Full Paper

Synthesis and Antibacterial Activity of a New Series of 3-[3-(Substituted Phenyl)-1-Isonicotinoyl-1*H*-Pyrazol-5-yl]-2*H*-Chromen-2-one Derivatives

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A novel series of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives **4a – k** have been synthesized by the reaction of 3-[2,3-dibromo-3-(substituted phenyl) propanoyl]-2*H*-chromen-2-one **3a – k** and isonicotinic acid hydrazide in the presence of triethylamine in absolute ethanol, characterized by spectral data and screened for their *in-vitro* antibacterial activity against Gram-positive and Gram-negative bacteria. Among the series, compounds **4e**, **4i**, and **4k** displayed an encouraging antibacterial activity profile as compared to the reference drug ampicillin against tested bacterial strains.

Keywords: Antibacterial activity / Coumarin / Isonicotinic acid hydrazide / Pyrazole

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Introduction

In recent years, microorganisms resistant to multiple antimicrobial agents have become a serious problem worldwide in the fight against infectious diseases. This has resulted in increased morbidity and mortality with an overall increase in healthcare costs. The increase in bacterial resistance has attracted considerable interest in the discovery and development of new classes of antibacterial agents preferably exhibiting chemical characteristics that clearly differ from those of the already existing drugs [1].

Despite numerous attempts in the search for more effective antimicrobials to develop a new structural prototype, the coumarins still remain as one of the most versatile class of compounds against microbes and, therefore, they are an important component among the molecules in drug discovery. Coumarin is an imperative scaffold, since several coumarin derivatives are known to be associated with multiple biological activities [2 – 5]. Coumarin-containing antibiotics (including novobiocin, chlorobiocin, and coumermycin) exert their potent antibacterial activity by inhibiting the function of the B-subunit of the enzyme DNA gyrase vital for the bacterium [6, 7]. The coumarins inhibit the ATPase activity of DNA gyrase by competing with ATP for binding to the B subunit of the enzyme [8, 9].

Several compounds containing a pyrazole ring system are reported to exhibit anti-inflammatory activity [10 – 12]. Much attention is given to pyrazoles as antimicrobial agents after the discovery of the natural pyrazole C-glycoside like pyrazofurin which demonstrates a broad spectrum of antimicrobial activity [13]. Consequently, several pyrazole derivatives that exhibited antimicrobial activity are reported by Tanitame and coworkers [14, 15]. Literature survey revealed that various compounds containing a pyrazole moiety are also found to possess antitumor [16], antimycobacterial [17, 18], and antileukemia [19] activities. Therefore, such medicinal properties associated with these two heterocycles render them as a useful

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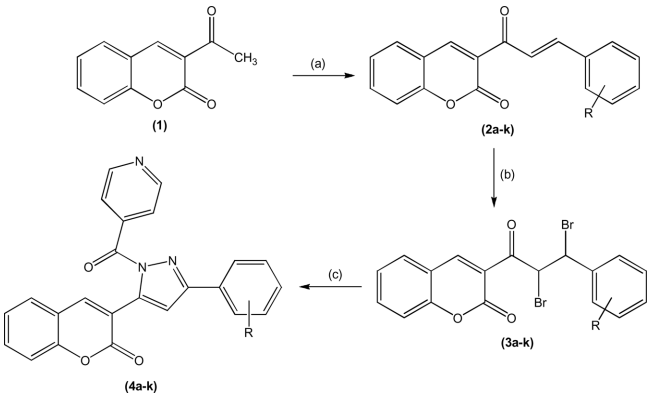


Reagents and conditions: (a) Ar-CHO, piperidine / *n*-butanol, reflux, 4 h; (b) Br_2 / $CHCl_3$, stirring, r.t., 12 h; (c) isonicotic acid hydrazide, TEA / ethanol, reflux, 12 h.

Scheme 1. Synthesis of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one.

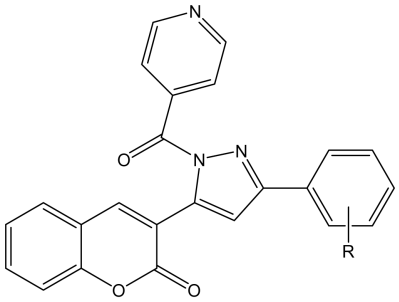
structural units in drug research. In view of these observations and in continuation of our research program on the synthesis of bi-heterocyclic compounds [20 – 23], we report herein the synthesis of some new 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives **4a – k**, for investigation of their antibacterial activity profile.

Results and discussion

Chemistry

The synthesis of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one **4a – k** was achieved through the versatile and efficient synthetic route outlined in Scheme 1. It is apparent from the scheme that the new target molecules possess both coumarin and pyrazole units. Reaction of 3-[2,3-dibromo-3-(substituted phenyl) propanoyl]-2*H*-chromen-2-one with isoniazid seemed to be a convenient route for the synthesis of desired molecules. Starting material 3-acetyl-2*H*-chromen-2-one **1** was synthesized by the reaction of salicylaldehyde with ethylacetoacetate in the presence of a catalytic amount of piperidine at room temperature following the literature procedure [24]. 3-[(2*E*)-3-substituted prop-2-enoyl]-2*H*-chromen-2-one (chalcones, **2a – k**) were obtained by Claisen – Schmidt condensation of 3-acetyl-2*H*-chromen-2-one **1** with various substituted benzaldehydes in the presence of a mixture of piperidine and *n*-butanol. Efforts to convert compounds **2a – k** into target molecules **4a-k** under a variety of conditions were not successful. Hence, an alternative method was adopted. This involved the bromination of chalcones **2a – k** and subsequent ring closure using isonicotinic acid hydrazide. Bromination of

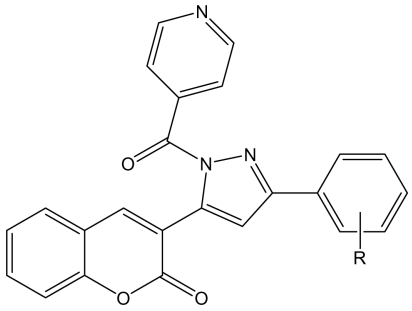
Table 1. Physicochemical data of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives **4a – k**.



Compound	R	Yield (%)	M.p. (°C)	R_f Value[a)]	Formula	M.W.
4a	-H	53	158 – 160	0.38	$C_{24}H_{15}N_3O_3$	393.42
4b	-4-OMe	83	120 – 122	0.27	$C_{25}H_{17}N_3O_4$	423.39
4c	-4-Cl	76	98 – 100	0.48	$C_{24}H_{14}ClN_3O_3$	427.86
4d	-4-NMe_2	70	148 – 150	0.46	$C_{26}H_{20}N_4O_3$	436.44
4e	-2,4-$(Cl)_2$	92	126 – 130	0.24	$C_{24}H_{13}Cl_2N_3O_3$	462.24
4f	-4-Me	74	90 – 92	0.36	$C_{25}H_{17}N_3O_{43}$	407.46
4g	-3-NO_2	67	100 – 102	0.25	$C_{24}H_{14}N_4O_5$	438.4
4h	-3-OMe	83	136 – 138	0.32	$C_{25}H_{17}N_3O_4$	423.43
4i	-4-F	64	190 – 192	0.29	$C_{24}H_{14}FN_3O_3$	411.35
4j	-2-NO_2	71	208 – 210	0.33	$C_{24}H_{14}N_4O_5$	438.39
4k	-4-OH	76	182 – 184	0.42	$C_{24}H_{15}N_3O_4$	409.36

[a)] All synthesized compounds were purified by column chromatography using chloroform / methanol (10: 1) as mobile phase and iodine vapors as visualizing agent.

Table 2. Spectral data of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives *4a – k*.

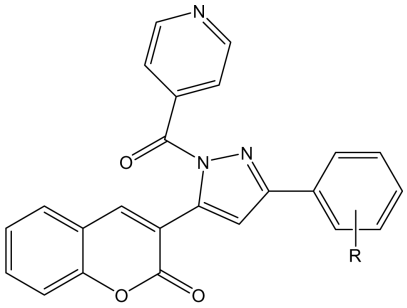


(4a-k)

Compound	R	UV (CH_3OH)	IR (KBr, cm^{-1})	^{1}H-NMR (DMSO-d_6, δ, ppm)	Elemental Analyses (CHN)
4a	-H	λ_{max} 289 (ε 14454)	1679.0 (coumarin, C=O), 1634.7 (amide C=O), 1566.9 (C=N), 1491.0 (C=C).	8.57 (s, 1H, 4-H of coumarin), 7.81 – 8.39 (m, 4H, Ar-H of pyridine), 7.48 (s, 1H, 4-H of pyrazole), 7.52 – 7.69 (m, 9H, Ar-H).	Anal. Calcd. for $C_{24}H_{15}N_3O_3$: C, 73.25; H, 3.87; N, 10.70. Found: C, 73.23; H, 3.90; N, 10.68.
4b	-4-OMe	λ_{max} 277 (ε 17116)	1681.9 (coumarin, C=O), 1624.7 (amide C=O), 1570.8 (C=N), 1490.0 (C=C).	8.18 (s, 1H, 4-H of coumarin), 7.84 – .02 (m, 4H, Ar-H of pyridine), 7.33 (s, 1H, 4-H of pyrazole), 7.41 – 7.68 (m, 8H, Ar-H), 3.86 (s, 3H, -OCH_3).	Anal. Calcd. for $C_{25}H_{17}N_3O_4$: C, 70.95; H, 4.08; N, 9.95. Found: C, 70.97; H, 4.09; N, 9.86.
4c	-4-Cl	λ_{max} 297 (ε 12350)	1682.0 (coumarin, C=O), 1614.4 (amide C=O), 1565.2 (C=N), 1486.7 (C=C).	8.7 (s, 1H, 4-H of coumarin), 7.52 – 8.05 (m, 4H, Ar-H of pyridine), 6.95 (s, 1H, 4-H of pyrazole), 7.03 – 7.48 (m, 8H, Ar-H).	Anal. Calcd. for $C_{24}H_{14}ClN_3O_3$: C, 67.40; H, 3.30; N, 9.80. Found: C, 67.45; H, 3.40; N, 9.72.
4e	-2,4-$(Cl)_2$	λ_{max} 285 (ε 10224)	1682.6 (coumarin, C=O), 1616.3 (amide C=O), 1566.1 (C=N), 1484.9 (C=C).	8.78 (s, 1H, 4-H of coumarin), 7.72 – 8.64 (m, 4H, Ar-H of pyridine), 6.85 (s, 1H, 4-H of pyrazole), 6.95 – 7.58 (m, 7H, Ar-H).	Anal. Calcd. for $C_{24}H_{13}Cl_2N_3O_3$: C, 62.33; H, 2.88; N, 9.11. Found: C, 62.42; H, 2.94; N, 9.05.
4f	-4-Me	λ_{max} 288 (ε 12217)	1715.4 (coumarin, C=O), 1672.7 (amide C=O), 1609.0 (C=N), 1552.9 (C=C).	8.57 (s, 1H, 4-H of coumarin), 7.81 – 8.39 (m, 4H, Ar-H of pyridine), 6.93 (s, 1H, 4-H of pyrazole), 7.37 – 7.69 (m, 8H, Ar-H), 1.59 (s, 3H, -CH_3).	Anal. Calcd. for $C_{25}H_{17}N_3O_3$: C, 73.74; H, 4.22; N, 10.30. Found: C, 73.75; H, 4.20; N, 10.38.
4g	-3-NO_2	λ_{max} 269 (ε 16454)	1685.2 (coumarin, C=O), 1611.1 (amide C=O), 1529.7 (C=N), 1449.3 (C=C).	8.37 (s, 1H, 4-H of coumarin), 7.71 – 8.29 (m, 4H, Ar-H of pyridine), 7.39 (s, 1H, 4-H of pyrazole), 7.45 – .69 (m, 8H, Ar-H).	Anal. Calcd. for $C_{24}H_{14}N_4O_5$: C, 65.73; H, 3.25; N, 12.80. Found: C, 65.75; H, 3.27; N, 12.84.
4h	-3-OMe	λ_{max} 285 (ε 15343)	1681.9 (coumarin, C=O), 1624.9 (amide C=O), 1570.8 (C=N), 1490.0 (C=C).	8.48 (s, 1H, 4-H of coumarin), 7.84 – 8.02 (m, 4H, Ar-H of pyridine), 7.19 (s, 1H, 4-H of pyrazole), 7.33 – 7.75 (m, 8H, Ar-H), 3.78 (s, 3H, -OCH_3).	Anal. Calcd. for $C_{25}H_{17}N_3O_4$: C, 70.90; H, 4.08; N, 9.95. Found: C, 70.85; H, 4.13; N, 9.86.
4i	-4-F	λ_{max} 276 (ε 19755)	1682.0 (coumarin, C=O), 1614.4 (amide C=O), 1565.2 (C=N), 1486.7 (C=C).	8.55 (s, 1H, 4-H of coumarin), 7.25 – 8.17 (m, 4H, Ar-H of pyridine), 6.77 (s, 1H, 4-H of pyrazole), 7.21 – 7.51 (m, 8H, Ar-H).	Anal. Calcd. for $C_{24}H_{14}FN_3O_3$: C, 70.08; H, 3.48; N, 10.25;. Found: C, 70.05; H, 3.50; N, 10.29.
4j	-2-NO_2	λ_{max} 276 (ε 18043)	1689.61 (coumarin, C=O), 1609.4 (amide C=O), 1552.3 (C=N), 1458.5 (C=C).	8.44 (s, 1H, 4-H of coumarin), 7.75 – 8.34 (m, 4H, Ar-H of pyridine), 7.31 (s, 1H, 4-H of pyrazole), 7.38 – 7.67 (m, 8H, Ar-H).	Anal. Calcd. for $C_{24}H_{14}N_4O_5$: C, 65.75; H, 3.22; N, 12.78;. Found: C, 65.79; H, 3.19; N, 12.68.
4k	-4-OH	λ_{max} 278 (ε 0679)	3208.0 (-OH), 1671.3 (coumarin, C=O), 1607.7 (amide C=O), 1559.6 (C=N), 1505.9 (C=C).	8.27 (s, 1H, 4-H of coumarin), 7.55 – 8.08 (m, 4H, Ar-H of pyridine), 6.93 (s, 1H, 4-H of pyrazole), 7.06 – 7.47 (m, 8H, Ar-H), 5.28 (s, 1H, -OH).	Anal. Calcd. for $C_{24}H_{15}N_3O_4$: C, 70.41; H, 3.69; N, 10.26;. Found: C, 70.45; H, 3.75; N, 10.21.

chalcones **2a–k** was carried out in chloroform using bromine in chloroform. The resulting dibromo compounds **3a–k** yielded on further treatment with isonicotinic acid hydrazide in the presence of triethylamine in absolute ethanol the desired compounds 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one **4a–k**. The structures of the synthesized compounds were established on the basis of physicochemical and elemental analysis and spectral data (IR and ^{1}H-NMR); they are summarized in Tables 1 and 2, respectively.

Table 3. *In-vitro* antibacterial activity of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one derivatives **4a–k** against selected strains (MIC in μg/mL).



(4a-k)

Compound	R	Gram-positive organisms		Gram-negative organisms	
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
4a	-H	64	>128	>128	>28
4b	-4-OMe	16	16	64	>128
4c	-4-Cl	1	4	8	4
4d	-4-NMe_2	32	>128	64	>128
4e	-2,4-$(Cl)_2$	0.5	0.5	0.5	2
4f	-4-Me	64	128	>128	>128
4g	-3-NO_2	2	2	0.5	4
4h	-3-OMe	16	32	32	>128
4i	-4-F	0.25	0.5	0.25	1
4j	-2-NO_2	4	2	1	2
4k	-4-OH	0.25	0.25	0.5	0.5
Ampicillin	–	0.5	0.5	0.5	0.5

Antibacterial activity

All newly synthesized compounds **4a–k** were evaluated for their *in-vitro* antibacterial activity against two Gram-positive bacteria, namely *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633), and two Gram-negative bacteria, namely *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) using the conventional agar-dilution method [25]. Ampicillin was used as a reference standard. The results of the *in-vitro* antibacterial activity screening of the test compounds are summarized in Table 3. Among the series, three compounds (**4e**, **4i**, and **4k**) exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria while the compounds **4c**, **4g**, and **4j** showed moderate antibacterial activity against the tested organisms. However, all other compounds in the series were found to have less or poor activity against both Gram-positive and Gram-negative bacteria as compared to standard. Minimum Inhibitory Concentration (MIC) was recorded as the lowest concentration of a compound to inhibit growth of the tested microorganisms. In comparing the MIC values with the standard (MIC = 0.5 μg/mL), compounds **4e**, **4i**, and **4k** exhibit the most potent *in-vitro* antibacterial activity against all evaluated organisms. Compounds **4e** (MIC = 0.5–2 μg/mL), **4i** (MIC = 0.25-1 μg/mL), and **4k** (MIC = 0.25–0.5 μg/mL) especially showed high antibacterial activity, while compounds **4c** (MIC = 1–8 μg/mL), **4g** (MIC = 0.5–4 μg/mL), and **4j** (MIC = 1–4 μg/mL) showed respectable antibacterial activity. The investigation of the structure-activity relationship revealed that the compounds with *p*-fluoro, *p*-hydroxy, *m*-nitro, *o*-nitro, and *o*, *p*-dichloro substituents at the third position in the aromatic ring of the pyrazole nucleus gave better results. They have emerged as active antibacterial agents.

Conclusion

Herein, we have described an efficient and convenient synthesis of a novel series of 3-[3-(substituted phenyl)-1-isonicotinoyl-1*H*-pyrazol-5-yl]-2*H*-chromen-2-one **4a – k**. These novel heterocyclic compounds containing both coumarin and pyrazole ring systems are prepared by the reaction of 3-[2,3-dibromo-3-(substituted phenyl)propanoyl]-2*H*-chromen-2-one **3a – k** with isonicotinic acid hydrazide in the presence of triethylamine in absolute ethanol. In general, the results of the *in-vitro* antibacterial activity tests are also encouraging as out of eleven compounds tested, compounds **4e**, **4i**, and **4k** exhibited an antibacterial activity which is comparable or even more potent than that of the reference drug. The MIC values of these novel compounds evidenced that the presence of fluorine, hydroxyl, nitro, and chlorine groups at the third position in the aromatic ring of the pyrazole nucleus gave rise to an increased antibacterial potency.

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The authors have declared no conflict of interest.

Experimental

Chemistry

All research chemicals were purchased from Sigma – Aldrich (St. Louis, MO, USA) or Lancaster Co. (Ward Hill, MA, USA) and used as such for the reactions. Solvents except laboratory reagent grade were dried and purified, when necessary, according to the literature. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates from Merck (Darmstadt, Germany). Melting points of synthesized compounds were determined in Thermonik melting point apparatus (Thermonik, Mumbai, India) and are uncorrected, UV spectra were recorded on Thermospectronic Spectrometer (Rochester, NY, USA) and IR spectra were recorded on Thermo Nicolet IR200 FT-IR Spectrometer (Madison, WI, USA) by using KBr pellets. The ^{1}H-NMR were recorded on Bruker AVANCE 300 (Bruker, Rheinstetten / Karlsruhe, Germany) using DMSO-d_6 as solvent. Chemical shifts are given in δ ppm units with respect to TMS as internal standard. The elemental analyses (C, H, N) of the compounds were performed on Heraus CHN-O rapid elemental analyzer (Heraeus, Hanau, Germany). Results of elemental analysis were within ± 0.4% of the theoretical values. The purity of compounds was examined by TLC on silica gel plate using chloroform and methanol (10 : 1) as mobile phase and iodine vapours as visualizing agent. The starting material 3-acetyl coumarin **1** was synthesized by a method reported earlier [21].

*General procedure of the preparation of 3-aryl-1-(3-coumarinyl)propan-1-ones **2a – k***

A mixture of 3-acetyl coumarin (**1**, 0.01 mol) and the various substituted aromatic aldehydes (0.012 mol) was dissolved in 10 mL of *n*-butanol under heating; then, 0.3 mL of glacial acetic acid and the same quantity of piperidine were added. The reaction mixture was refluxed for 4 h and then the solvent was removed in vacuum. The residue was triturated with 10 mL of ethanol until a precipitate formed. The precipitate was filtered off and crystallized from appropriate solvents.

*General procedure for the preparation of 3-[2,3-dibromo-3-(substituted phenyl)propanoyl]-2H-chromen-2-one derivatives **3a-k** [26]*

A 3-[(2*E*)-3-substituted prop-2-enoyl]-2*H*-chromen-2-one (**2a – k**, 0.01 mol) was dissolved in chloroform (100 mL) and bromine in chloroform (0.01 mol) was added dropwise with constant stirring. After the complete addition of the bromine solution, the reaction mixture was stirred for 12 h. Excess of chloroform was distilled off under reduced pressure. The thus obtained solid was filtered, dried, and washed from hot ethanol.

*General procedure for the synthesis of 3-[3-(substituted phenyl)-1-isonicotinoyl-1H-pyrazol-5-yl]-2H-chromen-2-one **4a – k***

A mixture of 3-[2,3-dibromo-3-(substituted phenyl)propanoyl]-2*H*-chromen-2-one (**3a – k**, 0.01 mol) and isoniazid (1.37 g, 0.01 mol) was dissolved in ethanol (150 mL) and triethylamine (10 mL). The reaction mixture was heated under reflux for 12 h. Excess of ethanol was distilled off under reduced pressure and the residue was triturated with ice-cold water. The precipitated solid was filtered, dried, and purified by column chromatography using silica gel (60-120) as stationary phase and chloroform / methanol (10 : 1) as mobile phase. The physicochemical, elemental, and spectral data of the synthesized compounds **4a – k** are summarized in Tables 1 and 2, respectively.

Antibacterial activity

Medium

The solid media Mueller – Hinton agar (MHA; beef infusion 300, g/L, casein acid hydrolysate 17.5, g/L, starch 1.5 g/L, agar 17, g/L, and distilled water 1000, mL, adjusted to pH 7.4) was used for testing the antibacterial activity.

Test microorganisms

Two Gram-positive bacteria, namely *Staphylococcus aureus* (ATCC-25923) and *Bacillus subtilis* (ATCC 6633) and two Gram-negative bacteria, namely *Escherichia coli* (ATCC-25922) and *Pseudomonas*

aeruginosa (ATCC-27853) were used for testing the antibacterial activity.

Minimum inhibitory concentration (MIC) [27]

The *in-vitro* antibacterial activity for newly synthesized compounds **4a – k** was evaluated using the conventional agar-dilution method [25]. Twofold serial dilutions of the compounds and reference drug (ampicilin) were prepared in MHA. 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 MHA was performed to obtain the required concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.05 μg/mL. The bacterial inocula were prepared by suspending 24 h-old bacterial colonies from MHA media in 0.85% saline. The inocula were adjusted to 0.5 McFarland Standard (1.5×10^8 CFU/mL) [28]. 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 of the prepared bacterial suspensions (10^4 CFU/spot) and incubated at 37°C for 24 h. At the end of the incubation period, the MIC was determined, which is the lowest concentration of the test compound that resulted in no visible growth on the plate. A control test was also performed with test medium supplemented with DMSO at the same dilutions as used in the experiment in order to ensure that the solvent had no influence on bacterial growth.

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