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Synthesis of New Antimicrobial Agents; Amide Derivatives of Pyranones and Pyridinones

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In this study, 4(1*H*)-pyridinones with an amide function in Position 2 of the pyridinone ring showed antimicrobial activity. The synthesis and the biological properties of some novel amide derivatives of pyran-4(1*H*)-one and 4(1*H*)-pyridinone have been reported. Amide derivatives of pyran-4(1*H*)-one and 4(1*H*)-pyridinone were prepared using two different methods. Antimicrobial activities were determined as MIC values using the microdilution broth method against the bacteria *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* and the fungi *Candida albicans*, *Candida krusei* and *Candida parapsilosis*. Compounds **6** and **9** exhibited the best inhibitor activity against *S. aureus*.

Key Words: Pyran-4(1*H*)-one, 4(1*H*)-pyridinone, antibacterial activity, antifungal agents.

Introduction

Some natural antibiotics contain a siderophore structure, e.g. albomycin. Most siderophores contain either hydroxamate or catechol groups, which are used to sequester iron[1]. A related structure, 3-hydroxypyranone, is present in kojic acid (5-hydroxy-2-hydroxymethyl-pyran-4(1*H*)-one), derivatives of which have been reported to exhibit antimicrobial activity[2,3]. This chelating ability is considered to play a significant role in this activity[4]. *In vitro* antibacterial and antifungal activities of hydroxy-4(1*H*)-pyridinone derivatives have been described[5,6]. A novel cephalosporin derivative with a 1,5-dihydroxy-4-pyridone-2-carboxyamide

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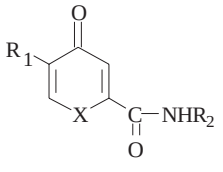
moiety, MT0703, displays excellent antibacterial activity against *P. aeruginosa* as well as *E. coli*, in both *in vitro* and *in vivo* conditions[7]. In addition, aminothiazolyloxyimino cephalosporins possessing 2-(5-hydroxy-4-pyridon-2-yl)ethenyl groups showed strong activity against *P. aeruginosa*[8]. The pyridone ring, which is a side chain of cephalosporin, plays a significant role in anti-pseudomonal activity[7,8].

In this study, we aimed to synthesize 5-benzyloxy-2-substituted-pyran-4(1*H*)-one (**4**,**7**), 5-benzyloxy-1-methyl-2-substituted-4(1*H*)-pyridinone (**5**,**8**) and 5-hydroxy-1-methyl-2-substituted-4(1*H*)-pyridinone (**6**,**9**) derivatives which have a pyridone ring as antimicrobial compounds. Related studies are given in the literature[7,8] (Figure).



R_1 = -$OCH_2C_6H_5$ or -OH
R_2 = -C_6H_5, 3-pyridyl
X = O or N-CH_3

Figure. Schematic representation of the synthesized of pyran-4(1*H*)-ones and 4(1*H*)-pyridinones derivatives (**4-9**).

Experimental

Chemistry

Kojic acid and the other chemicals were supplied from Across and Aldrich (England) and Fluka (England). Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus and were uncorrected. The IR spectra were recorded with a Perkin Elmer FT-IR Spectrometer 1720 X as a KBr disc (γ, cm^{-1}). ^{1}H-NMR spectra (DMSO-d_6) were recorded on a R24B Perkin-Elmer 60 MHz NMR and a Bruker AMX 400 MHz/ 52 MM NMR Spectrometer using TMS as an internal standard (chemical shift in δ, ppm). ^{13}C-NMR spectra were recorded on a Bruker AMX 400 MHz/ 52 MM NMR Spectrometer. Fast atom bombardment (FAB) mass spectra analyses were carried out by the Mass Spectrometry Facility, Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX. Mass spectra were recorded via VG Analytical ZAB-SE with Matrix m-nitrobenzylalcohol+Sodium and Xenon Gas @ 8 KV. Elemental analyses were performed by the Microanalytical Laboratories, Department of Chemistry, University of Manchester, Manchester, M13 9PL and the Scientific and Technical Research Council of Turkey (TÜBİTAK) (Leco CHNS-932 Elementary Analyzer). Elementary analyses for C, H and N are within ± 0.4% of the theoretical values.

5-Benzyloxy-2-hydroxymethyl-pyran-4(1*H*)-one (benzyl kojic acid) **(1)** was prepared using the procedure previously described by Thomas[9]: 82% yield as white needles, mp 130-131 °C (lit.[10], mp 131-133 °C).

5-Benzyloxy-pyran-4(1*H*)-one-2-carboxylic acid (benzyl comenic acid) (2).

1 (10.00 g, 43.2 mmol) was dissolved in acetone (500 mL) and cooled in an ice-bath to which was added Jones reagent (25 mL). The resulting brown mixture was left to stir for 1 h. The organic material was removed by filtration and the filtrate was evaporated to dryness. A pure compound (7.20 g, 68%) was obtained by recrystallization from methanol, mp 195-196 °C (lit.[11], mp 195-197 °C).

5-Benzyloxy-2-(2-thioxo-thiazolidine-3-carbonyl)-pyran-4(1*H*)-one (3).

2 (2.00 g, 8.13 mmol) was dissolved in dichloromethane (100 mL), and DCCI (1.84 g, 8.94 mmol) was added slowly. To this mixture, 2-mercapto-1,3-thiazolidine (1.06 g, 8.94 mmol) and a catalytic amount of DMAP (50 mg, 0.41 mmol) were added, and the colour of the reaction mixture turned a characteristic yellow. The mixture was stirred overnight at room temperature. The white precipitate, N,N′-dicyclohexylurea (DCU), was separated by filtration and the filtrate volume was made up to 200 mL by the addition of dichloromethane. The organic layer was washed three times with 0.1 M sodium hydroxide solution (100 mL) and water (100 mL), dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo* to yield the product as yellow oil. Column chromatography on silica gel (eluant: EtOAc) yielded the pure product as a bright yellow oil (2.00 g, 71%). IR (KBr) 1679 (amide C=O), 1646 (pyranone C=O), 1600 (pyranone C=C). ^{1}H-NMR ($CDCl_3$,60 MHz) δ 3.10 (t, 2H, J = 6.0 Hz, -CH_2N-), 4.35 (t, 2H, J = 6.0 Hz, -CH_2S-), 5.10 (s, 2H, -CH_2Ph), 6.30 (s, 1H, H^3), 7.30 (s, 5H, -CH_2*Ph*), 8.00 (s, 1H, H^6).

5-Benzyloxy-4-oxo-4H-pyran-2-carboxylic acid phenylamide (4).

Aniline (1.07 g, 10 mmol) was added into a solution in dichloromethane (100 mL) of **3** (2.00 g, 5.76 mmol), and the reaction mixture was refluxed overnight. The solution was washed with 0.1 M sodium hydroxide solution (3 x 50 mL) and water (3 x 50 mL), dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo* to yield a white powder. 5-Benzyloxy-4-oxo-4H-pyran-2-carboxylic acid phenylamide (**4**) (1.20 g, 65%) was obtained by recrystallization from chloroform-petroleum spirit, mp 241-2 °C. IR (KBr) 3330 (amide NH), 1682 (amide C=O), 1636 (pyranone C=O), 1548 (pyranone C=C). ^{1}H-NMR (DMSO-d_6, 60 MHz) δ 5.00 (s, 2H, -CH_2Ph), 6.95 (s, 1H, H^3), 7.35-7.70 (m, 10H, -NH*Ph* and -CH_2*Ph*), 8.15 (s, 1H, H^6). MS (FAB): m/z 322 (M^++H), 307 (100%). Anal. ($C_{19}H_{15}NO_4$) C, H, N.

5-Benzyloxy-4-oxo-4H-pyran-2-carboxylic acid pyridin-3-ylamide (7).

After dissolving **2** (2.00 g, 8.13 mmol) in DMF (30 mL), NMM (1.64 g, 16 mmol) and TBTU (2.74 g, 8.53 mmol) were added under nitrogen and the resulting solution was stirred at room temperature for 30 min. 3-Aminopyridine (1.53 g, 16 mmol) was added slowly to the reaction mixture and stirred at room temperature for 36 h. DMF was removed from the reaction mixture under high vacuum. The solid product was dissolved in dichloromethane (100 mL) and the resulting organic fraction was washed with 0.2 M HCl (3 x 20 mL), 0.2 M $NaHCO_3$ (3 x 20 mL) and water (1 x 50 mL) and then dried over anhydrous sodium sulphate. The solvent was evaporated to give a white solid product. Recrystallization from MeOH/$C_2H_5OC_2H_5$ furnished the 5-benzyloxy-4-oxo-4H-pyran-2-carboxylic acid pyridin-3-ylamide (**7**) as a white solid (1.85 g, 71%). Mp 230-1°C. IR (KBr) 3295 (amide NH), 1684 (amide C=O), 1638 (pyranone C=O), 1597 (pyranone C=C). ^{1}H-NMR (DMSO-d_6, 60 MHz) δ 4.90 (s, 2H, -CH_2Ph), 6.85 (s, 1H, H^3), 7.25-7.80 (m, 9H, pyridine and -CH_2*Ph*), 8.10 (s, 1H, H^6). MS (FAB): m/z 323 (M^++H, 100%). Anal. ($C_{18}H_{14}N_2O_4$) C, H, N.

General procedure for the preparation of 5-benzyloxy-1-methyl-2-substituted-4(1*H*)-pyridinone derivatives (5, 8).

Methylamine in methanol (20 mL, 40 mmol) was added to a solution of **4** or **7** (5 mmol) in methanol (10 mL). The reaction mixture was sealed in a thick-walled glass tube and stirred at 70 °C overnight. After removing the solvent, the residue was subjected to column chromatography on silica gel (eluant: $CHCl_3$: CH_3OH 90:10%) to give the pure 5-benzyloxy-1-methyl-2-substituted-4(1*H*)-pyridinone derivatives (**5**,**8**) as a white crystalline solid.

5-Benzyloxy-1-methyl-4(1*H*)-pyridinone-2-carboxylic acid phenylamide (5).

Yield: 74%, mp 212-3°C. IR (KBr) 3235 (amide NH), 1695 (amide C=O), 1608 (pyridinone C=O), 1544 (pyridinone C=C). ^{1}H-NMR (DMSO-d$_6$, 60 MHz) δ 3.60 (s, 3H, N-CH_3), 4.95 (s, 2H, -CH_2Ph), 6.35 (s, 1H, H^3), 7.30-7.80 (m, 10H, -NH*Ph* and -CH$_2$*Ph*), 7.60 (s, 1H, H^6). MS (FAB): m/z 335 (M$^+$+H, 100%). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

5-Benzyloxy-1-methyl-4(1*H*)-pyridinone-2-carboxylic acid pyridin-3-ylamide (8).

Yield: 75%, mp 220-2 °C. IR (KBr) 3219 (amide NH), 1667 (amide C=O), 1624 (pyridinone C=O), 1573 (pyridinone C=C). ^{1}H-NMR (DMSO-d$_6$, 60 MHz) δ 3.35 (s, 3H, N-CH_3), 5.05 (s, 2H, -CH_2Ph), 7.00 (s, 1H, H^3), 7.35-7.80 (m, 9H, pyridine and -CH$_2$*Ph*), 8.25 (s, 1H, H^6). MS (FAB): m/z 336 (M$^+$+H), 273 (100 %). Anal. ($C_{19}H_{17}N_3O_3$) C, H, N.

General procedure for debenzylation.

Solutions of compounds **5** or **8** in DMF (10 mL) were subjected to hydrogenolysis (12 psi) in the presence of 5% Pd/C (5-10% w/w) for 1 h. The mixtures were warmed and filtered. The filtrates were acidified using hydrogen chloride gas and rotary evaporated to yield a white powder. Recrystallization from MeOH/$C_2H_5OC_2H_5$ gave the pure products (**6** and **9**) as a white crystalline solid.

5-Hydroxy-1-methyl-4(1*H*)-pyridinone-2-carboxylic acid phenylamide hydrochloride (6). Yield: 63%, mp 226-8 °C. IR (KBr) 3300-3000 (broad amide NH and OH), 1678 (C=O amide), 1539 (pyridinone C=C). ^{1}H-NMR (DMSO-d$_6$, 400 MHz) δ 4.08 (s, 3H, N-CH_3), 5.00-6.00 (broad, -OH), 7.20 (t, 1H, J = 7.3 Hz, $H^{4'}$), 7.41 (t, 2H, J = 7.9 Hz, $H^{3'}$, $H^{5'}$), 7.58 (s, 1H, H^3), 7.74 (d, 2H, J = 7.8 Hz, $H^{2'}$, $H^{6'}$), 8.39 (s, 1H, H^6), 11.46 (s, 1H, -CO*NH*-). ^{13}C-NMR (DMSO-d$_6$, 400 MHz) δ 44.7 (N-*C*H$_3$), 113.1 C_3, 120.6 $C_{3',4'}$, 125.3 C_6, 129.3 $C_{5',6'}$, 133.2 $C_{2'}$, 138.1 $C_{1'}$, 142.2 C_5, 146.0 C_2, 159.2 C_4, 160.9 (-*C*ONH-). ^{13}C-DEPT (DMSO-d$_6$, 400 MHz) 44.7 (N-*C*H$_3$), 113.1, 120.6, 125.3, 129.3, 133.2. MS (FAB): 245 (M$^+$+H, 100%). Anal. ($C_{13}H_{12}N_2O_2$.HCl) C, H, N.

5-Hydroxy-1-methyl-4(1*H*)-pyridinone-2-carboxylic acid pyridin-3-ylamide dihydrochloride (9). Yield: 67%, mp 237-8 °C. IR (KBr) 3210-3000 (broad, amide NH and OH), 1701 (amide C=O), 1573 (pyridinone C=C). ^{1}H-NMR (DMSO-d$_6$, 400 MHz) δ 4.14 (s, 3H, N-CH_3), 5.00-6.50 (broad, -OH), 7.71 (s, 1H, H^3), 8.04 (dd, 1H, J_{AM} = 8.5 Hz, J_{MX} = 5.5 Hz, $H^{6'}$), 8.45 (s, 1H, H^6), 8.70-8.73 (m, 2H, $H^{4'}$, $H^{5'}$), 9.30 (d, 1H, J = 2.0 Hz, $H^{2'}$), 12.74 (s, 1H, -CON*H*.). ^{13}C-NMR (DMSO-d$_6$, 400 MHz) δ 45.1 (N-*C*H$_3$), 113.7 C_3, 127.5 C_6, 133.8 $C_{5'}$, 134.5 $C_{6'}$, 135.0 $C_{2'}$, 137.5 $C_{1'}$, 139.1 $C_{4'}$, 140.6 C_5, 146.4 C_2, 160.1 C_4, 160.6 (-*C*ONH-). ^{13}C-DEPT (DMSO-d$_6$, 400 MHz) 45.1 (N-*C*H$_3$), 113.7, 127.5, 133.8, 134.6, 135.0, 139.1. MS (FAB): m/z 230 (M$^+$+H, 100%). Anal. ($C_{12}H_{11}N_3O_2$.2HCl) C, H, N.

Microbiology

Minimal inhibitory concentrations (MICs) were determined by broth microdilution following the procedures reported by the National Committee for Clinical Laboratory Standards[12,13]. Fluconazole and ceftazidime were used as the reference compounds for fungi and bacteria, respectively. Two Gram-positive (*S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212) and two Gram-negative (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) bacteria were used as quality control strains[12]. For testing anti-yeast activities of the compounds, the following reference strains were tested: *C. albicans* ATCC 90018, *C. krusei* ATCC 6258 and

C. parapsilosis ATCC 22019 [13]. The MIC values of the compounds are presented in the table. The reference compounds were dissolved in sterile distilled water. The stock solutions of the synthesized compounds were prepared in dimethylsulfoxide (DMSO). The dilutions in the test medium were prepared at the required concentration of 512-0.5 μg/mL, and for reference compounds at 64-0.0625 μg/mL. The final inoculum densities were 5 x 10^5 cfu/mL for bacteria and 0.5-2.5 x 10^3 cfu/mL for fungi. MIC was defined as the lowest concentration of the compound that inhibited visible growth. It was established that dilution of DMSO lacked antimicrobial activity against any of the test micro-organisms.

Antibacterial activity assay

The cultures were grown on Mueller-Hinton Agar (MHA) (BBL, MD, USA) for all bacteria after 18-24 h of incubation at 35 °C. Before the assay, all of the bacteria were grown in Mueller-Hinton Broth (MHB) for 2-6 h. Then the bacterial suspensions were adjusted to 0.5 McFarland turbidity (1 x 10^8 cfu/mL). The microtiter plates were incubated at 35 °C and inspected visually after 18-24 h for bacteria. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity.

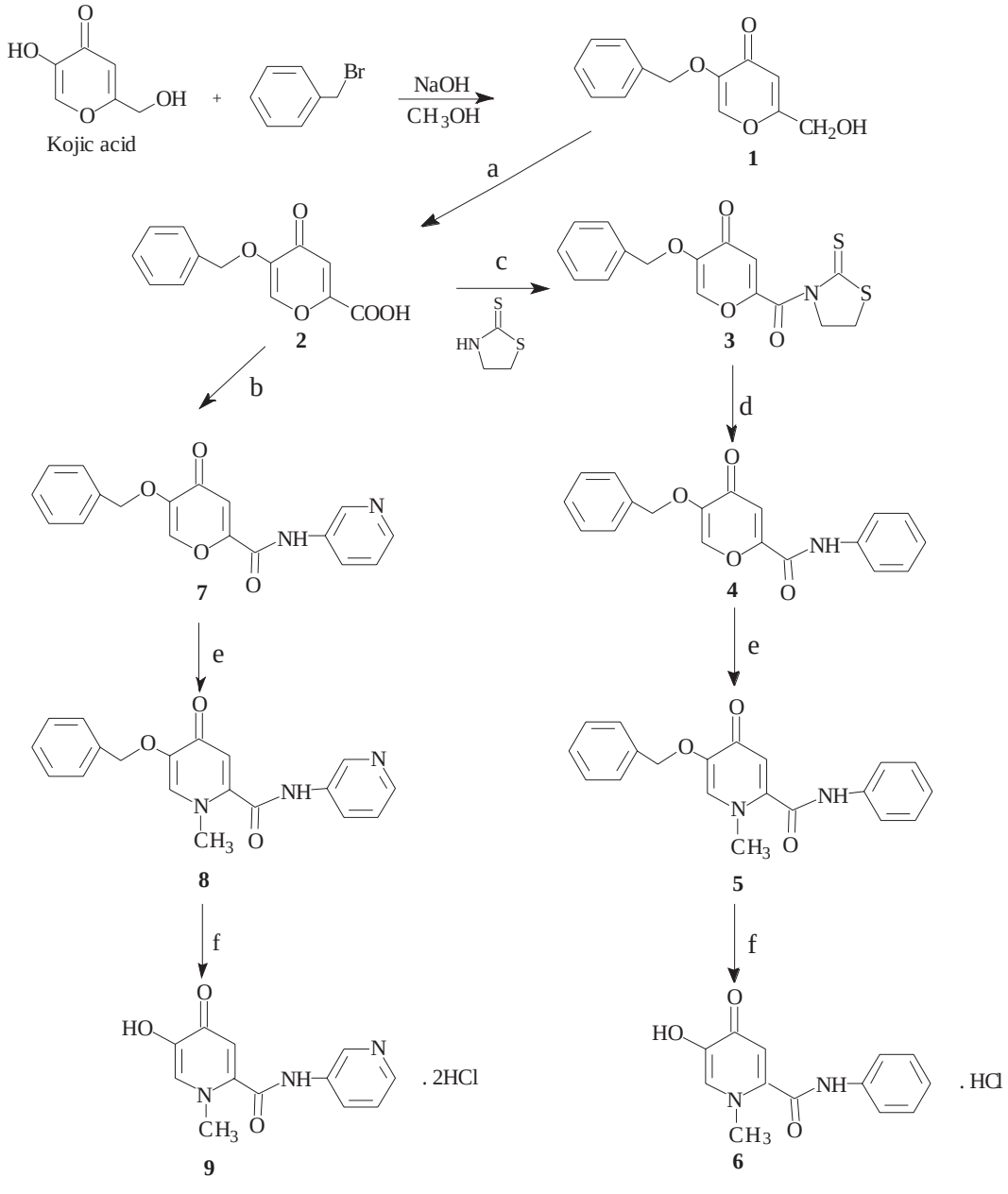
Antifungal activity assay

All fungi were cultivated in Sabouraud Dextrose Agar (Merck). RPMI-1640 medium (ICN-Flow, Aurora, OH-USA) with L-glutamin, buffered with 3-(*N*-morpholino)propanesulphonic acid (MOPS) (Buffer-ICN-Flow, Aurora, OH-USA) at pH = 7.4 was used as the culture medium. The microtiter plates were incubated at 35 °C and evaluated visually after 48 h. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity.

Table. Antibacterial and antifungal activities of the synthesized compounds (MIC in μg/mL).

Comp. No	MIC-μg/mL Bacteria				MIC-μg/mL Fungi		
	S. aureus ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 90018	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019
Kojic acid	256	256	128	128	128	128	128
1	256	128	128	128	128	128	128
2	256	256	128	128	128	128	128
4	128	128	256	256	128	128	128
5	128	128	256	256	128	128	128
6	16	64	128	128	128	64	128
7	128	64	256	256	128	128	128
8	256	128	256	128	128	128	128
9	32	64	256	256	128	64	128
Ceftazidime	2	-*	0.5	4			
Fluconazole					0.25	64	8

*No activity was observed against *E. faecalis*.



Reagents:

a: Jones reagent, b: TBTU, NMM, 3-aminopyridine/DCM, c: DCCI+DMAP/DCM, d: $C_6H_5NH_2$/DCM, e: NH_2CH_3/CH_3OH, f: H_2, Pd-C/HCl, DMF

Scheme. Synthesis of compounds **1**-**9**.

Results and Discussion

Chemistry

The synthetic approach to the two amide pyridinones and pyrones is outlined in Scheme. Kojic acid was refluxed with benzyl bromide in the presence of sodium hydroxide[6,14]. After benzylation, the protected kojic acid (**1**) was reacted with Jones reagent whereupon the primary alcohol was oxidized to the carboxylic acid[11]. For the synthesis of **4** and **7,** two different methods were used. In the first method, the benzylcomenic acid (**2**) was converted to an "activated" form (**3**) by coupling with 2-mercapto-1,3-thiazolidine using dicyclohexylcarbodiimide (DCCI) and 4-dimethylaminopyridine (DMAP) as an acylation catalyst[15]. The intermediate **3** was reacted with aniline to give **4**. In the second method, **2** was reacted with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), *N*-methylmorpholine (NMM) and 3-aminopyridine at room temperature to give **7** with high yield (71%)[16,17]. Derivatives of 4(1*H*)-pyridinone (**5**,**8**) were prepared from the pyran-4(1*H*)-one derivatives (**4**,**7**) by heating with methylamine according to **ANRORC** mechanism[18]. Catalytic hydrogenation was used for debenzylation[10]. The HCl salts of **6** and **9** were prepared by bubbling dry HCl gas in a *N*, *N*-dimethylformamide (DMF) solution of the base.

The structure of the synthesized compounds was assigned according to the reaction mechanism and was elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, ^{13}C-DEPT, FAB mass spectra and elemental analysis.

Antimicrobial Activity

Kojic acid and compounds **1**-**9** (except for **3**) were screened for their *in vitro* antimicrobial activity by the microdilution broth method as MIC values. Fluconazole and ceftazidime were used as the standard antifungal and antibacterial compounds, respectively. The inhibitory results are reported in the table. The screening data indicate that compounds **6** and **9**, with a catechol group on the 4(1*H*)-pyridinone ring, show the best antibacterial activity against *E. faecalis* and *S. aureus* among the synthesized compounds. Both have better antibacterial activities against Gram-positive bacteria than Gram-negative bacteria. The other compounds had no valuable inhibitory activity. Kojic acid, benzyl kojic acid, benzyl comenic acid and all intermediate amide compounds showed similar antifungal activity (MIC: 128 μg/mL). Their antifungal activity results were not encouraging, although most compounds manifested moderate antifungal activity. Compounds **6** and **9**, with an affinity to metal cations, have more potent activity against *C. krusei* than protected intermediate amide compounds (**4**,**5**,**7**,**8**). There is no difference in antimicrobial activity with the location of the phenyl or pyridine rings at position C-2.

In conclusion, some of the compounds of the amide series (**6**,**9**) proved to be promising antimicrobial agents. The 4(1*H*)-pyridinone ring, which is analogous to catechol and has chelating ability, is considered to play a significant role in antimicrobial activity. Further pharmacological investigation is needed in this area.

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