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ORIGINAL PAPER

Regioselective synthesis and antibacterial activity of 3-(cyanoacetyl)indole-based kojic acid derivatives

Robabeh Baharfar · Sakineh Asghari · Maryam Kiani

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Abstract A simple and facile protocol for the regioselective synthesis of diastereomeric 3-substituted indole derivatives is described by the three-component condensation of 3-(cyanoacetyl)indole, aldehydes, and kojic acid in the presence of ammonium acetate as catalyst. The synthesized compounds were tested for their antibacterial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. One compound showed good activity against *B. subtilis* compared with the standard drugs gentamicin and chloramphenicol.

Keywords Three-component reaction · 3-(Cyanoacetyl)indole · Kojic acid · Ammonium acetate · Antibacterial activities

Introduction

Multi-component reactions (MCRs) are of increasing importance in organic and medicinal chemistry [1, 2]. MCRs are reactions in which three or more reagents react in a one-pot procedure to give a single product [3]. These reactions have become increasingly popular during the last decade [4–6]. MCRs offer a wide range of advantages over single-component reactions such as high degree of atom economy, convergence, ease of execution, and a quick access to complex molecules [7].

Indole fragment is featured widely in broad ranges of pharmacologically and biologically active compounds [8–13]. Therefore, the synthesis and selective functionalization of indoles have been the focus of active research over the years [14–17]. In particular, 3-substituted indoles are privileged scaffolds in medicinal chemistry, which have been found in a fascinating array of bioactive natural products and pharmaceutical compounds [18–25]. These scaffolds are found in a number of biologically active compounds, especially with anti-cancer [26–29], anti-tumor [30], hypoglycemic [31], anti-inflammatory [32], analgesic [33, 34], and anti-pyretic activities [35].

Kojic acid is a natural product that has been isolated from various strains of microorganisms such as *Penicillium*, *Aspergillus*, and *Gluconacetobacter* [36]. Kojic acid and its derivatives have shown to possess various bioactivities such as anti-microbial [37, 38], cosmetic skin-whitening [39], anti-speck [40], pesticide and insecticide [41], anti-tumor [42], anti-diabetic [43], and anti-proliferative activities [44].

Therefore, in continuation of our previous work on the synthesis of novel biologically active heterocyclic compounds [45–47], we wish to report herein a simple protocol for the synthesis of novel 3-substituted indole and kojic acid derivatives **4a**–**4j** by the three-component condensation of 3-(cyanoacetyl)indole (**1**), benzaldehyde derivatives **2**, and kojic acid (**3a**) in the presence of ammonium acetate as catalyst (Scheme 1).

Also, antibacterial activities of 3-substituted indole derivatives **4a**–**4j** were investigated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*.

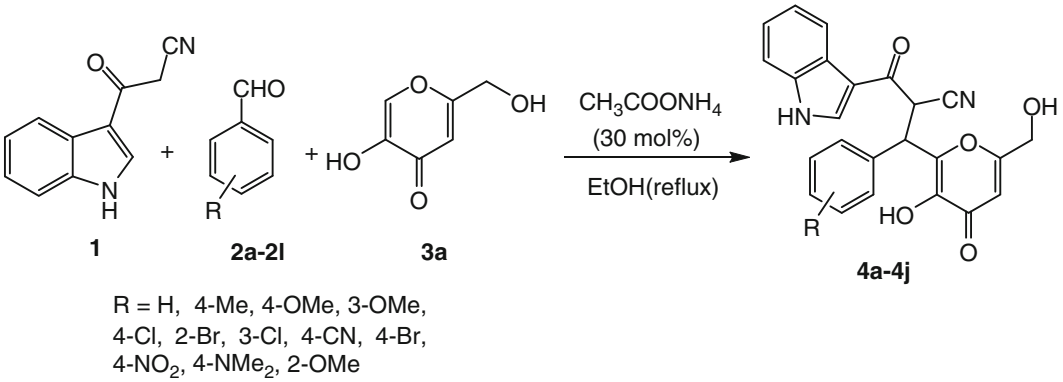
Results and discussion

Chemistry

In order to optimize the reaction conditions, the three-component reaction of 3-(cyanoacetyl)indole (**1**) with

R. Baharfar (✉) · S. Asghari · M. Kiani
Department of Chemistry, University of Mazandaran, P. O. Box 47415, Babolsar, Iran
e-mail: Baharfar@umz.ac.ir

Scheme 1



R = H, 4-Me, 4-OMe, 3-OMe, 4-Cl, 2-Br, 3-Cl, 4-CN, 4-Br, 4-NO_2, 4-NMe_2, 2-OMe

Table 1 Optimization of catalysts

Entry	Catalyst (mol%)	Time/h	Yield/%
1	No catalyst	24	NR[a]
2	Piperidine (30)	18	NR
3	DABCO (30)	18	40
4	CH_3COONH_4 (10)	18	20
5	CH_3COONH_4 (20)	18	55
6	CH_3COONH_4 (30)	18	95
7	CH_3COONH_4 (40)	18	95

[a] No reaction

Table 2 Optimization of solvent and temperature

Entry	Solvent	*T*/°C	Time/h	Yield/%
1	THF	r.t	24	NR
2	THF	Reflux	24	NR
3	1,4-Dioxane	r.t	24	NR
4	1,4-Dioxane	Reflux	24	NR
5	H_2O	r.t	24	NR
6	H_2O	Reflux	24	NR
7	MeOH	r.t	24	Trace
8	MeOH	Reflux	24	55
9	EtOH	r.t	24	Trace
10	EtOH	Reflux	18	95

benzaldehyde (**2a**) and kojic acid (**3a**) was investigated as model reaction. Initially, the reaction was carried out without any catalysts, but did not give any product (Table 1, entry 1). Also, when piperidine was used as the catalyst, the reaction did not proceed (Table 1, entry 2). The best result was obtained when the reaction was performed in the presence of ammonium acetate as catalyst. We also evaluated the amount of catalyst required for this transformation. It was found that when the amount of ammonium acetate increased from 10 to 20 mol% and 30 mol%, the yields increase from 20 to 55 % and 95 %, respectively. Using 30 mol% of ammonium acetate is sufficient to promote the reaction. Additional amounts of the catalyst did not improve the product yields (Table 1, entry 7).

To select the optimal solvent and temperature for this reaction, we performed the model reaction in different solvents, and at room temperature and at reflux conditions. It was observed that when using EtOH as the solvent at reflux condition, shorter reaction time and higher yield resulted (Table 2).

To study the scope the reaction, a series of substituted aldehydes **2a**–**2l** and kojic acid (**3a**) or some example of other compounds containing an enol group **3b**–**3d** were reacted with 3-(cyanoacetyl)indole (**1**) using ammonium acetate (30 mol% in boiling EtOH. Under the optimized reaction conditions, a series of 3-(cyanoacetyl)indole derivatives **4a**–**4j** were synthesized in excellent yields (Table 3). As presented in Table 3, the reaction of aldehydes with electron accepting groups leads to the highest yields and the shortest reaction times (entries 7 and 10), whereas the reaction of aldehydes **2k**, **2l** with strong electron releasing group (e.g., NMe_2) and the other OH acids **3b**–**3d** (entries 13–15) did not proceed, therefore no products could be isolated. The expected structures of the products **4m**–**4o** are depicted in Fig. 1.

As shown in Fig. 2, compounds **4a**–**4j** possess two chiral centers and therefore, they can exist as two diastereomeric pairs. For example, compound **4a** exists as two diastereomeric pairs, RR or its enantiomer SS, and RS or its enantiomer SR.

The ^{1}H NMR spectrum of **4a** shows existence of both two diastereomeric pairs. Unfortunately, all our attempts to separate the two diastereomeric pairs including recrystallization, flash and column chromatography by using

Table 3 Synthesis of three-substituted indole derivatives 4a–4j

Entry	Product	Aldehyde	OH acid	Time/h	M.p./°C	Yield/%	I/%[a]	Π/%[b]
1	**4a**	PhCHO (**2a**)	**3a**	18	236–238	95	56	39
2	**4b**	4-MeO-PhCHO (**2b**)	**3a**	28	232–234	91	53	38
3	**4c**	3-MeO-PhCHO (**2c**)	**3a**	24	211–213	80	47	33
4	**4d**	4-Me-PhCHO (**2d**)	**3a**	26	233–235	92	55	37
5	**4e**	4-Cl-PhCHO (**2e**)	**3a**	14	217–219	92	55	37
6	**4f**	3-Cl-PhCHO (**2f**)	**3a**	11	210–212	91	58	33
7	**4g**	4-CN-PhCHO (**2g**)	**3a**	8	235–237	97	60	37
8	**4h**	4-Br-PhCHO (**2h**)	**3a**	16	223–225	93	56	37
9	**4i**	2-Br-PhCHO (**2i**)	**3a**	13	219–221	90	58	32
10	**4j**	4-NO_2-PhCHO (**2j**)	**3a**	7	263–265	97	57	40
11	**4k**	4-Me_2N-PhCHO (**2k**)	**3a**	48	–	Trace	–	–
12	**4l**	2-MeO-PhCHO (**2l**)	**3a**	48	–	Trace	–	–
13	**4m**	**2a**	2,5-Dihydroxycyclohexa-2,5-diene-1,4-dione (**3b**)	48	–	NR	–	–
14	**4n**	**2a**	Phenol (**3c**)	48	–	Trace	–	–
15	**4o**	**2a**	4-Nitrophenol (**3d**)	48	–	Trace	–	–

[a, b] Determined by ^{1}H NMR spectroscopy

different ratios of various solvents have failed and we could only isolate the mixture of two diastereomers.

The structures and the purities of the products **4a–4j** were determined on the basis of their elemental analyses, mass spectra, ^{1}H and ^{13}C NMR and IR spectroscopic data. The ^{1}H NMR spectrum of **4a** in DMSO-d_6 for the first diastereomeric pair (RR/SS or RS/SR) exhibited two doublet of doublets at 4.07 and 4.25 ppm ($^2J_{HH}$ = 16.0 and $^3J_{HH}$ = 6.0 Hz) for CH_2 group (AB system), two doublets at 5.00 ppm for H_b and 5.74 ppm for H_a ($^3J_{HH}$ = 11.6 Hz), a triplet at 5.64 ppm ($^3J_{HH}$ = 6.4 Hz) for OH group and a singlet at 6.10 ppm for vinyl proton of kojic acid ring. The other signals at 7.16–7.50, 7.61, and 8.11 ppm consisted of characteristic resonances in the appropriate regions for nine aromatic protons along with three singlets at 8.91, 9.28, and 12.43 ppm for vinyl proton of indole, OH and NH groups, respectively. The proton decoupled ^{13}C NMR spectrum of **4a** showed 22 signals, which is in agreement with the proposed structure.

The ^{1}H NMR spectrum of **4a** in DMSO-d_6 for the second diastereomeric pair (RR/SS or RS/SR) exhibited two doublet of doublets at 4.44 and 4.48 ppm ($^2J_{HH}$ = 16.0 and $^3J_{HH}$ = 6.4 Hz) for CH_2 group (AB system), two doublets at 5.18 ppm ($^3J_{HH}$ = 10.8 Hz) and 5.61 ppm ($^3J_{HH}$ = 11.2 Hz) for H_b and H_a, a triplet at 5.79 ppm ($^3J_{HH}$ = 6.4 Hz) for OH group and a singlet at 6.36 ppm for vinyl proton of kojic acid ring. The other signals at 7.16–7.50, 7.52, and 8.05 consisted of characteristic resonances in the appropriate regions for aromatic protons along with three singlets at 8.75, 9.43, and 12.38 ppm for vinyl proton of indole, OH and NH groups, respectively. The proton decoupled ^{13}C NMR spectrum of **4a** showed 22 signals, which is in agreement with the proposed structure. The ^{1}H and ^{13}C NMR spectra of **4b–4j** were similar to those of **4a**, except to the substituted phenyl moiety that exhibited characteristic resonances in the appropriate regions of the spectra.

A plausible mechanism for the formation of products **4a–4j** is given in Scheme 2. The formation of these products can be rationalized by initial formation of intermediate **5** by Knoevenagel condensation of the aldehyde **2** and 3-(cyanoacetyl)indole. As shown in Scheme 2, the anion of kojic acid can attack to intermediate **5** in four routes: Michael-type addition (route a), direct addition (route b), C-alkylation (route c), or O-alkylation (route d). Among these four routes, the reaction proceeded regioselectively via route a, and led to intermediate **7**. Then, enolization and protonation of **7** in the reaction conditions result in final product **4**.

Antibacterial activity

The synthesized compounds were evaluated for their antibacterial activity against two Gram-positive *S. aureus* and *B. subtilis* and two Gram-negative bacterial strains *E. coli* and *P. aeruginosa*. Standard antibacterial drug gentamicin and chloramphenicol were also tested under similar conditions against these organisms (Table 4). Compound **4e** (*p*-chlorobenzaldehyde derivative) demonstrated excellent activity against *S. aureus* and *B. subtilis*, even more than standard compounds. Except compound **4f** (*m*-chlorobenzaldehyde derivative), none of the compounds did show antibacterial activities against *P. aeruginosa*. Also, compounds **4e**, **4f**, **4h**,

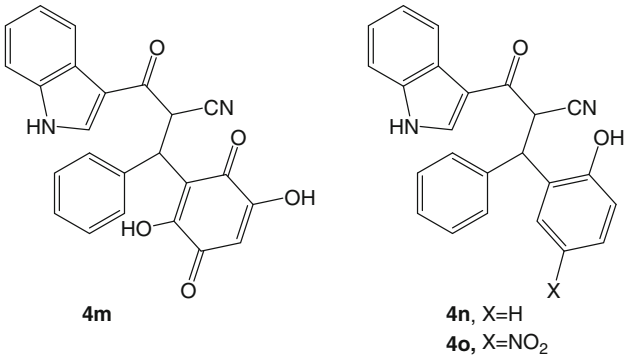


Fig. 1 The expected structures of the products **4m–4o**

4i, and **4j** (with Cl, Br, or NO_2 substituent) exhibited good activity against *E. coli*.

Experimental

All chemicals and reagents were purchased from Fluka and Merck and used without further purification. Melting points were measured on an Electrothermal 9100 apparatus. NMR spectra were recorded on a Bruker DRX-400 AVANCE instrument (400.1 MHz for ^{1}H, 100.6 MHz for ^{13}C) with DMSO-d_6 as solvent. Chemical shifts (δ) are given in parts per million (ppm) relative to TMS, and coupling constants (J) are reported in hertz (Hz). IR spectra were recorded on an FT-IR Bruker vector 22 spectrometer. Mass spectra were recorded on a Finnigan-Matt 8430 mass spectrometer operating at an ionization potential of 70 eV. Elemental analyses were carried on a Perkin-Elmer 2400II CHNS/O Elemental Analyzer. 3-(Cyanoacetyl)indole was prepared as reported in the literature [48].

General procedure for the synthesis of compounds ***4a–4j***

A mixture of 3-(cyanoacetyl)indole (1 mmol), benzaldehyde derivatives (1 mmol), kojic acid (1 mmol), and ammonium acetate (30 mol%) in 5 cm^3 ethanol was refluxed for appropriate time as indicated in Table 2. The completion of the reaction was indicated by the disappearance of the starting material in thin layer chromatography (EtOAc/*n*-hexane 18:2). After completion of the reaction, the product was recrystallized from methanol and washed with water.

3-[3-Hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-3-phenylpropanenitrile
(**4a**, $C_{24}H_{18}N_2O_5$)
White powder, 0.39 g (95 %); m.p.: 236-238 °C; $R_f = 0.4$ (*n*-hexane/ethyl acetate 1:9 v/v).

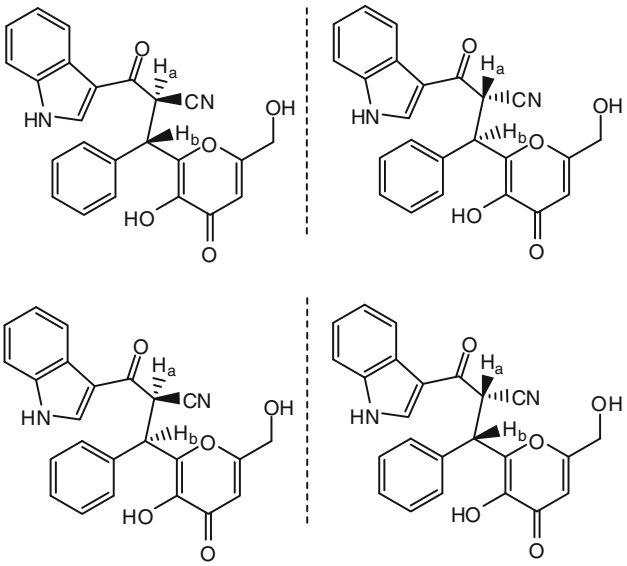
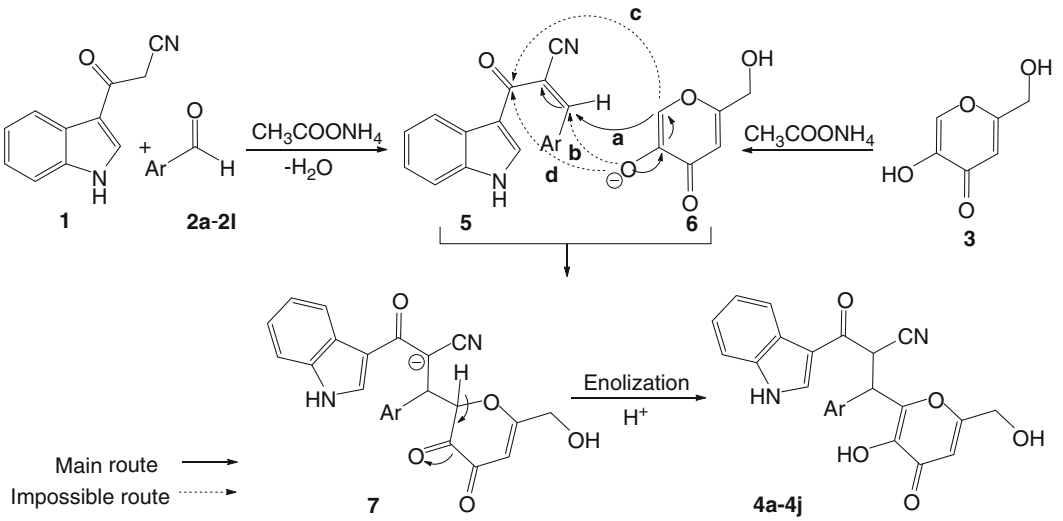


Fig. 2 Structures of two diastereomeric pairs of **4a** (I (RR/SS or RS/SR) and II (RR/SS or RS/SR))

4a-I (RR/SS or RS/SR): yield 56 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 4.07$ and 4.25 (2dd, AB system, 2H, $^{2}J_{HH} = 16.0$ Hz, $^{3}J_{HH} = 6.0$ Hz, CH_2), 5.00 (d, 1H, $^{3}J_{HH} = 11.6$ Hz, CH), 5.64 (t, 1H, $^{3}J_{HH} = 6.4$ Hz, OH), 5.74 (d, 1H, $^{3}J_{HH} = 11.6$ Hz, CH), 6.10 (s, 1H, CH_{kojic}), 7.16–7.50 (m, 7H, $7CH_{Ar}$), 7.61 (d, 1H, $^{3}J_{HH} = 7.2$ Hz, CH_{Ar}), 8.11 (d, 1H, $^{3}J_{HH} = 7.2$ Hz, CH_{Ar}), 8.91 (s, 1H, C*H*–NH), 9.28 (s, 1H, OH), 12.43 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 41.47$, 43.35 (2CH), 59.67 (CH_2), 109.26 (CH_{kojic}), 113.05 (CH_{indole}), 114.73, 118.10 ($2C_{indole}$), 121.63, 123.17, 124.19 ($3CH_{indole}$), 125.65 (C_{indole}), 128.29, 128.58, 129.19, 136.89 (5CH, C_{Ar}), 137.29 (CH_{indole}), 137.45 (CN), 142.14, 147.42, 167.30 ($3C_{kojic}$), 173.68, 182.66 (2CO) ppm.

4a-II (RR/SS or RS/SR): yield 39 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 4.44$ and 4.48 (2dd, AB system, 2H, $^{2}J_{HH} = 16.0$ Hz, $^{3}J_{HH} = 6.4$ Hz, CH_2), 5.18 (d, 1H, $^{3}J_{HH} = 10.8$ Hz, CH), 5.61 (d, 1H, $^{3}J_{HH} = 11.2$ Hz, CH), 5.79 (t, 1H, $^{3}J_{HH} = 6.4$ Hz, OH), 6.36 (s, 1H, CH_{kojic}), 7.16–7.50 (m, 7H, $7CH_{Ar}$), 7.52 (d, 1H, $^{3}J_{HH} = 7.6$ Hz, CH_{Ar}), 8.05 (d, 1H, $^{3}J_{HH} = 7.2$ Hz, CH_{Ar}), 8.75 (s, 1H, C*H*–NH), 9.43 (s, 1H, OH), 12.38 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 42.04$, 44.42 (2CH), 59.91 (CH_2), 109.38 (CH_{kojic}), 113.06 (CH_{indole}), 115.30, 118.33 ($2C_{indole}$), 121.68, 123.18, 124.20 ($3CH_{indole}$), 125.73 (C_{indole}), 128.30, 128.97, 129.36, 137.20 (5CH, C_{Ar}), 137.36 (CH_{indole}), 137.66 (CN), 142.45, 148.21, 168.17 ($3C_{kojic}$), 174.00, 183.55 (2CO) ppm; IR (KBr): $\bar{\nu} = 3{,}425$ (OH), 3,247 (NH), 2,250 (CN), 1,630 (C=O), 1,599 (C=C), 1,097 (C–O) cm^{-1}; MS (70 eV): *m/z* (%) = 414 ($M^{+\cdot}$, 8), 270 (M^{+}-

Scheme 2



C_9H_6NO, 29), 144 (M^+-$C_{15}H_{12}NO_4$, 100), 116 (M^+-$C_{16}H_{12}NO_5$, 66).

3-[3-Hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-3-(4-methoxyphenyl)-propanenitrile (**4b**, $C_{25}H_{20}N_2O_6$)

White powder, 0.40 g (91 %); m.p.: 232–234 °C; $R_f = 0.4$ (*n*-hexane/ethyl acetate 1:9 v/v).

4b-I (RR/SS or RS/SR): yield 53 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 3.77$ (s, 3H, CH_3), 4.04–4.26 (m, 2H, CH_2), 4.94 (d, 1H, $^3J_{HH} = 10.8$ Hz, CH), 5.55 (d, 1H, $^3J_{HH} = 10.8$ Hz, CH), 5.67–5.71 (m, 1H, OH), 6.09 (s, 1H, CH_{kojic}), 6.99 (d, 1H, $^3J_{HH} = 8.8$ Hz, CH_{Ar}), 7.19–7.53 (m, 6H, $6CH_{Ar}$), 8.09 (d, 1H, $^3J_{HH} = 7.6$ Hz, CH_{Ar}), 8.89 (s, 1H, C*H*–NH), 9.29 (s, 1H, OH), 12.42 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 41.54$, 42.66 (2CH), 55.42 (OCH_3), 59.69 (CH_2), 109.23 (CH_{kojic}), 113.04 (CH_{indole}), 114.53, 115.41 ($2C_{indole}$), 121.69, 123.17, 124.22 ($3CH_{indole}$), 125.66 (C_{indole}), 128.68, 129.59, 129.75, 130.11 (4CH, $2C_{Ar}$), 137.19 (CH_{indole}), 141.90 (CN), 148.57, 159.12, 167.20 ($3C_{kojic}$), 173.70, 182.78 (2CO) ppm.

4b-II (RR/SS or RS/SR): yield 38 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 3.62$ (s, 3H, CH_3), 4.39-4.50 (m, 2H, CH_2), 5.12 (d, 1H, $^3J_{HH} = 11.2$ Hz, CH), 5.70 (d, 1H, $^3J_{HH} = 11.6$ Hz, CH), 5.82 (t, 1H, $^3J_{HH} = 6.8$ Hz, OH), 6.35 (s, 1H, CH_{kojic}), 6.79 (d, 1H, $^3J_{HH} = 8.8$ Hz, CH_{Ar}), 7.19–7.53 (m, 6H, $6CH_{Ar}$), 8.06 (d, 1H, $^3J_{HH} = 7.2$ Hz, CH_{Ar}), 8.74 (s, 1H, C*H*–NH), 9.30 (s, 1H, OH), 12.41 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 42.30$, 43.66 (2CH), 55.59 (OCH_3), 59.92 (CH_2), 109.34 (CH_{kojic}), 113.05 (CH_{indole}), 114.68, 118.14 ($2C_{indole}$), 121.70, 123.18, 124.23 ($3CH_{indole}$), 125.67 (C_{indole}), 128.69, 129.60, 129.76, 130.12 (4CH, $2C_{Ar}$), 137.31 (CH_{indole}), 142.18 (CN), 148.58, 159.44, 168.08 ($3C_{kojic}$), 174.02, 182.79 (2CO) ppm; IR (KBr): $\bar{\nu} = 3{,}427$ (OH), 3,233 (NH), 2,259 (CN), 1,632 (C=O), 1,515 (C=C), 1,033 (C–O) cm^{-1}; MS (70 eV): *m/z* (%) = 444 ($M^{+\cdot}$, 5), 300 (M^+-C_9H_6NO, 27), 261 (M^+-$C_{11}H_7N_2O$, 25), 144 (M^+-$C_{16}H_{14}NO_5$, 100), 116 (M^+-$C_{17}H_{14}NO_6$, 20).

Table 4 Antibacterial activity of the complex using Kirby–Bauer technique (zone of growth inhibition/mm)

Product	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
4a	NE[a]	NE	15.0 ± 1.4	14.5 ± 1.4
4b	NE	NE	12.0 ± 1.4	14.0 ± 1.4
4c	NE	NE	13.0 ± 1.4	14.5 ± 1.4
4d	NE	NE	14.0 ± 1.4	12.5 ± 0.7
4e	11.5 ± 0.7	NE	22.0 ± 1.4	19.5 ± 0.7
4f	14.0 ± 1.4	9.5 ± 0.7	11.5 ± 0.7	14.0 ± 1.4
4g	NE	NE	11.0 ± 1.4	9.5 ± 0.7
4h	11.5 ± 0.7	NE	14.5 ± 0.7	17.0 ± 1.4
4i	13.0 ± 1.4	NE	14.0 ± 1.4	13.0 ± 0.7
4j	10.0 ± 1.4	NE	9.5 ± 0.7	12.5 ± 0.7
Gentamicin (10 μg/disc)	19.6 ± 1.1	15.6 ± 0.5	20.3 ± 1.5	26.0 ± 1.7
Chloramphenicol (30 μg/disc)	20.7 ± 1.5	NE	21.7 ± 0.6	22.3 ± 1.2

Concentration of compound: 20 mg cm^{-3}

Mueller–Hinton agar plate

[a] No effect

3-[3-Hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-3-(3-methoxyphenyl)-propanenitrile (**4c**, $C_{25}H_{20}N_2O_6$)

White powder, 0.35 g (80 %); m.p.: 211–213 °C; $R_f = 0.4$ (*n*-hexane/ethyl acetate 1:9 v/v).

4c-I (RR/SS or RS/SR): yield 47 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 3.81$ (s, 3H, CH_3), 4.08 and 4.24 (2dd, 2H, $^2J_{HH} = 16.0$ Hz, $^3J_{HH} = 5.6$ Hz, CH_2), 4.98 (d, 1H, $^3J_{HH} = 11.6$ Hz, CH), 5.64 (br s, 1H, OH), 5.74 (d, 1H, $^3J_{HH} = 12.0$ Hz, CH), 6.10 (s, 1H, CH_{kojic}), 6.73–7.29 (m, 6H, $6CH_{Ar}$), 7.51 (t, 1H, $^3J_{HH} = 8.4$ Hz, CH_{Ar}), 8.11 (d, 1H, $^3J_{HH} = 8.0$ Hz, CH_{Ar}), 8.92 (s, 1H, C*H*–NH), 9.28 (s, 1H, OH), 12.43 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 41.35$, 43.28 (2CH), 55.46 (CH_3), 59.71 (CH_2), 109.30 (CH_{kojic}), 113.06 (CH_{indole}), 113.45, 114.31 ($2C_{indole}$), 114.93, 118.07, 120.94 ($3CH_{indole}$), 121.64 (C_{indole}), 123.19, 124.20, 125.66, 130.26, 137.25, 137.36 (4CH, $2C_{Ar}$), 138.23 (CH_{indole}), 139.00 (CN), 147.28, 159.69, 167.24 ($3C_{kojic}$), 173.68, 182.66 (2CO) ppm.

4c-II (RR/SS or RS/SR): yield 33 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 3.66$ (s, 3H, CH_3), 4.40–4.51 (m, 2H, CH_2), 5.16 (d, 1H, $^3J_{HH} = 11.2$ Hz, CH), 5.61 (d, 1H, $^3J_{HH} = 11.2$ Hz, CH), 5.79 (t, 1H, $^3J_{HH} = 6.4$ Hz, OH), 6.37 (s, 1H, CH_{kojic}), 6.73–7.29 (m, 6H, $6CH_{Ar}$), 7.36 (t, 1H, $^3J_{HH} = 7.6$ Hz, CH_{Ar}), 8.07 (d, 1H, $^3J_{HH} = 8.0$ Hz, CH_{Ar}), 8.77 (s, 1H, C*H*–NH), 9.46 (s, 1H, OH), 12.40 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 41.93$, 44.33 (2CH), 55.60 (CH_3), 59.94 (CH_2), 109.43 (CH_{kojic}), 113.07 (CH_{indole}), 113.54, 114.73 ($2C_{indole}$), 115.33, 118.34, 121.26 ($3CH_{indole}$), 121.68 (C_{indole}), 123.20, 124.24, 125.72, 130.43, 137.29, 137.48 (4CH, $2C_{Ar}$), 138.24 (CH_{indole}), 139.01 (CN), 148.11, 159.95, 168.09 ($3C_{kojic}$), 174.01, 183.53 (2CO) ppm; IR (KBr): $\bar{\nu} = 3{,}421$ (OH), 3,224 (NH), 2,261 (CN), 1,633 (C=O), 1,596 (C=C), 1,042 (C–O) cm^{-1}; MS (70 eV): *m/z* (%) = 444 ($M^{+\cdot}$, 2), 262 (M^+-$C_{11}H_6N_2O$, 96), 144 (M^+-$C_{16}H_{14}NO_5$, 100), 116 (M^+-$C_{17}H_{14}NO_6$, 34).

3-[3-Hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-3-(4-methylphenyl)propanenitrile (**4d**, $C_{25}H_{20}N_2O_5$)

White powder, 0.39 g (92 %); m.p.: 233–235 °C; $R_f = 0.4$ (*n*-hexane/ethyl acetate 1:9 v/v).

4d-I (RR/SS or RS/SR): yield 55 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 2.32$ (s, 3H, CH_3), 4.03–4.27 (m, 2H, CH_2), 4.96 (d, 1H, $^3J_{HH} = 11.2$ Hz, CH), 5.59 (d, 1H, $^3J_{HH} = 11.2$ Hz, CH), 5.67 (t, 1H, $^3J_{HH} = 6.4$ Hz, OH), 6.09 (s, 1H, CH_{kojic}), 7.04–7.53 (m, 7H, $7CH_{Ar}$), 8.10 (d, 1H, $^3J_{HH} = 7.2$ Hz, CH_{Ar}), 8.90 (s, 1H, C*H*–NH), 9.27 (s, 1H, OH), 12.43 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 20.97$ (CH_3), 42.03, 44.00 (2CH), 59.65 (CH_2), 109.21 (CH_{kojic}), 113.04 (CH_{indole}), 114.72, 118.13 ($2C_{indole}$), 121.67, 123.21, 124.27 ($3CH_{indole}$), 125.62 (C_{indole}), 128.44, 129.75, 133.78, 134.60 (4CH, $2C_{Ar}$), 137.28 (CH_{indole}), 137.60 (CN), 141.93, 147.61, 167.27 ($3C_{kojic}$), 173.66, 182.66 (2CO) ppm.

4d-II (RR/SS or RS/SR): yield 37 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 2.14$ (s, 3H, CH_3), 4.41 and 4.47 (2dd, AB system, 2H, $^2J_{HH} = 16.0$ Hz, $^3J_{HH} = 6.0$ Hz, CH_2), 5.15 (d, 1H, $^3J_{HH} = 10.8$ Hz, CH), 5.71 (d, 1H, $^3J_{HH} = 11.6$ Hz, CH), 5.82 (t, 1H, $^3J_{HH} = 6.8$ Hz, OH), 6.35 (s, 1H, CH_{kojic}), 7.04–7.53 (m, 7H, $7CH_{Ar}$), 8.05 (d, 1H, $^3J_{HH} = 7.2$ Hz, CH_{Ar}), 8.77 (s, 1H, C*H*–NH), 9.28 (s, 1H, OH), 12.42 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 21.16$ (CH_3), 42.95, 44.07 (2CH), 59.89 (CH_2), 109.33 (CH_{kojic}), 113.05 (CH_{indole}), 115.37, 118.38 ($2C_{indole}$), 121.68, 123.22, 124.28 ($3CH_{indole}$), 125.69 (C_{indole}), 128.81, 129.91, 133.79, 134.61 (4CH, $2C_{Ar}$), 137.29 (CH_{indole}), 137.90 (CN), 141.94, 148.41, 168.14 ($3C_{kojic}$), 174.00, 183.65 (2CO) ppm; IR (KBr): $\bar{\nu} = 3{,}425$ (OH), 3,248 (NH), 2,260 (CN), 1,632 (C=O), 1,519 (C=C), 1,036 (C–O) cm^{-1}; MS: *m/z* (%) = 428 ($M^{+\cdot}$, 8), 284 (M^+-C_9H_6NO, 43), 245 (M^+-$C_{11}H_7N_2O$, 19), 144 (M^+-$C_{16}H_{14}NO_4$, 100), 116 (M^+-$C_{17}H_{14}NO_5$, 48).

3-(4-Chlorophenyl)-3-[3-hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)propanenitrile (**4e**, $C_{27}H_{17}ClN_2O_5$)

White powder, 0.41 g (92 %); m.p.: 217–219 °C; $R_f = 0.4$ (*n*-hexane/ethyl acetate 1:9 v/v).

4e-I (RR/SS or RS/SR): yield 55 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 4.05$–4.28 (m, 2H, CH_2), 5.04 (d, 1H, $^3J_{HH} = 11.6$ Hz, CH), 5.77 (d, 1H, $^3J_{HH} = 11.6$ Hz, CH), 5.69 (br s, 1H, OH), 6.13 (s, 1H, CH_{kojic}), 7.21–7.67 (m, 7H, $7CH_{Ar}$), 8.11 (d, 1H, $^3J_{HH} = 7.6$ Hz, CH_{Ar}), 8.89 (s, 1H, C*H*–NH), 9.39 (s, 1H, OH), 12.45 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 41.42$, 42.70 (2CH), 59.66 (CH_2), 109.31 (CH_{kojic}), 113.08 (CH_{indole}), 114.69, 117.94 ($2C_{indole}$), 121.62, 123.23, 124.25 ($3CH_{indole}$), 125.61 (C_{indole}), 129.23, 130.46, 130.87, 133.08 (4CH, $2C_{Ar}$), 135.81 (CH_{indole}), 137.36 (CN), 142.23, 146.92, 167.42 ($3C_{kojic}$), 173.70, 182.44 (2CO) ppm.

4e-II (RR/SS or RS/SR): yield 37 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): $\delta = 4.41$–4.51 (m, 2H, CH_2), 5.20 (d, 1H, $^3J_{HH} = 10.8$ Hz, CH), 5.63 (d, 1H, $^3J_{HH} = 10.8$ Hz, CH), 5.84 (br s, 1H, OH), 6.39 (s, 1H, CH_{kojic}), 7.21–7.67 (m, 7H, $7CH_{Ar}$), 8.07 (d, 1H, $^3J_{HH} = 7.6$ Hz, CH_{Ar}), 8.76 (s, 1H, C*H*–NH), 9.55 (s, 1H, OH), 12.44 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): $\delta = 41.88$, 43.76 (2CH), 59.89 (CH_2), 109.44 (CH_{kojic}), 113.09 (CH_{indole}), 115.22, 118.23 ($2C_{indole}$), 121.67, 123.28, 124.32 ($3CH_{indole}$), 125.69 (C_{indole}),

129.37, 130.47, 130.88, 133.40 (4CH, $2C_{Ar}$), 136.57 (CH_{indole}), 137.43 (CN), 142.54, 147.65, 168.27 ($3C_{kojic}$), 174.03, 183.27 (2CO) ppm; IR (KBr): $\overline{\nu}$ = 3,417 (OH), 3,239 (NH), 2,259 (CN), 1,632 (C=O), 1,594 (C=C), 1,035 (C–O) cm^{-1}; MS (70 eV): *m/z* (%) = 448 ($M^{+\cdot}$, 9), 450 ($M^{+\cdot}$+2, 3), 304 (M^{+}-C_9H_6NO, 26), 306 (M^{+}+2-C_9H_6NO, 14), 265 (M^{+}-$C_{11}H_7N_2O$, 14), 267 (M^{+}+2-$C_{11}H_7N_2O$, 6), 144 (M^{+}-$C_{15}H_{11}ClNO_4$, 100), 116 (M^{+}-$C_{16}H_{11}ClNO_5$, 33).

3-(3-Chlorophenyl)-3-[3-hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-propanenitrile (**4f**, $C_{27}H_{17}ClN_2O_5$)

White powder, 0.40 g (91 %); m.p.: 210–212 °C; R_f = 0.4 (*n*-hexane/ethyl acetate 1:9 v/v).

4f-I (RR/SS or RS/SR): yield 58 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.07 and 4.25 (2dd, AB system, 2H, $^2J_{HH}$ = 16.0 Hz, $^3J_{HH}$ = 6.0 Hz, CH_2), 5.04 (d, 1H, $^3J_{HH}$ = 11.6 Hz, CH), 5.65 (t, 1H, $^3J_{HH}$ = 6.4 Hz, OH), 5.81 (d, 1H, $^3J_{HH}$ = 11.6 Hz, CH), 6.13 (s, 1H, CH_{kojic}), 7.20–7.76 (m, 7H, $7CH_{Ar}$), 8.11 (d, 1H, $^3J_{HH}$ = 7.2 Hz, CH_{Ar}), 8.94 (d, 1H, $^3J_{HH}$ = 2.8 Hz, C*H*–NH), 9.42 (s, 1H, OH), 12.46 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.36, 42.86 (2CH), 59.68 (CH_2), 109.37 (CH_{kojic}), 113.10 (CH_{indole}), 114.70, 117.90 ($2C_{indole}$), 121.62, 123.22, 124.24 ($3CH_{indole}$), 125.64 (C_{indole}), 127.45, 128.33, 128.58, 131.12, 133.72, 137.31 (4CH, $2C_{Ar}$), 137.55 (CH_{indole}), 139.22 (CN), 142.36, 146.70, 167.40 ($3C_{kojic}$), 173.69, 182.34 (2CO) ppm.

4f-II (RR/SS or RS/SR): yield 33 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.45–4.52 (m, 2H, CH_2), 5.20 (d, 1H, $^3J_{HH}$ = 10.8 Hz, CH), 5.67 (d, 1H, $^3J_{HH}$ = 10.8 Hz, CH), 5.80 (br s, 1H, OH), 6.39 (s, 1H, CH_{kojic}), 7.20–7.76 (m, 7H, $7CH_{Ar}$), 8.07 (d, 1H, $^3J_{HH}$ = 7.6 Hz, CH_{Ar}), 8.79 (d, 1H, $^3J_{HH}$ = 2.8 Hz, C*H*–NH), 9.59 (s, 1H, OH), 12.44 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.65, 44.00 (2CH), 59.91 (CH_2), 109.51 (CH_{kojic}), 113.11 (CH_{indole}), 115.14, 118.21 ($2C_{indole}$), 121.67, 123.27, 124.30 ($3CH_{indole}$), 125.71 (C_{indole}), 128.00, 128.45, 128.73, 131.26, 133.97, 137.37 (4CH, $2C_{Ar}$), 137.56 (CH_{indole}), 139.94 (CN), 142.68, 147.42, 168.24 ($3C_{kojic}$), 174.00, 183.15 (2CO) ppm; IR (KBr): $\overline{\nu}$ = 3,481 (OH), 3,293 (NH), 2,248 (CN), 1,633 (C=O), 1,579 (C=C), 1,088 (C–O) cm^{-1}; MS: *m/z* (%) = 448 ($M^{+\cdot}$, 3), 450 ($M^{+\cdot}$+2, 1), 304 ($M^{+\cdot}$-C_9H_6NO, 8), 306 ($M^{+\cdot}$+2-C_9H_6NO, 9), 144 (M^{+}-$C_{15}H_{11}ClNO_4$, 100), 116 (M^{+}-$C_{16}H_{11}ClNO_5$, 30).

3-[3-Hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-3-(4-isocyanophenyl)-propanenitrile (**4g**, $C_{25}H_{17}N_3O_5$)

White powder, 0.42 g (97 %); m.p.: 235–237 °C; R_f = 0.4 (*n*-hexane/ethyl acetate 1:9 v/v).

4g-I (RR/SS or RS/SR): yield 60 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.06 and 4.24 (2dd, AB system, 2H, $^2J_{HH}$ = 16.0 Hz, $^3J_{HH}$ = 6.4 Hz, CH_2), 5.11 (d, 1H, $^3J_{HH}$ = 11.6 Hz, CH), 5.64 (t, 1H, $^3J_{HH}$ = 6.4 Hz, OH), 5.82 (d, 1H, $^3J_{HH}$ = 11.6 Hz, CH), 6.12 (s, 1H, CH_{kojic}), 7.20–7.30 (m, 2H, $2CH_{Ar}$), 7.50–7.55 (m, 1H, CH_{Ar}), 7.84 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{Ar}$), 7.95 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{Ar}$), 8.10 (d, 1H, $^3J_{HH}$ = 8.0 Hz, CH_{Ar}), 8.90 (s, 1H, C*H*–NH), 9.47 (s, 1H, OH), 12.45 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.23, 43.08 (2CH), 59.63 (CH_2), 109.37 (CH_{kojic}), 111.29 (CH_{indole}), 113.11, 114.64 ($2C_{indole}$), 117.78, 118.77, 121.59 ($3CH_{indole}$), 123.25 (C_{indole}), 124.27, 125.61, 129.62, 133.20 (4CH, $2C_{Ar}$), 137.30 (CH_{indole}), 137.51, 142.33 (2 CN), 142.87, 146.21, 167.51 ($3C_{kojic}$), 173.66, 182.13 (2CO) ppm.

4g-II (RR/SS or RS/SR): yield 37 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.43–4.46 (m, 2H, CH_2), 5.27 (d, 1H, $^3J_{HH}$ = 10.8 Hz, CH), 5.69 (d, 1H, $^3J_{HH}$ = 10.8 Hz, CH), 5.79 (t, 1H, $^3J_{HH}$ = 6.4 Hz, OH), 6.37 (s, 1H, CH_{kojic}), 7.20–7.30 (m, 2H, $2CH_{Ar}$), 7.50–7.55 (m, 1H, CH_{Ar}), 7.64 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{Ar}$), 7.76 (d, 2H, $^3J_{HH}$ = 8.4 Hz, $2CH_{Ar}$), 8.04 (d, 1H, $^3J_{HH}$ = 8.0 Hz, CH_{Ar}), 8.77 (s, 1H, C*H*–NH), 9.63 (s, 1H, OH), 12.44 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.44, 44.25 (2CH), 59.86 (CH_2), 109.49 (CH_{kojic}), 111.57 (CH_{indole}), 113.12, 115.00 ($2C_{indole}$), 118.06, 118.99, 121.65 ($3CH_{indole}$), 123.29 (C_{indole}), 124.33, 125.70, 130.10, 133.34 (4CH, $2C_{Ar}$), 137.37 (CH_{indole}), 137.52, 142.54 (2CN), 142.96, 146.90, 168.35 ($3C_{kojic}$), 173.97, 182.92 (2CO) ppm; IR (KBr): $\overline{\nu}$ = 3,221 (OH, NH), 2,231 (CN), 1,656, 1,626 (C=O), 1,585 (C=C), 1,042 (C–O) cm^{-1}; MS: *m/z* (%) = 439 ($M^{+\cdot}$, 3), 295 (M^{+}-C_9H_6NO, 7), 144 (M^{+}-$C_{16}H_{11}N_2O_4$, 100), 116 (M^{+}-$C_{17}H_{11}N_2O_5$, 21).

3-(4-Bromophenyl)-3-[3-hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-propanenitrile (**4h**, $C_{24}H_{17}BrN_2O_5$)

White powder, 0.45 g (93 %); m.p.: 223–225 °C; R_f = 0.4 (*n*-hexane/ethyl acetate 1:9 v/v).

4h-I (RR/SS or RS/SR): yield 56 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.06 and 4.25 (2d, AB system, 2H, $^2J_{HH}$ = 15.2 Hz, CH_2), 5.01 (d, 1H, $^3J_{HH}$ = 9.2 Hz, CH), 5.78 (br s, 2H, 1CH, OH), 6.11 (s, 1H, CH_{kojic}), 7.21–7.69 (m, 7H, $7CH_{Ar}$), 8.10 (d, 1H, $^3J_{HH}$ = 7.6 Hz, CH_{Ar}), 8.89 (s, 1H, C*H*–NH), 9.38 (s, 1H, OH), 12.44 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.30, 42.69 (2CH), 59.65 (CH_2), 109.31 (CH_{kojic}), 113.09 (CH_{indole}), 114.69, 117.92 ($2C_{indole}$), 121.65, 123.25, 124.30 ($3CH_{indole}$), 125.63 (C_{indole}), 130.78, 131.20, 132.15, 136.27 (4CH, $2C_{Ar}$), 137.03 (CH_{indole}), 137.30 (CN), 142.25, 146.84, 167.39 ($3C_{kojic}$), 173.68, 182.39 (2CO) ppm.

4h-II (RR/SS or RS/SR): yield 37 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.38–4.53 (m, 2H, CH_2),

5.18 (d, 1H, $^{3}J_{HH}$ = 10.8 Hz, CH), 5.62 (d, 1H, $^{3}J_{HH}$ = 10.8 Hz, CH), 5.60–5.63 (m, 1H, OH), 6.37 (s, 1H, CH_{kojic}), 7.21–7.69 (m, 7H, $7CH_{Ar}$), 8.06 (d, 1H, $^{3}J_{HH}$ = 7.6 Hz, CH_{Ar}), 8.77 (s, 1H, C*H*–NH), 9.39 (s, 1H, OH), 12.43 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.76, 43.81 (2CH), 59.88 (CH_2), 109.42 (CH_{kojic}), 113.10 (CH_{indole}), 115.19, 118.23 ($2C_{indole}$), 121.96, 123.26, 124.31 ($3CH_{indole}$), 125.72 (C_{indole}), 130.79, 131.21, 132.28, 136.28 (4CH, $2C_{Ar}$), 137.04 (CH_{indole}), 137.31 (CN), 142.56, 147.54, 168.26 ($3C_{kojic}$), 174.00, 183.22 (2CO) ppm; IR (KBr): $\bar{\nu}$ = 3,414 (OH), 3,239 (NH), 2,258 (CN), 1,632 (C=O), 1,593 (C=C), 1,034 (C–O) cm^{-1}; MS: *m/z* (%) = 492 ($M^{+\cdot}$, 1), 494 ($M^{+\cdot}$+2, 1), 308 (M^{+}-$C_{11}H_7N_2O$, 15), 310 (M^{+}+2-$C_{11}H_7N_2O$, 19), 144 (M^{+}-$C_{15}H_{11}BrNO_4$, 100), 116 (M^{+}-$C_{16}H_{11}BrNO_5$, 23).

3-(2-Bromophenyl)-3-[3-hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-propanenitrile (**4i**, $C_{24}H_{17}BrN_2O_5$)

White powder, 0.44 g (90 %); m.p.: 219–221 °C; R_f = 0.4 (*n*-hexane/ethyl acetate 1:9 v/v).

4i-I (RR/SS or RS/SR): yield 58 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.05 and 4.25 (2dd, AB system, 2H, $^{2}J_{HH}$ = 16.0 Hz, $^{3}J_{HH}$ = 6.0 Hz, CH_2), 5.63–5.77 (m, 3H, 2CH, OH), 6.12 (s, 1H, CH_{kojic}), 7.12–8.28 (m, 8H, $8CH_{Ar}$), 8.86 (s, 1H, C*H*–NH), 9.29 (s, 1H, OH), 12.42 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 42.03, 42.28 (2CH), 59.65 (CH_2), 109.23 (CH_{kojic}), 113.07 (CH_{indole}), 114.68, 117.66 ($2C_{indole}$), 121.68, 123.13, 124.22 ($3CH_{indole}$), 125.69 (C_{indole}), 128.42, 129.90, 130.48, 133.19, 133.55, 136.02 (4CH, $2C_{Ar}$), 137.03 (CH_{indole}), 137.28 (CN), 142.78, 146.34, 167.52 ($3C_{kojic}$), 173.71, 182.29 (2CO) ppm.

4i-II (RR/SS or RS/SR): yield 32 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.35 and 4.43 (2dd, AB system, 2H, $^{2}J_{HH}$ = 16.0 Hz, $^{3}J_{HH}$ = 6.0 Hz, CH_2), 5.63–5.77 (m, 3H, 2CH, OH), 6.36 (s, 1H, CH_{kojic}), 7.12–8.28 (m, 8H, $8CH_{Ar}$), 8.73 (s, 1H, C*H*–NH), 9.43 (s, 1H, OH), 12.37 (s, 1H, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 42.21, 42.85 (2CH), 59.68 (CH_2), 109.24 (CH_{kojic}), 113.11 (CH_{indole}), 114.98, 117.86 ($2C_{indole}$), 121.84, 123.19, 124.28 ($3CH_{indole}$), 125.73 (C_{indole}), 128.74, 130.18, 130.76, 133.50, 133.65, 136.89 (4CH, $2C_{Ar}$), 137.22 (CH_{indole}), 137.35 (CN), 143.14, 146.37, 168.40 ($3C_{kojic}$), 174.08, 183.07 (2CO) ppm; IR (KBr): $\bar{\nu}$ = 3,426 (OH), 3,242 (NH), 2,257 (CN), 1,655, 1,626 (C=O), 1,599 (C=C), 1,036 (C–O) cm^{-1}; MS: *m/z* (%) = 492 (M^{+}, 4), 494 ($M^{+\cdot}$+2, 4), 347 (M^{+}-C_9H_6NO, 12), 349 (M^{+}+2-C_9H_6NO, 14), 144 (M^{+}-$C_{15}H_{11}BrNO_4$, 100), 116 (M^{+}-$C_{16}H_{11}BrNO_5$, 10).

3-[3-Hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]-2-(1H-indol-3-ylcarbonyl)-3-(4-nitrophenyl)propanenitrile (**4j**, $C_{24}H_{17}N_3O_7$)

White powder, 0.44 g (97 %); m.p.: 263–265 °C; R_f = 0.4 (*n*-hexane/ethyl acetate 1:9 v/v).

4j-I (RR/SS or RS/SR): yield 57 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.06 and 4.26 (2dd, 2H, $^{2}J_{HH}$ = 16.0 Hz, $^{3}J_{HH}$ = 6.0 Hz, CH_2), 5.19 (d, 1H, $^{3}J_{HH}$ = 11.6 Hz, CH), 5.64 (t, 1H, $^{3}J_{HH}$ = 6.4 Hz, OH), 5.87 (d, 1H, $^{3}J_{HH}$ = 11.6 Hz, CH), 6.13 (s, 1H, CH_{kojic}), 7.26–7.31 (m, 2H, $2CH_{Ar}$), 7.54 (d, 1H, $^{3}J_{HH}$ = 6.4 Hz, CH_{Ar}), 7.93 (d, 2H, $^{3}J_{HH}$ = 8.8 Hz, $2CH_{Ar}$), 8.11 (d, 1H, $^{3}J_{HH}$ = 7.2 Hz, CH_{Ar}), 8.32 (d, 1H, $^{3}J_{HH}$ = 8.8 Hz, CH_{Ar}), 8.91 (d, 1H, $^{3}J_{HH}$ = 3.2 Hz, C*H*–NH), 9.50 (s, 1H, OH), 12.46 (d, 1H, $^{3}J_{HH}$ = 3.2 Hz, NH) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 41.24, 42.81 (2CH), 59.63 (CH_2), 109.36 (CH_{kojic}), 113.12 (CH_{indole}), 114.63, 117.73 ($2C_{indole}$), 121.59, 123.25, 124.37 ($3CH_{indole}$), 125.62 (C_{indole}), 129.96, 130.46, 137.20, 137.38 (4CH, $2C_{Ar}$), 142.62 (CH_{indole}), 144.26 (CN), 146.00, 147.49, 167.57 ($3C_{kojic}$), 173.65, 182.03 (2CO) ppm.

4j-II (RR/SS or RS/SR): yield 40 %; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 4.44–4.50 (m, 2H, CH_2), 5.34 (d, 1H, $^{3}J_{HH}$ = 10.8 Hz, CH), 5.74 (d, 1H, $^{3}J_{HH}$ = 10.8 Hz, CH), 5.79 (t, 1H, $^{3}J_{HH}$ = 6.8 Hz, OH), 6.38 (s, 1H, CH_{kojic}), 7.26–7.31 (m, 2H, $2CH_{Ar}$), 7.51 (d, 1H, $^{3}J_{HH}$ = 7.6 Hz, CH_{Ar}), 7.74 (d, 2H, $^{3}J_{HH}$ = 8.8 Hz, $2CH_{Ar}$), 8.04 (d, 1H, $^{3}J_{HH}$ = 7.2 Hz, CH_{Ar}), 8.14 (d, 2H, $^{3}J_{HH}$ = 8.8 Hz, $2CH_{Ar}$), 8.80 (d, 1H, $^{3}J_{HH}$ = 3.2, C*H*–NH), 9.67 (s, 1H, OH), 12.44 (d, 1H, $^{3}J_{HH}$ = 2.8 Hz, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 41.43, 44.01 (2CH), 59.85 (CH_2), 109.48 (CH_{kojic}), 113.13 (CH_{indole}), 114.98, 118.03 ($2C_{indole}$), 121.65, 124.27, 124.49 ($3CH_{indole}$), 125.71 (C_{indole}), 129.97, 130.47, 137.31, 137.52 (4CH, $2C_{Ar}$), 142.94 (CH_{indole}), 144.87 (CN), 146.69, 147.82, 168.42 ($3C_{kojic}$), 173.96, 182.85 (2CO) ppm; IR (KBr): $\bar{\nu}$ = 3,222 (NH, OH), 2,252 (CN), 1,656, 1,626 (C=O), 1,586 (C=C), 1,044 (C–O) cm^{-1}; MS: *m/z* (%) = 459 ($M^{+\cdot}$, 1), 315 ($M^{+\cdot}$-C_9H_6NO, 3), 144 (M^{+}-$C_{15}H_{11}N_2O_6$, 100), 116 (M^{+}-$C_{16}H_{11}N_2O_7$, 21).

General procedure for evaluation of antibacterial activity

Antibacterial activities of the synthesized 3-(cyanoacetyl)indole derivatives **4a–j** were assayed using Kirby–Bauer disc diffusion method, where a filter disc was impregnated with the compounds and placed on the surface of inoculated agar plates [49]. The synthesized compounds were dissolved into DMSO to achieve 20 mg cm^{-3} solution then filter sterilized using a 0.22-μm Ministart (Sartorius).

The antibacterial activities of the synthesized compounds were investigated against four bacterial species. Test organisms included *E. coli* PTCC 1330, *P. aeruginosa* PTCC 1074, *S. aureus* ATCC 35923, and *B. subtilis* PTCC 102 [50]. Late exponential phase of the bacteria was prepared by inoculating 1 % (v/v) of the cultures into the fresh Muller–Hinton broth (Merck) and incubating in an orbital shaker at 37 °C and 100 rpm overnight. Before using the cultures, they were standardized with a final cell density of approximately 10^8 cfu cm^{-3}. Muller–Hinton agar (Merck) was prepared and inoculated with the standardized cultures of the test organisms and then spread as uniformly as possible throughout the entire media. Sterile paper discs (6 mm diameter, Padtan, Iran) were impregnated with 20 mm^3 of the compound solution then allowed to dry. The impregnated disc was introduced on the upper layer of the seeded agar plate and incubated at 37 °C for 24 h. The antibacterial activities of the synthesized compounds were compared with known antibiotic gentamicin (10 μg/disc) and chloramphenicol (30 μg/disc) as positive control and DMSO (20 mm^3/disc) as negative control. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (mm) on the surface of plates, and the results were reported as mean ± SD after three repeats.

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