

Full Paper

Design and Synthesis of Novel Benzopyran-2-one Derivatives of Expected Antimicrobial Activity through DNA Gyrase-B Inhibition

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In an attempt to find a new class of antibacterial agents, we have synthesized thirty new coumarin (2*H*-benzopyran-2-one) analogues. These derivatives include substituted azetidin-2-ones (β -lactam) **3a–f**, pyrrolidin-2-ones **4a–f**, 2*H*-1,3,4-oxadiazoles **5a–f**, and thiazolidin-4-ones **6a–f** attached to 4-phenyl-2*H*-benzopyran-2-one through an oxyacetamido or an oxymethyl bridge. The target compounds were synthesized starting from 2-oxo-4-phenyl-2*H*-benzo[b]pyran-7-yl-oxy-acetic acid hydrazides **2a–f**. The new compounds were evaluated as DNA gyrase-B inhibitors through molecular modeling and docking techniques using the Molsoft ICM 3.4-8C program. The synthesized compounds were also screened for antibacterial activity against four different species of Gram-positive and Gram-negative bacteria; as well as screening against *C. albicans* for antifungal activity. The molecular modeling data were in accordance with the antimicrobial screening results.

Keywords: Antimicrobial activity / Coumarin / DNA gyrase-B inhibitor / 2*H*-benzopyran-2-one / Molecular modeling

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Introduction

Antibiotic resistance is a major problem worldwide and, therefore, it is essential to develop new antibacterial drugs. In DNA replication, one group of enzymes has proved to be an effective target for therapeutic agents, which are topoisomerase enzymes. DNA gyrase – a type II topoisomerase – is found in all bacteria and controls the topological state of DNA [1]. DNA gyrase consists of two subunits GyrA (875 amino acids) and GyrB (804 amino acids) with the active species being a heterotetramer A_2B_2 [2]. Mechanistic studies have revealed the steps that are involved in the gyrase-supercoiling reaction [3]. This process involves the wrapping of DNA around the A_2B_2 com-

plex, cleavage of this DNA on both strands, and the passage of a segment of DNA through the double strand break. Re-ligation of the break results in the introduction of two negative supercoils. These processes require the binding and hydrolysis of ATP [4]. Inhibition of DNA gyrase blocks relaxation of supercoiled DNA, relaxation being a requirement for transcription and replication. DNA gyrase is a selective target for antibacterial agents, such as the most studied quinolone and coumarin antibiotics. Quinolone drugs (*e.g.* ciprofloxacin) affect the protein subunit GyrA, and coumarins (*e.g.* novobiocin) act on GyrB [5, 6].

The synthesis and antibacterial activity of azetidin-2-ones as new antimicrobial agents against multidrug-resistant pathogens were reported [7, 8]. Furthermore, a number of substituted pyrrolidinone, 1,3,4-oxadiazoles, and thiazolidin-4-ones were found to exhibit appreciable antimicrobial and antifungal activities [9–14]. These observations prompted us to incorporate the β -lactam (azetidin-2-one), pyrrolidin-2-one, 2*H*-1,3,4-oxadiazole, or thiazolidin-4-one moieties with the coumarin nucleus to synthesize new derivatives of expected antimicrobial

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Abbreviation: Internal Coordinate Mechanics (ICM)

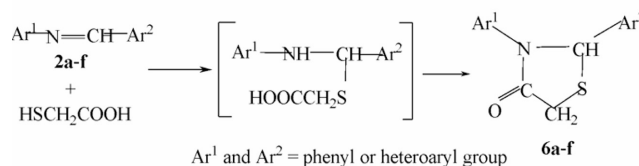
activity potentially through inhibition of DNA gyrase-B enzyme. Molecular docking represents one of the growing applications in computational biology, where in molecular modeling techniques are used to predict how macromolecules (typically a protein) interact with other molecules (may be other proteins, nucleic acids, or small drug-like molecules). In many cases, molecular modeling results give a reasonable prediction of the biological activity a drug molecule may possess. So, to understand the obtained microbiological data on a structural basis, we evaluated the scoring functions of the gyrase-B inhibitors (Novobiocin, and the newly synthesized compounds) through molecular modeling and docking techniques using Molsoft ICM 3.4-8C program. ICM stands for “Internal Coordinate Mechanics” – a program originally aimed at energy optimization of several biopolymers with respect to an arbitrary subset of internal coordinates such as bond lengths, bond angles, torsion angles, and phase angles [15–17].

Results and discussion

Chemistry

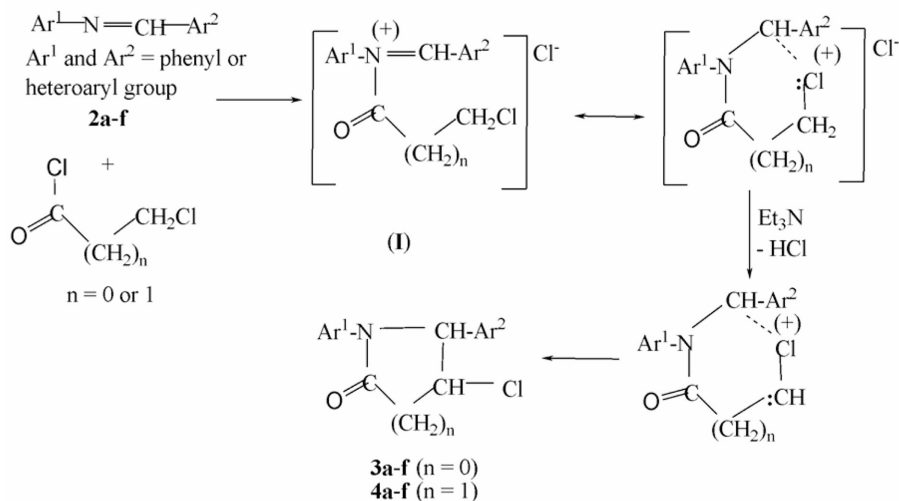
The key starting materials (2-oxo-4-phenyl-2H-benzo[b]pyran-7-yl) oxyacetic acid arylidene hydrazides **2a–f** were obtained in good yields upon condensation of (2-oxo-4-phenyl-2H-benzo[b]pyran-7-yl) oxyacetic acid hydrazide **1** with different aromatic aldehydes in acetic acid [18]. Reaction of the arylidene derivatives **2a–f** with two different acid chlorides namely chloroacetyl chloride and 3-chloropropionyl chloride in presence of triethylamine gave azetidin-2-one (monocyclic β -lactam) deriv-

atives **3a–f** and pyrrolidin-2-one derivatives **4a–f**, respectively. Studies on the mechanism of azetidin-2-one (β -lactam) formation [19] concluded that the salt (**I**) is formed as an intermediate which undergoes dehydrohalogenation in the presence of triethylamine to produce **3a–f** (see Equation 1 and Scheme 1). In the intermediate (**I**), a partial positive charge should reside on the carbon atom of the imine which becomes later C-4 of the azetidin-2-one (β -lactam) **3a–f**. It is reasonable to expect that the atom carrying a free pair of electrons would form a loose bond with this carbon and generate a transition state. This would assure the ease of formation of β -lactam by bringing the appropriate carbon atoms C-3 and C-4 close to bond forming distance. Similarly, pyrrolidin-2-one derivatives **4a–f** were formed. In another approach, treatment of arylidene derivatives **2a–f** with acetic anhydride and anhydrous sodium acetate gave 2H-1,3,4-oxadiazoles **5a–f** through acetylation with concomitant cyclization (Scheme 1). Finally, the reaction of **2a–f** with mercaptoacetic acid gave the corresponding 2,3-diaryl-1,3-thiazolidin-4-ones **6a–f** (see Equation 2 and Scheme 1). Both ana-

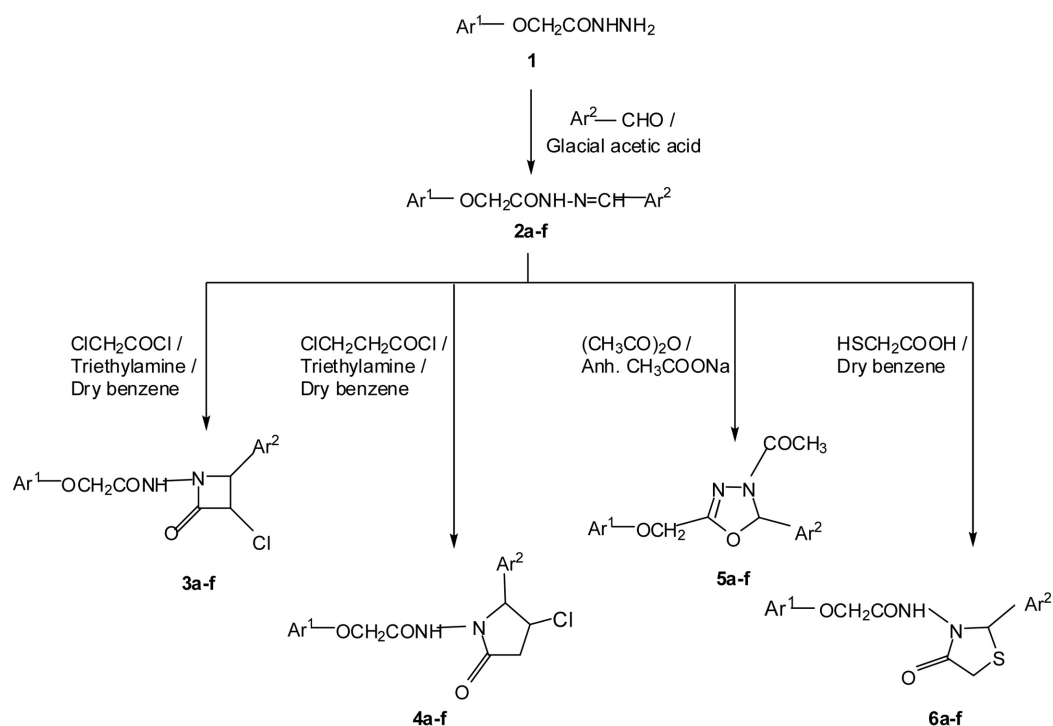
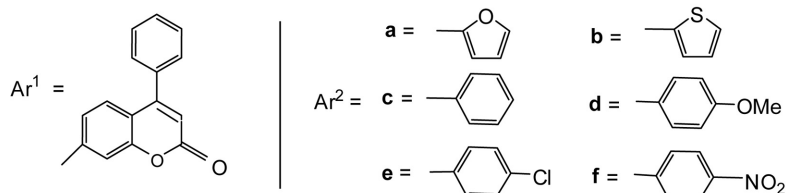


Equation 2. Mechanism of 4-thiazolidinones formation.

lytical and spectroscopic data of all the synthesized compounds are in full agreement with the proposed structures.



Equation 1. Mechanism of β -lactam and pyrrolidin-2-ones formation.

**Legend for derivatives 2-6:****Scheme 1.** Mechanisms of formation of compounds **3a–f**, **4a–f**, and **6a–f**.**Antimicrobial screening**

All the 30 newly synthesized compounds were tested *in vitro* for their antibacterial activity (10 mg/mL) using the agar diffusion method [20] against *Bacillus subtilis*, *Sarcina lutea*, and *Staphylococcus aureus* as representatives of Gram-positive bacteria, *Escherichia coli* as representative of Gram-negative bacteria, and the fungus *Candida albicans*. The antimicrobial activity of the newly synthesized compounds is reflected as zone of growth inhibition of the tested microorganisms (measured in mm) (Table 1). Among the arylidene hydrazides **2a–f**, compound **2f** was the most active derivative giving a significant activity against *Bacillus subtilis* (20 mm), *Sarcina lutea* (15 mm), and *Staphylococcus aureus* (20 mm) as Gram-positive bacteria, *Escherichia coli* (15 mm) as Gram-negative bacteria and also against the fungus *Candida albicans* (15 mm). On

the other hand, compound **2c** showed only slight activity against *Bacillus subtilis* (8 mm). As for the β -lactam derivatives **3a–f**, compound **3f** gave good activity against *Staphylococcus aureus* (20 mm) and slight activity against *Escherichia coli* (9 mm), while compound **3c** was only slightly active against *Bacillus subtilis* (9 mm). On the other hand, screening of pyrrolidin-2-ones **4a–f** revealed four derivatives possessing antimicrobial activity. Compound **4f** gave moderate activity against *Bacillus subtilis* (15 mm), while **4a** and **4c** showed also moderate activity against *Staphylococcus aureus* (15 and 12 mm, respectively). Finally, **4c** and **4e** showed slight activity against *Sarcina lutea* (8 mm for both). Of the 2H-1,3,4-oxadiazoles **5a–f**, **5a**, and **5d** showed slight activity against *Staphylococcus aureus* (9 mm) and *Sarcina lutea* (9 mm), respectively. Finally, **6a** was the only 1,3-thiazolidin-4-one deriv-

Table 1. Antimicrobial activity of the tested benzopyran-2-one analogues.

Com- pound ^{a)}	<i>Bacillus</i> <i>subtilis</i> (G + ve) ^{b, c)}	<i>Sarcina</i> <i>lutea</i> (G+ve) ^{c)}	<i>Staph.</i> <i>aureus</i> (G+ve)	<i>E.coli</i> (G-ve)	<i>C. albicans</i> (fungus)
2c	8	–	–	–	–
2f	20	15	20	15	15
3c	9	–	–	–	–
3f	–	–	20	9	–
4a	7	–	15	–	–
4c	–	8	12	–	–
4e	–	8	–	–	–
4f	15	–	–	–	–
5a	–	9	–	–	–
5d	–	–	9	–	–
6a	8	10	–	8	–
Tetra- cycline	30	40	36	34	–
Ampho- tercin B	–	–	–	–	30

^{a)} All the newly prepared compounds were subjected to microbiological evaluation. Yet, only those compounds that showed antimicrobial activity are enlisted in the table.

^{b)} The antimicrobial activity of the newly synthesized compounds against test organisms is expressed in terms of zone of growth inhibition (measured in mm).

^{c)} Test solutions of the benzopyran-2-one analogues were prepared in a concentration of 10 mg/ml.

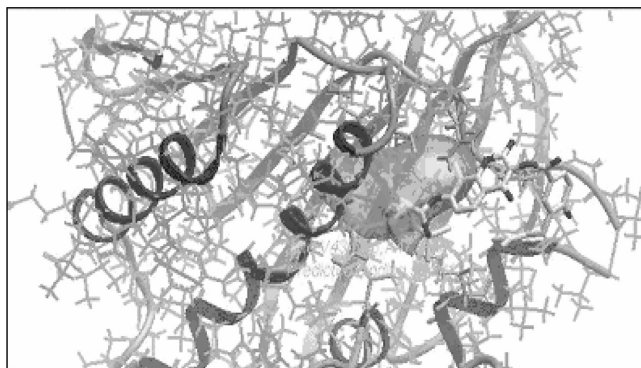
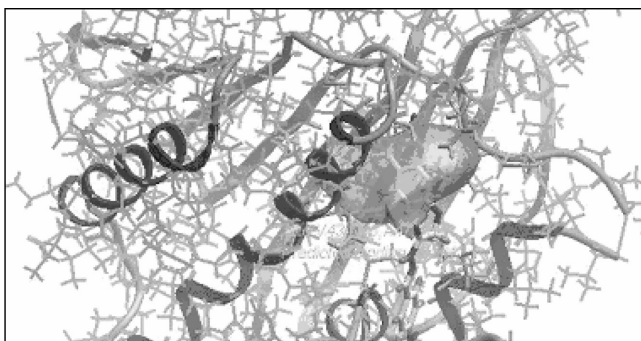
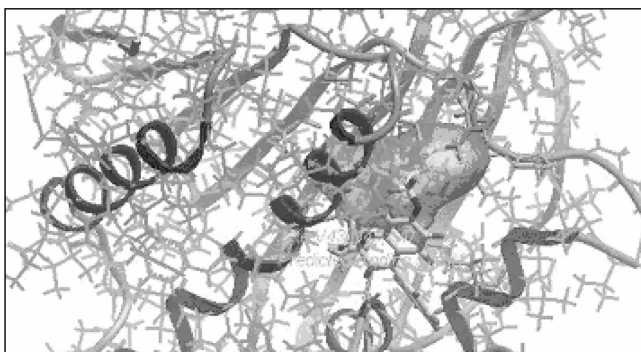
active possessing antimicrobial efficacy with slight activity against *Bacillus subtilis* (8 mm), *Sarcina lutea* (10 mm) and *Escherichia coli* (8 mm).

Drug-modeling studies

To pre-assess the potential microbiological behavior of our new coumarin derivatives on structural basis, the scoring functions are used to estimate or predict the binding affinity / biological activity of the DNA gyrase-B inhibitor (Novobiocin, coumarin derivative antibiotic), and the newly synthesized compounds (five different classes of coumarin derivatives) through molecular modeling and docking techniques using Molsoft ICM 3.4-8C program [21–23] in order to predict the binding geometry for each binder.

The goal is to have an adequate 3D-model of the receptor pocket to dock a ligand. Usually, a good start is to try to dock the known ligand to the receptor model, then to dock the tested compounds to determine ICM scores. ICM scores of –32 and lower are generally considered good scores but this usually depends on the receptor.

In order to compare the binding affinity of the newly synthesized coumarin analogues, we docked all the derivatives **2–6 (a–f)** into the empty binding site of the experimentally-known bacterial topoisomerase with its bound

**Figure 1.** The docking solution of Novobiocin within gyrase-B binding pocket. (ICM score –123).**Figure 2.** The docking solution of **2f** within gyrase-B binding pocket. (ICM score –80.44).**Figure 3.** The docking solution of **5d** within gyrase-B binding pocket. (ICM score –93.36).

inhibitor novobiocin. In these simulations, the ligand is fully flexible (free translation, rotation of functional groups about single bonds). Figures 1–3 show the docking solutions with the highest predicted binding affinity for gyrase B with the different coumarin derivatives.

As shown in Tables 1 and 2 and Figs. 1–3 the following results can be drawn:

ICM score of novobiocin is –123.72 (Fig. 1).

Table 2. ICM Scores of novobiocin and the tested benzopyran-2-one analogues.

N°	ICM scores	N°	ICM scores	N°	ICM scores
Novobiocin	–123.72	3e	–87.16	5d	–93.36
2a	–73.48	3f	–89.96	5e	–91.08
2b	–75.61	4a	–89.07	5f	–92.68
2c	–78.19	4b	–85.93	6a	–77.30
2d	–79.07	4c	–86.78	6b	–79.97
2e	–76.52	4d	–83.42	6c	–78.91
2f	–80.44	4e	–87.22	6d	–86.37
3a	–85.69	4f	–86.94	6e	–84.20
3b	–84.08	5a	–87.29	6f	–83.20
3c	–84.53	5b	–88.91		
3d	–87.08	5c	–87.90		

ICM scores of oxyacetic acid arylidene hydrazide **2a–f** range from –73.48 to –80.44. Compound **2f**, the biologically most active with antimicrobial efficacy against all the five tested microorganisms (Table 1), possessed the lowest ICM score –80.44 (Fig. 2).

ICM scores of azetidin-2-one **3a–f** range from –84.08 to –89.96. Within this series, the biologically most active compound was **3f** which showed the lowest ICM score –89.96.

ICM scores of pyrrolidin-2-one **4a–f** range from –83.42 to –89.07. The biologically most active compound **4a** displayed the lowest ICM score –89.07, while compounds **4f** and **4c**, with good activity, possessed ICM score of –86.90 and –86.78, respectively.

ICM scores of 2H-1,3,4-oxadiazole **5a–f** range from –87.29 to –93.36 where the biologically most active compounds **5d** (Fig. 3, lowest ICM score of all the new compounds) and **5a** possessed the lowest and highest ICM scores, respectively (–93.36 and –87.29).

ICM scores of 1,3-thiazolidin-4-one **6a–f** range from –77.30 to –86.37, but the antibacterially most active compound **6a** had the highest ICM score (–77.30).

Conclusions

From the literature and our own studies we knew the importance of the coumarin structure for inhibition of gyrase B [5]. In our study, we focused on substitution in position 7 of the coumarin moiety. Reaction of **2** with chloroacetyl chloride or 3-chloropropionyl chloride to give azetidin-2-ones **3a–f** and pyrrolidin-2-ones **4a–f** abolishes antifungal activity and decreases antibacterial activity against Gram-negative bacteria, while retaining the antibacterial activity against Gram-positive bacteria. Cyclization of **2** to give 2H-1,3,4-oxadiazoles **5a–f** abol-

ishes antifungal and antibacterial activity against Gram-negative bacteria and decreases antibacterial activity against Gram-positive bacteria. Reaction of **2** with mercaptoacetic acid to give 1,3-thiazolidin-4-ones **6a–f** abolishes antifungal activity and decreases antibacterial activity against Gram-positive and Gram-negative bacteria. Substitution with *p*-nitro phenyl moiety gives good activity, while substitution with phenyl moiety shows moderate activity as reflected in the screening data of **2f** versus **2c**, **3f** versus **3c**, and **4f** versus **4c**.

In many cases, the experimental findings are in good agreement with predicted binding affinities obtained by molecular docking studies. Yet, there are some derivatives (**5a** and **6a**) that showed promising *in-vitro* antibacterial activity not typically coinciding with their overall ICM scores among other members of the chemical series. The aforementioned infers that the antibacterial activity can not merely be attributed to those parameters measured during the molecular modeling study which reflects the necessity of performing the biological screening.

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The authors have declared no conflict of interest.

Experimental

Chemistry

All melting points are uncorrected and determined by the open capillary method using a Gallenkamp melting point apparatus (MFB-595-010M; Weiss-Gallenkamp, London, UK). Microanalyses were carried out at the microanalytical unit, Faculty of Science, Cairo University. Infrared spectra were determined (KBr) using Shimadzu Infrared Spectrometer (IR-435; Shimadzu, Japan) and FT-IR 1650 (Perkin Elmer, USA). ¹H-NMR Spectra were carried out using Joel, FX 90Q, NMR Spectrometer at 200 MHz and Fourier transform EM-390, 300 MHz NMR Spectrometer (JEOL, Tokyo, Japan). Mass spectra were carried out using Finnigan SSQ 7000 Gas Chromatograph-Mass spectrometer (Thermo Electron Corporation, Bremen, Germany). TLC was carried out using Art. 5735, DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents were CCl₄:CH₃OH (9 : 1) and the spots were visualized by UV 366 and 254 nm.

General method for preparation of (2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy) acetic acid arylidene hydrazides **2a–f**

A mixture of **1** (3.10 g, 10 mmol) and the appropriate aromatic aldehyde (10 mmol) in acetic acid (20 mL) was refluxed for 6 h. The excess solvent was then removed under reduced pressure.

The precipitate formed after cooling was collected by filtration and crystallized from ethanol to give **2a–f**.

1-[(Furan-2-yl)methylene]-2-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetohydrazide 2a

The general method was adopted to prepare **2a** employing furfuraldehyde. Yield: 85%; mp.: 254–256°C. IR (cm⁻¹): 3150 (NH), 1731, 1683 (2 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 5.22 (s, 2H, OCH₂), 6.23 (s, 1H, H-3 coumarin), 6.60–7.90 (m, 11H, furan and ArH), 8.20 (s, 1H, CH=N), 11.20 (s, 1H, NH exch.). Anal. Calcd. for C₂₂H₁₆N₂O₅ (388.37): C, 68.04; H, 4.15; N, 7.21. Found: C, 68.02; H, 4.16; N, 6.99.

1-[(Thiophen-2-yl)methylene]-2-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)aceto-hydrazide 2b

The general method was adopted to prepare **2b** employing thiophene-2-carbaldehyde. Yield: 86%; mp.: 241–243°C. IR (cm⁻¹): 3200 (NH), 1730, 1680 (2 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 4.94 (s, 2H, OCH₂), 6.25 (s, 1H, H-3), 6.07–7.73 (m, 11H, thiophene and Ar), 8.00 (s, 1H, CH=N), 11.20 (s, 1H, NH exch.). Anal. Calcd. for C₂₂H₁₆N₂O₄S (404.44): C, 56.34; H, 3.99; N, 6.92. Found: C, 65.33; H, 4.04; N, 7.18.

1-(Benzylidene)-2-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetohydrazide 2c

The general method was adopted to prepare **2c** employing benzaldehyde. Yield: 85%; mp.: 222–223°C. IR (cm⁻¹): 3150 (NH), 1756, 1708 (2 C=O). ¹H-NMR (CDCl₃) δ ppm: 4.69 (s, 2H, OCH₂), 6.24 (s, 1H, H-3), 6.75–7.65 (m, 13H, Ar), 8.95 (s, 1H, CH=N), 10.20 (s, 1H, NH exch.). Anal. Calcd. for C₂₄H₁₈N₂O₄ (398.41): C, 72.35; H, 4.55; N, 7.03. Found: C, 72.71; H, 4.56; N, 6.93.

1-(4-Methoxybenzylidene)-2-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetohydrazide 2d

The general method was adopted to prepare **2d** employing 4-methoxybenzaldehyde. Yield: 90%; mp.: 228–230°C. IR (cm⁻¹): 3200 (NH), 1730, 1680 (2 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 3.80 (s, 3H, OCH₃), 5.20 (s, 2H, OCH₂), 6.22 (s, 1H, H-3), 6.80–7.90 (m, 12H, Ar), 8.20 (s, 1H, CH=N), 11.20 (s, 1H, NH exch.). MS: m/z (%) = 428 (49) [M⁺]. Anal. Calcd. for C₂₅H₂₀N₂O₅ (428.25): C, 70.08; H, 4.71; N, 6.54. Found: C, 70.11; H, 5.20; N, 6.39.

1-(4-Chlorobenzylidene)-2-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetohydrazide 2e

The general method was adopted to prepare **2e** employing 4-chlorobenzaldehyde. Yield: 90%; mp.: 242–244°C. IR (cm⁻¹): 3150 (NH), 1729, 1685 (2 C=O). ¹H-NMR (CDCl₃) δ ppm: 5.23 (s, 2H, OCH₂), 6.24 (s, 1H, H-3), 6.80–7.80 (m, 12H, Ar), 8.80 (s, 1H, CH=N), 10.00 (s, 1H, NH exch.). MS: m/z (%) = 434 (16) [M⁺ + 2], 432 (41) [M⁺]. Anal. Calcd. for C₂₄H₁₇ClN₂O₄ (432.86): C, 66.59; H, 3.96; N, 6.47. Found: C, 66.60; H, 4.00; N, 6.42.

1-(4-Nitrobenzylidene)-2-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetohydrazide 2f

The general method was adopted to prepare **2f** employing 4-nitrobenzaldehyde. Yield: 95%; mp.: 225–228°C. IR (cm⁻¹): 3200 (NH), 1756, 1685 (2 C=O), 1609 (NH bending). ¹H-NMR (DMSO-d₆) δ ppm: 4.93 (s, 2H, OCH₂), 6.24 (s, 1H, H-3), 6.80–7.95 (m, 12H, Ar), 8.30 (s, 1H, CH=N), 10.20 (s, 1H, NH exch.). Anal. Calcd. for

C₂₄H₁₇N₃O₆ (443.42): C, 65.01; H, 3.86; N, 9.48. Found: C, 64.96; H, 3.90; N, 9.60.

General method for preparation of 4-aryl-3-chloro-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido]azetidin-2-ones 3a–f

To a mixture of the appropriate arylidene derivative **2a–f** (10 mmol) and triethylamine (2 mL, 20 mmol) in dry benzene (50 mL), monochloroacetyl chloride (2.2 mL, 20 mmol) was added dropwise with cooling. The reaction mixture was stirred at room temperature for 30 min and then refluxed for 3 h. The solid which precipitated upon cooling was filtered off and crystallized from chloroform / methanol.

3-Chloro-4-(furan-2-yl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido]azetidin-2-one 3a

Yield: 45%; mp.: 116–118°C. IR (cm⁻¹): 3200 (NH), 1750, 1700, 1650 (3 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 4.16 (s, 2H, OCH₂), 4.90 (d, J = 7 Hz, 1H, Ar-CH β-lactam), 5.10 (d, J = 7 Hz, 1H, Cl-CH β-lactam), 6.25 (s, 1H, H-3), 7.00–7.95 (m, 11H, furan and Ar), 10.30 (s, 1H, NH exch.). Anal. Calcd. for C₂₄H₁₇ClN₂O₆ (464.86): C, 62.01; H, 3.69; N, 6.03. Found: C, 62.09; H, 3.57; N 6.11.

3-Chloro-4-(thiophen-2-yl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido]azetidin-2-one 3b

Yield: 60%; mp.: 120–122°C. IR (cm⁻¹): 3200 (NH), 1750, 1700, 1650 (3 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 4.24 (s, 2H, OCH₂), 4.94 (d, J = 7 Hz, 1H, Ar-CH β-lactam), 5.20 (d, J = 7 Hz, 1H, Cl-CH β-lactam), 6.24 (s, 1H, H-3), 7.07–7.72 (m, 11H, thiophene and Ar), 10.00 (s, 1H, NH exch.). Anal. Calcd. for C₂₄H₁₇ClN₂O₅S (480.92): C, 59.94; H, 3.56; N, 5.82. Found: C, 59.90; H, 3.30; N, 5.80.

3-Chloro-4-phenyl-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido]azetidin-2-one 3c

Yield: 50%; mp.: 128–130°C. IR (cm⁻¹): 3300 (NH), 1750, 1710, 1610 (3 C=O). ¹H-NMR (CHCl₃-d₆) δ ppm: 4.77 (s, 2H, OCH₂), 5.10 (d, J = 7 Hz, 1H, Ar-CH β-lactam), 5.30 (d, J = 7 Hz, 1H, Cl-CH β-lactam), 6.30 (s, 1H, H-3), 6.90–7.95 (m, 13H, Ar), 10.03 (s, 1H, NH exch.). Anal. Calcd. for C₂₆H₁₉ClN₂O₅ (474.90): C, 65.76; H, 4.03; N, 5.90. Found: C, 65.63; H, 4.19; N, 5.70.

3-Chloro-4-(4-methoxyphenyl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido]azetidin-2-one 3d

Yield: 50%; mp.: 187–188°C. IR (cm⁻¹): 3200 (NH), 1750, 1700, 1650 (3 C=O). ¹H-NMR (CHCl₃-d₆) δ ppm: 3.80 (s, 3H, OCH₃), 4.26 (s, 2H, OCH₂), 4.95 (d, J = 7 Hz, 1H, Ar-CH β-lactam), 5.40 (d, J = 7 Hz, 1H, Cl-CH β-lactam), 6.30 (s, 1H, H-3), 6.85–7.90 (m, 12H, Ar), 10.00 (s, 1H, NH exch.). Anal. Calcd. for C₂₇H₂₁ClN₂O₆ (504.93): C, 64.23; H, 4.19; N, 5.55. Found: C, 63.98; H, 4.40; N, 5.62.

3-Chloro-4-(4-chlorophenyl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido]azetidin-2-one 3e

Yield: 55%; mp.: 194–195°C. IR (cm⁻¹): 3224 (NH), 1754, 1708, 1650 (3 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 4.17 (s, 2H, OCH₂), 4.79 (d, J = 7.2 Hz, 1H, Ar-CH β-lactam), 4.95 (d, J = 7.2 Hz, 1H, Cl-CH β-lactam), 6.27 (s, 1H, H-3), 6.99–7.57 (m, 12H, Ar), 10.39 (s, 1H, NH exch.). MS: m/z (%) = 509 (0.98) [M⁺]. Anal. Calcd. for C₂₆H₁₈Cl₂N₂O₅

(509.35): C, 61.31; H, 3.56; N, 5.50. Found: C, 61.10; H, 3.60; N, 5.45.

3-Chloro-4-(4-nitrophenyl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido] azetidin-2-one 3f

Yield: 65%; mp.: 156–157°C. IR (cm⁻¹): 3200 (NH), 1750, 1703, 1624 (3 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 4.93 (s, 2H, OCH₂), 5.20 (d, J = 7 Hz, 1H, Ar-CH β-lactam), 5.90 (d, J = 7 Hz, 1H, Cl-CH β-lactam), 6.24 (s, 1H, H-3), 6.98–7.90 (m, 12H, Ar), 10.10 (s, 1H, NH exch.). MS: m/z (%) = 521 (0.33) [M⁺+2], 519 (0.25) [M⁺]. Anal. Calcd. for C₂₆H₁₈ClN₃O₇ (519.90): C, 60.07; H, 3.49; N, 8.08. Found: C, 60.01; H, 3.37; N, 8.10.

General method for preparation of 5-aryl-4-chloro-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido] pyrrolidin-2-one 4a–f

As described above for 3a–f, using 3-chloropropionyl chloride. The solid obtained was crystallized from chloroform / ether.

4-Chloro-5-(furan-2-yl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido] pyrrolidin-2-one 4a

Yield: 50%; mp.: 124–126°C. IR (cm⁻¹): 3200 (NH), 1756, 1700, 1650 (3 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 3.91 (d, J = 2.2 Hz, 2H, CH₂ pyrrol.), 4.24 (q, 1H, Cl-CH pyrrol.), 4.94 (s, 2H, OCH₂), 5.10 (d, J = 4 Hz, 1H, Ar-CH pyrrol.), 6.25 (s, 1H, H-3), 7.07–7.90 (m, 11H, furan and Ar), 9.60 (s, 1H, NH exch.). Anal. Calcd. for C₂₅H₁₉ClN₂O₆ (478.89): C, 62.70; H, 4.00; N, 5.85. Found: C, 62.90; H, 3.94; N, 5.90.

4-Chloro-5-(thiophen-2-yl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido] pyrrolidin-2-one 4b

Yield: 55%; mp.: 132–134°C. IR (cm⁻¹): 3200 (NH), 1750, 1700, 1650 (3 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 3.90 (d, J = 2.2 Hz, 2H, CH₂ pyrrol.), 4.24 (q, 1H, Cl-CH pyrrol.), 4.95–5.20 (m, 3H, OCH₂ and Ar-CH pyrrol.), 6.23 (s, 1H, H-3), 7.06–7.90 (m, 11H, thiophene and Ar), 9.60 (s, 1H, NH exch.). Anal. Calcd. for C₂₅H₁₉ClN₂O₅S (494.95): C, 60.67; H, 3.87; N, 5.66. Found: C, 60.89; H, 4.10; N, 5.62.

4-Chloro-5-phenyl-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido]pyrrolidin-2-one 4c

Yield: 50%; mp.: 78–80°C. IR (cm⁻¹): 3275 (NH), 1756, 1708, 1640 (3 C=O). ¹H-NMR (CDCl₃) δ ppm: 3.75 (d, J = 2.2 Hz, 2H, CH₂ pyrrol.), 4.25 (q, 1H, Cl-CH pyrrol.), 4.68 (s, 2H, OCH₂), 4.80 (d, J = 4.4 Hz, 1H, Ar-CH pyrrol.), 6.21 (s, 1H, H-3), 6.74–6.91 (m, 4H, Ar), 7.33–7.56 (m, 9H, Ar), 10.10 (s, 1H, NH exch.). Anal. Calcd. for C₂₇H₂₁ClN₂O₅ (488.93): C, 66.33; H, 4.33; N, 5.73. Found: C, 66.39; H, 4.36; N, 5.53.

4-Chloro-5-(4-methoxyphenyl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido] pyrrolidin-2-one 4d

Yield: 50%; mp.: 169–170°C. IR (cm⁻¹): 3275 (NH), 1756, 1708, 1640 (3 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 3.53 (d, J = 2.0 Hz, 2H, CH₂ pyrrol.), 3.87 (s, 3H, OCH₃), 4.20 (q, 1H, Cl-CH pyrrol.), 4.80–5.20 (m, 3H, OCH₂ and Ar-CH pyrrol.), 6.24 (s, 1H, H-3), 6.90–7.75 (m, 12H, Ar), 10.30 (s, 1H, NH exch.). Anal. Calcd. for C₂₈H₂₃ClN₂O₆ (518.95): C, 64.80; H, 4.47; N, 5.40. Found: C, 64.68; H, 4.55; N, 5.66.

4-Chloro-5-(4-chlorophenyl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido] pyrrolidin-2-one 4e

Yield: 55%; mp.: 130–132°C. IR (cm⁻¹): 3200 (NH), 1756, 1708, 1650 (3 C=O). ¹H-NMR (CDCl₃) δ ppm: 3.49 (d, J = 2 Hz, 2H, CH₂ pyrrol.), 4.33 (q, 1H, Cl-CH pyrrol.), 4.69 (s, 2H, OCH₂), 4.81 (d, J = 4 Hz, 1H, Ar-CH pyrrol.), 6.24 (s, 1H, H-3), 6.83–6.95 (m, 3H, Ar), 7.42–7.52 (m, 9H, Ar), 10.10 (s, 1H, NH exch.). MS: m/z (%) = 509 (0.98) [M⁺]. Anal. Calcd. for C₂₇H₂₀Cl₂N₂O₅ (523.37): C, 61.96; H, 3.85; N, 5.35. Found: C, 61.93; H, 3.91; N, 5.15.

4-Chloro-5-(4-nitrophenyl)-1-[(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxy)acetamido] pyrrolidin-2-one 4f

Yield: 55%; mp.: 140–142°C. IR (cm⁻¹): 3275 (NH), 1750, 1710, 1640 (3 C=O). ¹H-NMR (CDCl₃) δ ppm: 3.80 (d, J = 2.2 Hz, 2H, CH₂ pyrrol.), 4.30 (q, 1H, Cl-CH pyrrol.), 4.68 (s, 2H, OCH₂), 4.90 (d, J = 4 Hz, 1H, Ar-CH pyrrol.), 6.21 (s, 1H, H-3), 7.40–7.80 (m, 8H, Ar), 8.10 (d, J = 8 Hz, 2H, Ar), 8.40 (d, J = 8 Hz, 2H, Ar), 10.20 (s, 1H, NH exch.). Anal. Calcd. for C₂₇H₂₀ClN₃O₇ (533.37): C, 60.74; H, 3.78; N, 7.87. Found: C, 60.60; H, 3.60; N 7.44.

General method for preparation of 3-Acetyl-2-aryl-5-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxymethyl)-1,3,4-(2H)-oxadiazole 5a–f

A mixture of 2a–f (10 mmol), acetic anhydride (15 mL), and anhydrous sodium acetate (0.1 g) was refluxed for 1 h. The solution was cooled and poured onto ice cold water and stirred for 1 h. The solid was filtered off, washed with water, dried, and crystallized from methanol.

3-Acetyl-2-(furan-2-yl)-5-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxymethyl)-1,3,4-(2H)-oxadiazole 5a

Yield: 75%; mp.: 127–128°C. IR (cm⁻¹): 1739, 1693 (2 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 2.20 (s, 3H, COCH₃), 4.81 (s, 2H, OCH₂), 6.22 (s, 1H, H-3), 6.87–7.02 (m, 4H, oxadiazole and furan), 7.30–7.80 (m, 8H, Ar). Anal. Calcd. for C₂₄H₁₈N₂O₆ (430.42): C, 66.97; H, 4.22; N, 6.51. Found: C, 66.47; H, 3.80; N, 6.80.

3-Acetyl-2-(thiophen-2-yl)-5-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxymethyl)-1,3,4-(2H)-oxadiazole 5b

Yield: 70%; mp.: 120–122°C. IR (cm⁻¹): 1750, 1708 (2 C=O). ¹H-NMR (CDCl₃) δ ppm: 2.25 (s, 3H, COCH₃), 4.75 (s, 2H, OCH₂), 6.24 (s, 1H, H-3), 6.82–6.95 (m, 4H, oxadiazole and thiophene), 7.39–7.60 (m, 8H, Ar). Anal. Calcd. for C₂₄H₁₈N₂O₅S (446.48): C, 64.56; H, 4.03; N, 6.27. Found: C, 64.59; H, 3.80; N, 6.13.

3-Acetyl-2-phenyl-5-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxymethyl)-1,3,4-(2H)-oxadiazole 5c

Yield: 70%; mp.: 182–184°C. IR (cm⁻¹): 1750, 1700 (2 C=O). ¹H-NMR (CDCl₃) δ ppm: 2.40 (s, 3H, COCH₃), 4.82 (s, 2H, OCH₂), 6.22 (s, 1H, H-3), 6.99 (s, 1H, oxadiazole), 7.31–7.50 (m, 13H, Ar). Anal. Calcd. for C₂₆H₂₀N₂O₅ (440.46): C, 70.90; H, 4.58; N, 6.36. Found: C, 71.15; H, 4.69; N, 6.39.

3-Acetyl-2-(4-methoxyphenyl)-5-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxymethyl)-1,3,4-(2H)-oxadiazole 5d

Yield: 75%; mp.: 185–187°C. IR (cm⁻¹): 1754, 1708 (2 C=O). ¹H-NMR (DMSO-d₆) δ ppm: 2.25 (s, 3H, COCH₃), 3.70 (s, 3H, OCH₃), 4.95 (s, 2H, OCH₂), 6.26 (s, 1H, H-3), 6.89 (s, 1H, oxadiazole), 7.09–

7.83 (m, 12H, Ar). Anal. Calcd. for $C_{27}H_{22}N_2O_6$ (470.48): C, 68.93; H, 4.71; N, 5.95. Found: C, 68.84; H, 4.80; N, 5.84.

3-Acetyl-2-(4-chlorophenyl)-5-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxymethyl)-1,3,4-(2H)-oxadiazole 5e

Yield: 75%; mp.: 138–140°C. IR (cm^{-1}): 1754, 1712 (2 C=O). 1H -NMR ($CDCl_3$) δ ppm: 2.40 (s, 3H, $COCH_3$), 4.93 (s, 2H, OCH_2), 6.25 (s, 1H, H-3), 6.90 (d, $J = 13.4$ Hz, 2H, Ar), 7.08 (s, 1H, oxadiazole), 7.40 (d, $J = 13.4$ Hz, 2H, Ar), 7.55 (broad, 8H, Ar). MS: m/z (%) = 476 (0.99) [$M^+ + 2$], 474 (1.13) [M^+]. Anal. Calcd. for $C_{26}H_{19}ClN_2O_5$ (474.90): C, 65.76; H, 4.03; N, 5.90. Found: C, 65.80; H, 4.10; N, 5.76.

3-Acetyl-2-(4-nitrophenyl)-5-(2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxymethyl)-1,3,4-(2H)-oxadiazole 5f

Yield: 70%; mp.: 143–145°C. IR (cm^{-1}): 1754, 1708 (2 C=O). 1H -NMR ($DMSO-d_6$) δ ppm: 2.20 (s, 3H, $COCH_3$), 4.94 (s, 2H, OCH_2), 6.25 (s, 1H, H-3), 6.90 (s, 1H, oxadiazole), 7.20–7.95 (m, 12H, Ar). Anal. Calcd. for $C_{26}H_{19}N_3O_7$ (485.45): C, 64.33; H, 3.95; N, 8.66. Found: C, 64.20; H, 3.92; N, 8.78.

General method for preparation of 2-aryl-3-[2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxyacetamido]-thiazolidin-4-one 6a–f

Method A: A mixture of **2a–f** (10 mmol) and thioglycolic acid (2.76 g, 2.1 mL, 30 mmol) in dry benzene (100 mL) was refluxed for 72 h using a Dean–Stark apparatus. The solution was evaporated under reduced pressure and the residue washed with 5% $NaHCO_3$ solution followed by water, dried and crystallized from $CHCl_3$ /ether.

Method B: To a solution of **2a–f** (10 mmol) in dry dioxane (15 mL), mercaptoacetic acid (0.91 g, 0.7 mL, 10 mmol) and anhydrous $ZnCl_2$ (0.2 g) was added and the mixture heated to reflux for 10 h. The solvent was removed under reduced pressure and the residue washed with 5% $NaHCO_3$ (3 \times 25 mL) followed by water, dried and crystallized from $CHCl_3$ /ether.

2-(Furan-2-yl)-3-[2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxyacetamido]-thiazolidin-4-one 6a

Yield: 60%; mp.: 125–127°C. IR (cm^{-1}): 3200 (NH), 1730–1720, 1681 (3 C=O). 1H -NMR ($DMSO-d_6$) δ ppm: 3.84 (s, 2H, CH_2 thiazol.), 5.22 (s, 2H, CH_2O), 6.24 (s, 1H, H-3), 6.60–6.90 (m, 3H, furan), 7.00 (s, 1H, CH thiazol.), 7.30–7.92 (m, 8H, Ar), 11.60 (s, 1H, NH exch.). Anal. Calcd. for $C_{24}H_{18}N_2O_6S$ (462.48): C, 62.33; H, 3.92; N, 6.06. Found: C, 62.54; H, 3.72; N, 6.31.

3-[2-Oxo-4-phenyl-2H-benzo[b]pyran-7-yloxyacetamido]-2-(thiophen-2-yl)-thiazolidin-4-one 6b

Yield: 65%; mp.: 122–123°C. IR (cm^{-1}): 3200 (NH), 1756, 1708, 1660 (3 C=O). 1H -NMR ($CDCl_3$) δ ppm: 4.28 (s, 2H, CH_2 thiazol.), 4.69 (s, 2H, CH_2O), 6.24 (s, 2H, H-3 and CH thiazol.), 6.80–6.95 (m, 3H, thiophene), 7.32–7.52 (m, 8H, Ar), 10.00 (s, 1H, NH exch.). MS: m/z (%) = 480 (10.28) [$M^+ + 2$]. Anal. Calcd. for $C_{24}H_{18}N_2O_5S_2$ (478.54): C, 60.24; H, 3.79; N, 5.85. Found: C, 60.54; H, 3.89; N, 5.75.

3-[2-Oxo-4-phenyl-2H-benzo[b]pyran-7-yloxyacetamido]-2-phenyl-thiazolidin-4-one 6c

Yield: 60%; mp.: 152–156°C. IR (cm^{-1}): 3286 (NH), 1754–1712, 1660 (3 C=O). 1H -NMR ($CDCl_3$) δ ppm: 3.92 (s, 2H, CH_2 thiazol.), 4.62 (s, 2H, CH_2O), 5.95 (s, 1H, H-3), 6.18 (s, 1H, CH thiazol.), 6.77–8.10 (m, 13H, Ar), 10.00 (s, 1H, NH exch.). Anal. Calcd. for $C_{26}H_{20}N_2O_5S$ (472.52): C, 66.09; H, 4.27; N, 5.93. Found: C, 66.00; H, 4.20; N, 6.11.

2-(4-Methoxyphenyl)-3-[2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxyacetamido]-thiazolidin-4-one 6d

Yield: 60%; mp.: 114–116°C. IR (cm^{-1}): 3200 (NH), 1750–1718, 1660 (3 C=O). 1H -NMR ($CDCl_3$) δ ppm: 3.80 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2 thiazol.), 5.01 (s, 2H, CH_2O), 6.24 (s, 2H, H-3 and CH thiazol.), 7.20–7.90 (m, 12H, Ar), 9.90 (s, 1H, NH exch.). Anal. Calcd. for $C_{27}H_{22}N_2O_6S$ (502.54): C, 64.53; H, 4.41; N, 5.57. Found: C, 64.20; H, 4.53; N, 5.16.

2-(4-Chlorophenyl)-3-[2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxyacetamido]-thiazolidin-4-one 6e

Yield: 70%; mp.: 128–130°C. IR (cm^{-1}): 3150 (NH), 1750, 1700, 1660 (3 C=O). 1H -NMR ($DMSO-d_6$) δ ppm: 4.13 (s, 2H, CH_2 thiazol.), 4.83 (s, 2H, CH_2O), 6.23 (s, 2H, H-3 and CH thiazol.), 6.96 (d, $J = 8.8$ Hz, 2H, Ar), 7.36 (d, $J = 8.0$ Hz, 2H, Ar), 7.54 (m, 8H, Ar), 10.00 (s, 1H, NH exch.). Anal. Calcd. for $C_{26}H_{19}ClN_2O_5S$ (506.96): C, 61.60; H, 3.78; N, 5.53. Found: C, 61.82; H, 3.99; N, 5.76.

2-(4-Nitrophenyl)-3-[2-oxo-4-phenyl-2H-benzo[b]pyran-7-yloxyacetamido]-thiazolidin-4-one 6f

Yield: 70%; mp.: 132–134°C. IR (cm^{-1}): 3200 (NH), 1754, 1708, 1660 (3 C=O). 1H -NMR ($DMSO-d_6$) δ ppm: 4.12 (s, 2H, CH_2 thiazol.), 4.79 (s, 2H, CH_2O), 6.24 (s, 1H, H-3), 6.40 (s, 1H, CH thiazol.), 7.03 (d, $J = 8.0$ Hz, 2H, Ar), 7.33 (d, $J = 8.0$ Hz, 2H, Ar), 7.56 (m, 8H, Ar), 10.39 (s, 1H, NH exch.). Anal. Calcd. for $C_{26}H_{19}N_3O_7S$ (517.51): C, 60.34; H, 3.70; N, 8.12. Found: C, 60.30; H, 3.80; N, 7.76.

Antimicrobial activity

Test organisms

The antimicrobial activity of 30 novel compounds **2–6a–f** was evaluated *in vitro* against *Bacillus subtilis*, *Sarcina lutea*, and *Staphylococcus aureus* (as representative examples of Gram-positive bacteria), *Escherichia coli* (as representative of Gram-negative bacteria), and the fungus *Candida albicans* (Table 1).

Method – Agar plate disc diffusion technique

Agar plate disc diffusion technique has been used as general method [20].

Test materials of 6 mm in diameter sterilized Whatman filter paper discs were impregnated with 10 mg/mL solution of the test compound and standard (tetracycline and amphotericin B) dissolved in DMF and allowed to air-dry. The discs were applied to the surface of nutrient agar plates seeded with the test organism (each plate contains 15 mL of the agar medium previously seeded with 0.2 mL of 18 h growth culture in liquid media for each organism). The incubated plates were incubated at 37°C for 48 h and the inhibition zone was measured in mm around each disc. Each experiment was repeated three times and the results are the average of the three runs. Discs impregnated with DMF were used as a control.

Drug-modeling studies

Generation of ligand and enzyme structures

The crystal structure of enzyme (DNA GYRASE-B) with its bound inhibitor (Novobiocin, (3R, 4S, 5S, 6R)-5-hydroxy-6-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]carbonylamino-8-methyl-2-oxochromen-7-yl]oxy-3-methoxy-2,2-dimethyl-oxan-4-yl) carbamate) **1AJ6** was downloaded through the Protein Data Bank PDB/ RCSB site and saved as *.pdb file [24].

A set of coumarin analogues synthesized to inhibit DNA GYRASE-B was compiled by us earlier. All compounds were built in ChemDraw Ultra version 8.0.3 and their energy minimized through Chem3D Ultra version 8.0.3/MM2, Jop Type: minimum RMS Gradient of 0.100, and saved as MDL MolFile (*.mol).

Docking using Molsoft ICM 3.4-8C program

The novel energy-minimized benzopyran-2-one analogues were docked into the active site of DNA Gyrase-B crystal structure using ICM-Pro software version 3.4-8C. ICM-Pro scores the binding of a ligand to a receptor based upon the comparison of a series of small molecule / protein interactions that have been reported in the PDB database. A rigid receptor / flexible ligand approach was adopted that uses five potential energy maps combining hydrophobicity, electrostatics, hydrogen bond formation, and two van-der-Waals parameters. In all cases, the program's default parameters were used.

References

- [1] R. Menzel, M. Gellert, *Adv. Pharmacol.* **1994**, 29A, 201–225.
- [2] A. Maxwell, M. Gellert, *Adv. Protein Chem.* **1986**, 38, 69–107.
- [3] S. C. Kampranis, A. D. Bates, A. Maxwell, *Proc. Natl. Acad. Sci. U. S. A.* **1999**, 96, 8414–8419.
- [4] L. Brino, A. Urzhumtsev, M. Mousli, C. Bronner, *et al.*, *J. Biol. Chem.* **2000**, 275, 9468–9475.
- [5] R. H. Flatman, A. Eustaquio, S. M. Li, L. Heide, A. Maxwell, *Antimicrob Agents Chemother.* **2006**, 50, 1136–1142.
- [6] A. Maxwell, *Trends Microbiol* **1997**, 5, 102–109.
- [7] F. Broccolo, G. Cainelli, G. Caltabiano, C. E. Cocuzza, *et al.*, *J. Med. Chem.* **2006**, 49, 2804–2811.
- [8] F. Benfatti, G. Cardillo, L. Gentilucci, A. Tolomelli, *Bioorg. Med. Chem. Lett.* **2007**, 17, 1946–1950.
- [9] M. Cherry, C. G. Daly, D. Mitchell, J. Highfield, *J. Clin. Periodontol.* **2007**, 34, 148–155.
- [10] M. E. Hensler, G. Bernstein, V. Nizet, A. Nefzi, *Bioorg. Med. Chem. Lett.* **2006**, 16, 5073–5079.
- [11] A. I. Eid, F. A. Ragab, S. L. El-Ansary, S. M. El-Gazayerly, F. E. Mourad, *Arch. Pharm.* **1994**, 327, 211–213.
- [12] A. A. Farghaly, P. Vanelle, H. S. El-Kashef, *Heterocycl. Comm.* **2005**, 11, 255–262.
- [13] M. Cacic, M. Trkovnik, F. Cacic, E. Has-Schon, *Molecules* **2006**, 11, 134–147.
- [14] M. Sayyed, S. Mokle, M. Bokhare, A. Mankar, *et al.*, *ARKIVOC* **2006**, ii, 187–192.
- [15] R. P. Tomko, C. B. Johansson, M. Totrov, R. Abagyan, *et al.*, *Exp. Cell Res.* **2000**, 255, 47–55.
- [16] I. Kufareya, L. Budagyan, E. Raush, M. Totrov, R. Abagyan, *Proteins* **2007**, 67, 400–417.
- [17] C. N. Cavasotto, M. A. Ortiz, R. A. Abagyan, F. J. Piedrafita, *Bioorg. Med. Chem. Lett.* **2006**, 16, 1969–1974.
- [18] A. S. Hamed, S. L. El-Ansary, D. A. Abou El-Ella, N. A. Farag, *Bull. Fac. Pharm. Cairo Univ.* **2005**, 43, 73–86.
- [19] A. K. Bose, Y. H. Chlang, M. S. Manhas, *Tetrahedron Lett.* **1972**, 40, 4091–4094.
- [20] C. H. Collins, *Microbiol. Methods* (Butterworths: London) **1967**, 364.
- [21] D. A. Ostrov, J. A. Hernández Parda, P. E. Corsino, K. A. Finton, T. C. Le N. Rowe, *Antimicrob Agents Chemother.* **2007**, 51, 3688–3698.
- [22] M. Oblak, S. G. Grdadolink, M. Kotnik, R. Jerala, *et al.*, *Bioorg. Med. Chem. Lett.* **2005**, 15, 5207–5210.
- [23] H. J. Boehm, M. Boehringer, D. Bur, H. Gmuender, *et al.*, *J. Med. Chem.* **2000**, 43, 2664–2674.
- [24] G. A. Holdgate, A. Tunnicliffe, W. H. Ward, S. A. Weston, *et al.*, *Biochemistry* **1997**, 36, 9663–9673.