplicate in the case of a significant difference in the results, and the average inhibition diameter was measured in mm/mg sample. The results are presented in Table 1.

Molecular docking study

Molecular docking studies were conducted in this work in order to obtain precise predictions on optimized conformation for both the new synthesized derivatives (as ligand) and their target receptor protein to form a stable complex. The cocrystallized topoisomerase II was downloaded from protein data bank (Code 2XCS), the docking studies were carried out by London dG force, and refinement of the results was realized using force field energy. The preparation of the synthesized compounds for docking was achieved via their 3D structure built by Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc., Montreal, Quebec, Canada). Specific procedures were taken into account before docking including 3D protonation of the structures, running conformational analysis using systemic search, selecting the least energetic conformer and applying the same docking protocol used with ligand.

Conclusion

In conclusion, simple synthetic routes were successfully established to design new pyrazole derivatives having 1,5,10,10a-tetrahydrobenzo[*g*]quinoline-3-carbonitrile moiety. The structures of the identified compounds were in agreement with the spectral data. They were also subjected to in vitro screening to test their antibacterial activity against gram-positive and gram-negative bacteria. The findings demonstrated that all compounds displayed good to excellent antibacterial activity against the tested bacterial strains. In fact, compounds **4b** and **3b** possess excellent antibacterial activity against all the bacteria exceeding that of the standard drug ampicillin. In addition, compound **4d** exhibited similar antibacterial activity to ampicillin against *Bacillus subtilis* and Staphylococcus *aureus* bacteria. Mainly, compounds **3d**, **5b**, **5d**, **6b** showed interesting antibacterial activity against gram-positive and gram-negative bacteria. The structure–activity relationship studies suggested that the presence of the chlorine atom at the para position of the phenyl attached to the pyrazole moiety affects meaningfully the antibacterial activity of the synthesized compounds (**3b**, **4b**, **5b**, and **6b**) in comparison with other compounds bearing hydrogen atom, methoxy and nitro groups. The conducted molecular docking studies ascertained that the most active antibacterial compounds possessed good energy binding scores within the active site of topoisomerase II DNA gyrase enzymes (PDB ID: 2XCS), suggesting that they can act by the inhibition of DNA replication.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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