Full Paper

Synthesis and Antibacterial Activity of Quinolone-Based Compounds Containing a Coumarin Moiety

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A new series of quinolone-based compounds containing a coumarin moiety have been synthesized and studied for their antibacterial activity against a panel of *gram*-positive and *gram*-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). The results of the antibacterial evaluation of *N*-[2-(coumarin-3-yl)ethyl]piperazinyl quinolone derivatives in comparison with parent quinolones (norfloxacin, ciprofloxacin, and enoxacin) indicated that *N*-[2-(coumarin-3-yl)-2-oxoethyl]ciprofloxacin derivative (compound **8b**) showed comparable or more potent antibacterial activity with respect to the reference drugs against the test strains. Generally, in both *gram*-positive and *gram*-negative bacteria, better results are obtained with cyclopropyl at the N-1 position of the quinolone ring and 2-oxo- on the ethyl spacer of coumarin and piperazine rings.

Keywords: Antibacterial activity / Coumarin / Quinolones / Synthesis

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Introduction

The emergence of multidrug-resistant *gram*-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) have made treatment of infectious diseases difficult and have, over the last decades, become a serious medical problem. As pathogenic bacteria continuously evolve mechanisms of resistance to currently used antibacterials, so the discovery of novel and potent antibacterial drugs is the best way to overcome bacterial resistance and develop effective therapies [1].

Since nalidixic acid was discovered in 1962, numerous quinolone derivatives have been synthesized to improve their antibacterial activities. Thus, the quinolones have evolved from agents used solely for the treatment of urinary tract infections to molecules with potent activity against a wide spectrum of significant bacterial pathogens [2]. The important strategies in quinolone research during the last few years include improving the pharmacokinetic properties, increasing the activity against *gram*-positive cocci and anaerobes [3 – 6].

As targets, the quinolones have two type-II topoisomerases: DNA gyrase and DNA topoisomerase IV, both required for cell growth and division [7, 8]. The primary target of the quinolones depends on the bacteria and seems to be DNA gyrase in most *gram*-negative microorganisms and topoisomerase IV in *Staphylococcus aureus* and *Streptococcus pneumoniae* [3, 7 – 10].

Besides the quinolones, other naturally occurring bacterial DNA gyrase inhibitors, such as the coumarins, which include novobiocin **1** (Fig. 1) and structurally related compounds, clorobiocin **2**, and RU 79115 **3** have also been known as antibacterial agents [11, 12]. The coumarins inhibit ATPase activity of DNA gyrase by competing with ATP for binding to the B subunit of the enzyme. However, due to their toxicity in eukaryotes, their poor water solubility, and their low activity against *gram*-nega-

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Abbreviations: methicillin-resistant *Staphylococcus aureus* (MRSA); minimum inhibitory concentration (MIC)

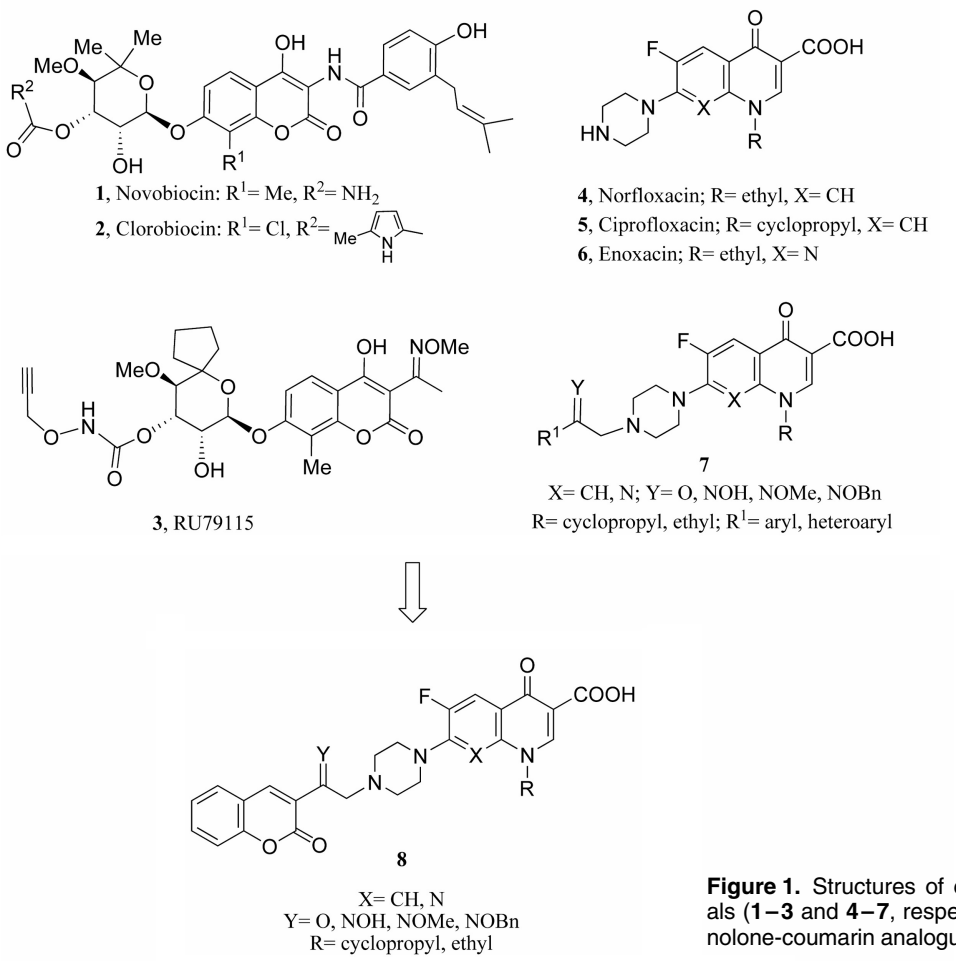


Figure 1. Structures of coumarin and quinolone antibacterials (**1–3** and **4–7**, respectively) and the newly designed quinolone-coumarin analogues **8**.

tive bacteria, no pharmaceutically useful drug has, so far, been derived from the coumarins [11]. However, renewed interest in coumarin antibiotics came from their potent *gram*-positive antibacterial activity and, especially, against methicillin-resistant strains of staphylococci species (MRSA and MRSE) which are currently one of the major concerns in treatment of bacterial infections [13].

The structure-activity relationship studies of quinolones have been extensively investigated and the substituent at the C-7 position has a great impact of modulating potency, spectrum, and pharmacokinetics [3, 14–17]. Recently, we have synthesized novel *N*-substituted 7-piperazinyl quinolones **7** (Fig. 1) differing from norfloxacin **4**, ciprofloxacin **5**, or enoxacin **6**, solely by the linkage of various 2-aryl-2-oxoethyl and 2-aryl-2-oxyiminoethyl groups to the piperazinyl residue at C-7 of the parent drug with *in-vitro* antibacterial activity comparable or higher than reference drugs [18–21].

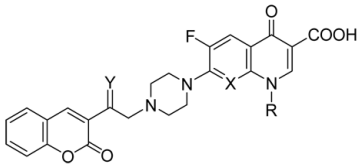
In the current study, in continuation of our work on *N*-substituted piperazinyl quinolone series, we aimed to combine the structural features of our promising antibacterial *N*-(2-arylethyl) piperazinyl quinolones **7** and coumarin antibacterial drug, RU 79115 **3**. Thus here, we wish to report the synthesis and antibacterial activity of *N*-[2-(coumarin-3-yl)ethyl]piperazinyl quinolones **8** (Fig. 1).

Results and discussion

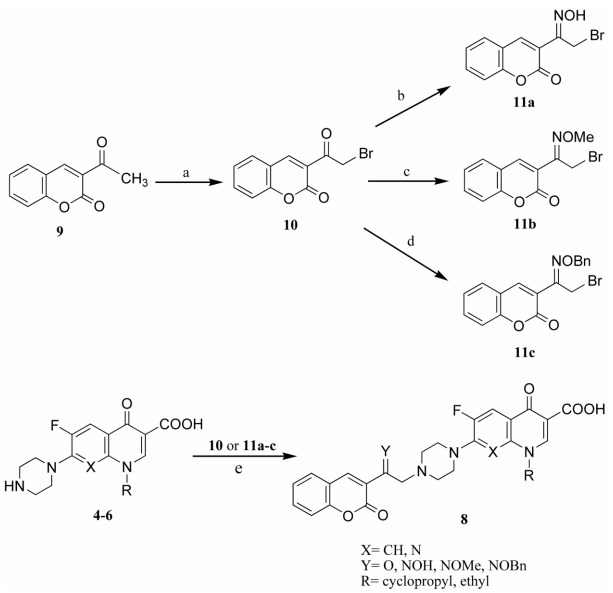
Chemistry

The synthesis of *N*-[2-(coumarin-3-yl)ethyl]piperazinyl quinolones **8** was achieved through the versatile and efficient synthetic route outlined in Scheme 1. The starting compound 3-acetylcoumarin **9** was converted to 3-(bromoacetyl)coumarin **10** by refluxing with Br_2 in $CHCl_3$ [22]. Compound **10** was converted to 3-(bromoacetyl)coumarin oxime **11a** by stirring with 3 equivalents of hydroxylamine hydrochloride in methanol at 22–25°C. Similarly, the 3-(bromoacetyl)coumarin oxime ethers **11b, c** were prepared by reaction of compound **10** with

Table 1. Structures and physicochemical data of compounds **8a–l**.



Compound	X	Y	R	Mp. (°C)	Reaction Time (h)	Yield (%)	Formula	M.W.
8a	CH	O	Et	212–214	12	54	$C_{27}H_{24}FN_3O_6$	505.49
8b	CH	O	*c*-Pr	211–213	12	70	$C_{28}H_{24}FN_3O_6$	517.51
8c	N	O	Et	215–216	6	57	$C_{26}H_{23}FN_4O_6$	506.48
8d	CH	NOH	Et	151–152	12	96	$C_{27}H_{25}FN_4O_6$	520.51
8e	CH	NOH	*c*-Pr	157–158	12	85	$C_{28}H_{25}FN_4O_6$	532.52
8f	N	NOH	Et	155–157	12	88	$C_{26}H_{24}FN_5O_6$	521.5
8g	CH	$NOCH_3$	Et	211–212	72	73	$C_{28}H_{27}FN_4O_6$	534.54
8h	CH	$NOCH_3$	*c*-Pr	138–140	72	66	$C_{29}H_{27}FN_4O_6$	546.55
8i	N	$NOCH_3$	Et	216–218	24	90	$C_{27}H_{26}FN_5O_6$	535.52
8j	CH	NOBn	Et	227–228	72	93	$C_{34}H_{31}FN_4O_6$	610.63
8k	CH	NOBn	*c*-Pr	180–181	72	83	$C_{35}H_{31}FN_4O_6$	622.64
8l	N	NOBn	Et	170–171	48	91	$C_{33}H_{30}FN_5O_6$	611.62



Reagents and conditions: (a) Br_2, $CHCl_3$, 22-25°C, and then reflux; (b) hydroxylamine hydrochloride, MeOH, 22–25°C; (c) methoxy amine hydrochloride, MeOH, 22–25°C; (d) *O*-benzyl hydroxylamine hydrochloride, MeOH, 22–25°C; (e) DMF, $NaHCO_3$, 22–25°C.

Scheme 1. Synthesis route of compounds **8a–l**.

methoxy amine hydrochloride or *O*-benzylhydroxylamine hydrochloride [18–20]. Reaction of quinolones (**4**, **5**, or **6**) with α-bromoketone **10** or α-bromo oxime derivatives **11a–c** in DMF, in the presence of $NaHCO_3$ at 22–25°C afforded corresponding ketones **8a–c** and oxime derivatives **8d–l**, respectively (Table 1) [18–20].

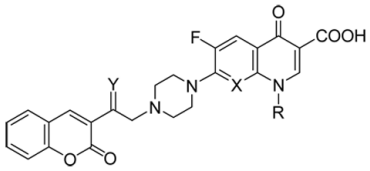
Antibacterial activity

The newly synthesized compounds **8a–l** were evaluated for their *in-vitro* antibacterial activity against *Staphylococcus aureus* ATCC 6538p, methicillin-resistant *Staphylococcus aureus* (MRSA I and MRSA II, clinical isolates), *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, and *Pseudomonas aeruginosa* ATCC 9027 using conventional agar-dilution method [23]. The MIC (minimum inhibitory concentration) values were determined by comparison to norfloxacin **4**, ciprofloxacin **5**, and enoxacin **6** as reference drugs. The MICs (μg/mL) obtained for compounds **8a–l** are presented in Table 2.

The MIC values of the test derivatives indicate that most compounds exhibit good activity against *gram*-positives including MRSA and *gram*-negative bacteria.

Antibacterial screening of compounds **8a–l** against staphylococci reveals that compounds **8b** and **8h** exhibit the most potent *in-vitro* antibacterial activity against staphylococci and comparable activity (MIC = 0.19–0.39 μg/mL) with respect to the compounds **4–6** (MIC = 0.19–0.78 μg/mL). In addition, the activities of compounds **8a**, **8c**, **8e**, and **8g** against staphylococci were respectable (MIC = 0.78–1.65 μg/mL). Most tested compounds had appreciable *in-vitro* activity (MIC < 0.78 μg/mL) against *B. subtilis*, but were less active than compounds **4–6**. All compounds did not show any improvement of activity against *gram*-negative bacteria in comparison to **4–6**. However, the most active compound **8b** showed comparable activity against *gram*-negative bacteria, with respect to **4–6**.

Table 2. *In-vitro* antibacterial activities of compounds **8a–l** against selected strains (MICs in μg/mL).



Compound	X	Y	R	Gram-positive organisms					Gram-negative organisms		
				S. a.[a]	*MRSA I*[b]	*MRSA II*[b]	*S. e.*[c]	*B. s.*[d]	*E. c.*[e]	*K. p.*[f]	*P. a.*[g]
8a	CH	O	Et	0.78	1.56	1.56	0.39	0.39	0.049	0.025	1.56
8b	CH	O	c-Pr	0.19	0.39	0.39	0.049	0.049	0.013	0.003	0.39
8c	N	O	Et	0.78	0.78	0.78	0.39	0.39	0.049	0.049	3.13
8d	CH	NOH	Et	6.25	6.25	6.25	3.13	1.56	0.39	0.39	25
8e	CH	NOH	c-Pr	0.78	1.56	1.56	0.39	0.19	0.049	0.025	3.13
8f	N	NOH	Et	3.13	3.13	3.13	1.56	0.78	0.78	0.39	12.5
8g	CH	$NOCH_3$	Et	0.78	1.56	1.56	1.56	0.39	1.56	0.78	50
8h	CH	$NOCH_3$	c-Pr	0.39	0.39	0.39	0.39	0.098	0.39	0.19	50
8i	N	$NOCH_3$	Et	3.13	3.13	3.13	6.25	0.78	6.25	1.56	>100
8j	CH	NOBn	Et	50	>100	>100	100	100	100	12.5	>100
8k	CH	NOBn	c-Pr	12.5	25	25	1.56	0.78	1.56	0.78	>100
8l	N	NOBn	Et	50	>100	>100	100	100	100	12.5	>100
4 Norfloxacin				0.39	0.78	0.78	0.049	0.098	0.049	0.025	1.56
5 Ciprofloxacin				0.19	0.39	0.39	0.025	0.025	0.013	0.003	0.39
6 Enoxacin				0.39	0.78	0.78	0.098	0.19	0.098	0.049	1.56

[a] *S. a.*: *Staphylococcus aureus* ATCC 6538p.
[b] MRSA I and II: methicillin-resistant *Staphylococcus aureus* (clinical isolates I and II).
[c] *S. e.*: *Staphylococcus epidermidis* ATCC 12228.
[d] *B. s.*: *Bacillus subtilis* ATCC 6633.
[e] *E. c.*: *Escherichia coli* ATCC 8739.
[f] *K. p.*: *Klebsiella pneumoniae* ATCC 10031.
[g] *P. a.*: *Pseudomonas aeruginosa* ATCC 9027

The MIC values of the ketones, oximes, and oxime ethers indicate that the most active compounds in each series were ciprofloxacin derivatives (R = cyclopropyl, X = CH), while enoxacin derivatives and norfloxacin derivatives exhibit equal activity against most strains. These results reveal the impact of cyclopropyl substituent at N-1 position in all series. Moreover, the alteration of ketone to an unsubstituted or substituted oxime group could not improve the overall activity against most strains. Generally, in both *gram*-positive and *gram*-negative bacteria, better results are obtained with cyclopropyl at N-1 and 2-oxo- on the ethyl spacer of coumarin and piperazine (compound **8b**).

According to structure-activity relationships for the quinolones, the spectrum of antibacterial coverage and the overall pharmacokinetics largely depend upon the C-7 substitution [14–18]. On the other hand, Shen *et al*. [24, 25] have suggested along with their cooperative drug-enzyme-DNA-binding model, that the 7-position is related to drug-enzyme interactions. Whereas the great majority of the new quinolones under development or in clinical use is incorporated with piperazine or pyrrolidine bearing small substitution (e. g. methyl), a few of the quinolones are substituted at C-7 with bulky substituent on the cyclic amine. Recently, we identified a series of *N*-substituted piperazinyl quinolones **7** in which the N-4 hydrogen of piperazinyl group of norfloxacin **4**, ciprofloxacin **5**, and enoxacin **6** is replaced with various 2-oxoethyl or 2-oxyiminoethyl moieties and display *in-vitro* antibacterial activity comparable or higher than respective parent quinolones [18–21]. Therefore, our strategy to achieve a better antimicrobial profile has focused on introducing new functionality on the piperazine ring. In the present study, structure **7** was used as starting point for chemical manipulations. So, twelve new analogs **8a–l** were prepared by replacing the aryl with a coumarin ring on 2-oxoethyl or 2-oxyiminoethyl moieties. These molecules **8**, (Fig. 1) carry the structural features of 7-

piperazinylquinolones **7** and RU 79115 **3**, (well known inhibitor of DNA gyrase by binding of the coumarin moiety to the B subunit of gyrase). The studies of the antibacterial activities of these compounds show that for a molecule to exhibit considerable activity against both *gram*-positive and *gram*-negative bacteria, the correct combination of the substituents in the molecule is very essential. Amongst the compounds studied here, compound **8b** exhibits promising antibacterial activity. Although these restricted series of quinolone-coumarin hybrid molecules could not show high synergistic or additive effects with respect to the parent quinolones, further tuning of the molecules could be reached by modifying the substituents at the different positions of the coumarin ring to improve the activity. However, in the absence of structural information on the complex of quinolones with DNA gyrase it is difficult to rationalize these results at the molecular level. In addition to the target enzymes other factors such as bacterial penetration and efflux systems may play an important role in defining the SAR.

In conclusion, some of the new *N*-[2-(coumarin-3-yl)ethyl]piperazinyl quinolones **8** containing a carbonyl-related functional groups (ketone, oxime, *O*-methyloxime, and *O*-benzyloxime) on the ethyl spacer showed considerable antibacterial activity and modification of the position 8 and N-1 substituent on the quinolone ring, and ethyl spacer functionality produced relatively major changes in terms of activity. In general, the results of antibacterial evaluation of the test compounds in comparison with the reference drugs indicated that compound **8b** showed comparable or more potent antibacterial activity with respect to the reference drugs **4–6** against all tested species.

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The authors have declared no conflict of interest.

Experimental

Chemical reagents and all solvents used in this study were purchased from Merck AG (Darmstadt, Germany). The starting materials **4–6** and **9** were purchased from Aldrich Chemical (Steinheim, Germany). The 3-(bromoacetyl)coumarin **10** was prepared according to the literature [22]. Melting points were determined in open glass capillaries using Bibby Stuart Scientific SMP3 apparatus (Bibby Sterlin Ltd., U.K.) and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks; Shimadzu, Tokyo, Japan). ^{1}H-NMR spectra were recorded using a Bruker 500 spectrometer (Bruker Bioscience, Billerica, MA, USA), and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. Elemental analyses were carried out on a HERAEUS CHN-O rapid elemental analyzer (Heraeus GmbH, Hanau, Germany) for C, H and N, and the results are within ± 0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC (Merck).

3-(Bromoacetyl)coumarin oxime ***11a***

A solution of **10** (267 mg, 1.0 mmol) and hydroxylamine hydrochloride (209 mg, 3.0 mmol) in methanol (10 mL) was stirred at 22–25°C overnight. Then, water (25 mL) was added and the precipitate was filtered and washed with water to give compound **11a** (240 mg). Yield 85%; mp. 185–187°C; IR (KBr, cm^{-1}) 1740, 1723, 1610, 1361, 1258, 955, 835, 760; ^{1}H-NMR (500 MHz, DMSO-d_6) 4.55 (s, 2H, CH_2-Br), 7.42 (dt, 1H, H-6 coumarin, *J* = 7.85 and 0.82 Hz), 7.47 (d, 1H, H-8 coumarin, *J* = 6.94 Hz), 7.69 (dt, 1H, H-7 coumarin, *J* = 8.66 and 1.55 Hz), 7.88 (dd, 1H, H-5 coumarin, *J* = 7.66 and 1.39 Hz), 8.29 (s, 1H, H-4 coumarin), 12.41 (s, 1H, oxime).

3-(Bromoacetyl)coumarin-O-methyloxime ***11b***

To a stirred solution of **10** (534 mg, 2.0 mmol) in MeOH (16 mL) at 22–25°C, was added 25% solution of *O*-methylhydroxyl ammonium chloride in diluted HCl (1002 mg, 3.0 mmol). After 20 h stirring at 22–25°C, the precipitated white solid was filtered off, washed with cold methanol, and dried to give **11b** (518 mg). Yield 87%; mp. 152–153°C; IR (KBr, cm^{-1}) 1723, 1630, 1606, 1572, 1459, 1434, 1363, 1242, 1163, 1094, 1043, 1001, 886, 765; ^{1}H-NMR (500 MHz, DMSO-d_6) 4.12 (s, 3H, CH_3), 4.64 (s, 2H, CH_2-Br), 7.37 (t, 1H, H-6 coumarin, *J* = 7.39 Hz), 7.41 (d, 1H, H-8 coumarin, *J* = 8.67 Hz), 7.60–7.66 (m, 2H, H-5 and H-7 coumarin), 8.05 (s, 1H, H-4 coumarin).

3-(Bromoacetyl)coumarin-O-benzyloxime ***11c***

A solution of **10** (534 mg, 2.0 mmol) and *O*-benzyl hydroxylamine hydrochloride (479 mg, 3.0 mmol) in methanol (16 mL) was stirred at 22–25°C overnight. The resulting suspension was cooled (0–4°C) and the precipitated white solid was filtered off, washed with cold methanol, and dried to give **11c** (550 mg). Yield 74%; mp. 103–104°C; IR (KBr, cm^{-1}) 1715, 1621, 1607, 1450, 1362, 1239, 1165, 1094, 1051, 881, 765, 734; ^{1}H-NMR (500 MHz, DMSO-d_6) 4.68 (s, 2H, CH_2-Br), 5.36 (s, 2H, O-CH_2-Ph), 7.35–7.48 (m, 7H, H-6 coumarin, H-8 coumarin and phenyl), 7.58–7.64 (m, 2H, H-5 and H-7 coumarin), 8.00 (s, 1H, H-4 coumarin).

General procedure for the synthesis of compounds ***8a–l***

A mixture of 3-(bromoacetyl)coumarin **10** or 3-(bromoacetyl)coumarin oxime derivatives **11a–c** (0.55 mmol), quinolone **4–6** (0.5 mmol), and $NaHCO_3$ (0.5 mmol) in DMF (5 mL), was stirred at 22–25°C for 6–72 h. After consumption of quinolone, water (20 mL) was added and the precipitate was filtered, washed with water, and crystallized from methanol-chloroform (9 : 1) to give compound **8a–l**.

Compound ***8a***

IR (KBr, cm^{-1}) 3440, 1728, 1628, 1610, 1559, 1480, 1453, 1384, 1261, 1190, 966, 762; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.41 (t, 3H, CH_3, *J* = 7.13 Hz), 2.70–2.78 (m, 4H, piperazine), 3.32–3.39 (m, 4H, piperazine), 3.93 (s, 2H, $COCH_2$), 4.59 (q, 2H, CH_2-CH_3, *J* = 7.12 Hz), 7.19 (d, 1H, H-8 quinolone, *J* = 7.27 Hz), 7.41 (t, 1H, H-6 coumarin, *J* = 7.49 Hz), 7.49 (d, 1H, H-8 coumarin, *J* = 8.31 Hz),

7.76 (dt, 1H, H-7 coumarin, *J* = 6.99 and 1.54 Hz), 7.92 (d, 1H, H-5 quinolone, *J* = 13.31 Hz), 7.97 (dd, 1H, H-5 coumarin, *J* = 7.80 and 1.38 Hz), 8.69 (s, 1H, H-4 coumarin), 8.96 (s, 1H, H-2 quinolone), 15.41 (s, 1H, COOH).

*Compound **8b***

IR (KBr, cm^{-1}) 3449, 1731, 1690, 1628, 1610, 1560, 1475, 1261, 1185, 964, 761; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.16–1.21 (m, 2H, cyclopropyl), 1.29–1.34 (m, 2H, cyclopropyl), 2.70–2.79 (m, 4H, piperazine), 3.32–3.38 (m, 4H, piperazine), 3.80–3.88 (m, 1H, cyclopropyl), 3.95 (s, 2H, $COCH_2$), 7.44 (t, 1H, H-6 coumarin, *J* = 7.76 Hz), 7.49 (d, 1H, H-8 coumarin, *J* = 8.35 Hz), 7.57 (d, 1H, H-8 quinolone, *J* = 7.42 Hz), 7.77 (dt, 1H, H-7 coumarin, *J* = 7.79 and 1.46 Hz), 7.91 (d, 1H, H-5 quinolone, *J* = 13.25 Hz), 7.97 (dd, 1H, H-5 coumarin, *J* = 6.59 and 1.36 Hz), 8.67 and 8.69 (two s, 2H, H-2 quinolone and H-4 coumarin), 15.20 (s, 1H, COOH).

*Compound **8c***

IR (KBr, cm^{-1}) 3433, 1735, 1688, 1630, 1610, 1560, 1466, 1444, 1263, 959, 809, 748; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.39 (t, 3H, CH_3, *J* = 7.00 Hz), 2.69–2.77 (m, 4H, piperazine), 3.80–3.87 (m, 4H, piperazine), 3.93 (s, 2H, $COCH_2$), 4.49 (q, 2H, CH_2-CH_3, *J* = 7.06 Hz), 7.44 (t, 1H, H-6 coumarin, *J* = 7.40 Hz), 7.48 (d, 1H, H-8 coumarin, *J* = 8.34 Hz), 7.76 (dt, 1H, H-7 coumarin, *J* = 7.86 and 1.43 Hz), 7.97 (dd, 1H, H-5 coumarin, *J* = 7.75 and 1.13 Hz), 8.09 (d, 1H, H-5 quinolone, *J* = 13.55 Hz), 8.68 (s, 1H, H-4 coumarin), 8.98 (s, 1H, H-2 quinolone), 15.33 (s, 1H, COOH).

*Compound **8d***

IR (KBr, cm^{-1}) 3443, 1719, 1669, 1629, 1484, 1473, 1387, 1258; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.39 (t, 3H, CH_3, *J* = 7.07 Hz), 2.62–2.68 (m, 4H, piperazine), 3.22–3.30 (m, 4H, piperazine), 3.46 (s, 2H, CNOH-CH_2), 4.56 (q, 2H, CH_2-CH_3, *J* = 7.17 Hz), 7.15 (d, 1H, H-8 quinolone, *J* = 7.26 Hz), 7.40 (t, 1H, H-6 coumarin, *J* = 7.75 Hz), 7.45 (d, 1H, H-8 coumarin, *J* = 8.30 Hz), 7.66 (dt, 1H, H-7 coumarin, *J* = 7.75 and 1.46 Hz), 7.77 (dd, 1H, H-5 coumarin, *J* = 7.73 and 1.19 Hz), 7.91 (d, 1H, H-5 quinolone, *J* = 13.28 Hz), 8.15 (s, 1H, H-4 coumarin), 8.94 (s, 1H, H-2 quinolone), 11.31 (s, 1H, oxime), 15.35 (s, 1H, COOH).

*Compound **8e***

IR (KBr, cm^{-1}) 3428, 1720, 1627, 1455, 1385, 1337, 1261, 760; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.10–1.20 (m, 2H, cyclopropyl), 1.25–1.35 (m, 2H, cyclopropyl), 2.59–2.70 (m, 4H, piperazine), 3.09–3.42 (m, 4H, piperazine), 3.74 (s, 2H, CNOH-CH_2), 3.76–3.90 (m, 1H, cyclopropyl), 7.00 (d, 1H, H-8 quinolone, *J* = 8.32 Hz), 7.40 (t, 1H, H-6 coumarin, *J* = 7.62 Hz), 7.56 (d, 1H, H-8 coumarin, *J* = 7.34 Hz), 7.66 (dt, 1H, H-7 coumarin, *J* = 7.33 and 1.26 Hz), 7.77 (d, 1H, H-5 coumarin, *J* = 7.69 Hz), 7.91 (d, 1H, H-5 quinolone, *J* = 13.47 Hz), 8.14 (s, 1H, H-4 coumarin), 8.66 (s, 1H, H-2 quinolone), 11.31 (s, 1H, oxime), 15.21 (s, 1H, COOH).

*Compound **8f***

IR (KBr, cm^{-1}) 3431, 1716, 1630, 1444, 1372, 1262, 809, 762; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.39 (t, 3H, CH_3, *J* = 7.18 Hz), 2.67–2.73 (m, 4H, piperazine), 3.80–3.89 (m, 4H, piperazine), 3.72 (s, 2H, CNOH-CH_2), 4.51 (q, 2H, CH_2-CH_3, *J* = 7.15 Hz), 7.38 (t, 1H, H-6 coumarin, *J* = 7.83 Hz), 7.39 (d, 1H, H-8 coumarin, *J* = 7.76 Hz), 7.53 (dt, 1H, H-7 coumarin, *J* = 7.70 and 1.48 Hz), 7.77 (dd, 1H, H-5 coumarin, *J* = 7.70 and 1.17 Hz), 8.11 (d, 1H, H-5 quinolone, *J* = 13.48 Hz), 8.15 (s, 1H, H-4 coumarin), 8.99 (s, 1H, H-2 quinolone), 11.30 (s, 1H, oxime), 15.32 (s, 1H, COOH).

*Compound **8g***

IR (KBr, cm^{-1}) 3453, 1731, 1629, 1517, 1474, 1452, 1384, 1258, 1045, 1011, 888, 768; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.58 (t, 3H, CH_3, *J* = 7.25 Hz), 2.69–2.75 (m, 4H, piperazine), 3.15–3.23 (m, 4H, piperazine), 3.92 (s, 2H, C-CH_2-N), 4.04 (s, 1H, OCH_3), 4.31 (q, 2H, CH_2-CH_3, *J* = 7.24 Hz), 6.78 (d, 1H, H-8 quinolone, *J* = 6.82 Hz), 7.34 (t, 1H, H-6 coumarin, *J* = 6.90 Hz), 7.39 (d, 1H, H-8 coumarin, *J* = 8.60 Hz), 7.55–7.61 (m, 2H, H-5 and H-7 coumarin), 7.94 (s, 1H, H-4 coumarin), 8.06 (d, 1H, H-5 quinolone, *J* = 13.06 Hz), 8.69 (s, 1H, H-2 quinolone), 15.12 (s, 1H, COOH).

*Compound **8h***

IR (KBr, cm^{-1}) 3454, 1728, 1627, 1608, 1492, 1456, 1337, 1258, 1046, 889, 760; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.17–1.22 (m, 2H, cyclopropyl), 1.35–1.42 (m, 2H, cyclopropyl), 2.68–2.76 (m, 4H, piperazine), 3.17–3.25 (m, 4H, piperazine), 3.47–3.56 (m, 1H, cyclopropyl), 3.93 (s, 2H, C-CH_2-N), 4.04 (s, 3H, O-CH_3), 7.29 (d, 1H, H-8 quinolone), 7.34 (t, 1H, H-6 coumarin, *J* = 7.52 Hz), 7.39 (d, 1H, H-8 coumarin, *J* = 8.62 Hz), 7.56–7.62 (m, 2H, H-5 and H-7 coumarin), 7.94 (s, 1H, H-4 coumarin), 8.01 (d, 1H, H-5 quinolone, *J* = 13.08 Hz), 8.78 (s, 1H, H-2 quinolone), 15.05 (s, 1H, COOH).

*Compound **8i***

IR (KBr, cm^{-1}) 3444, 1739, 1630, 1468, 1262, 1126, 1040, 1006, 885, 806, 747; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.50 (t, 3H, CH_3, *J* = 7.18 Hz), 2.63–2.68 (m, 4H, piperazine), 3.72–3.78 (m, 4H, piperazine), 3.90 (s, 2H, C-CH_2-N), 4.03 (s, 3H, O-CH_3), 4.40 (q, 2H, CH_2-CH_3, *J* = 7.19 Hz), 7.35 (dt, 1H, H-6 coumarin, *J* = 7.71 and 0.86 Hz), 7.40 (d, 1H, H-8 coumarin, *J* = 8.35 Hz), 7.57–7.63 (m, 2H, H-5 and H-7 coumarin), 7.95 (s, 1H, H-4 coumarin), 8.10 (d, 1H, H-5 quinolone, *J* = 13.36 Hz), 8.71 (s, 1H, H-2 quinolone), 15.08 (s, 1H, COOH).

*Compound **8j***

IR (KBr, cm^{-1}) 3428, 2826, 1727, 1627, 1480, 1361, 1302, 1257, 1131, 1006, 909, 750; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.58 (t, 3H, CH_3, *J* = 7.19 Hz), 2.65–2.72 (m, 4H, piperazine), 3.14–3.22 (m, 4H, piperazine), 3.98 (s, 2H, C-CH_2-N), 4.30 (q, 2H, CH_2-CH_3, *J* = 7.22 Hz), 5.28 (s, 2H, O-CH_2-Ph), 6.77 (d, 1H, H-8 quinolone , *J* = 6.75 Hz), 7.34 (t, 1H, H-6 coumarin, *J* = 7.59 Hz), 7.35–7.47 (m, 6H, H-8 coumarin and phenyl), 7.54–7.61 (m, 2H, H-5 and H-7 coumarin), 7.89 (s, 1H, H-4 coumarin), 8.06 (d, 1H, H-5 quinolone, *J* = 13.04 Hz), 8.68 (s, 1H, H-2 quinolone), 15.11 (s, 1H, COOH).

*Compound **8k***

IR (KBr, cm^{-1}) 3433, 1727, 1627, 1496, 1467, 1337, 1257, 1040, 1011, 880, 757, 720; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.17–1.22 (m, 2H, cyclopropyl), 1.35–1.41 (m, 2H, cyclopropyl), 2.66–2.74 (m, 4H, piperazine), 3.17–3.23 (m, 4H, piperazine), 3.48–3.54 (m, 1H, cyclopropyl), 3.98 (s, 2H, C-CH_2-N), 5.28 (s, 2H, O-CH_2-Ph), 7.26–7.47 (m, 8H, H-8 quinolone, H-6 coumarin, H-8 coumarin and phenyl), 7.54–7.61 (m, 2H, H-5 and H-7 coumarin), 7.90 (s, 1H, H-4 coumarin), 8.02 (d, 1H, H-5 quinolone, *J* = 13.08 Hz), 8.79 (s, 1H, H-2 quinolone), 15.05 (s, 1H, COOH).

*Compound **8l***

IR (KBr, cm^{-1}) 3440, 1731, 1631, 1608, 1468, 1358, 1259, 1127, 997, 808, 752; ^{1}H-NMR (500 MHz, DMSO-d_6) 1.49 (t, 3H, CH_3, J = 7.05 Hz), 2.55–2.71 (m, 4H, piperazine), 3.70–3.77 (m, 4H, piperazine), 3.95 (s, 2H, C-CH_2-N), 4.39 (q, 2H, CH_2-CH_3, J = 7.08 Hz), 5.26 (s, 2H, O-CH_2-Ph), 7.30–7.45 (m, 7H, H-6 coumarin, H-8 coumarin and phenyl), 7.54–7.61 (m, 2H, H-5 and H-7 coumarin), 7.90 (s, 1H, H-4 coumarin), 8.09 (d, 1H, H-5 quinolone, J = 13.31 Hz), 8.70 (s, 1H, H-2 quinolone), 15.08 (s, 1H, COOH).

Antibacterial activity

Compounds **8a–l** were evaluated for their antibacterial activity using conventional agar-dilution method [23]. Twofold serial dilutions of the compounds and reference drugs **4–6** were prepared in Mueller–Hinton agar. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with melted Mueller–Hinton agar was performed to obtain the required concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.19, 0.098, 0.049, 0.025, 0.013, 0.006, 0.003, and 0.0015 μg/mL. The bacteria inocula were prepared by suspending overnight colonies from Mueller–Hinton agar media in 0.85% saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standard (1.5×10^8 CFU/mL). The suspensions were then diluted in 0.85% saline to give 10^7 CFU/mL. Petri dishes were spot-inoculated with 1 μL of each prepared bacterial suspension (10^4 CFU/spot) and incubated at 35–37°C for 18 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

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