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Microwave-Assisted Synthesis of New Series Some Acetyl Coumarin Derivatives and Studying of Some their Pharmacological Activities

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Abstract: Development of potent and effective antimicrobial drugs is one of the more pressing goals of current research in chemistry. A green protocol has been used to synthesize a novel series of coumarin derivatives **(3a,b)**, **(5a,b)**, **(7a-c)** and **(8a--c)** in the shorter reaction time, higher yields and simple operations, when compared with the conventional heating method. Structures of the new products were confirmed on the basis of spectroscopic data (FT-IR, 1D-NMR). The synthesized compounds **(3a,b)** and **(7a-c)** were screened for the antimicrobial activity against gram positive and gram negative bacteria.

Keywords: Coumarinylchalcones, Coumarinyl pyrimidines, microwave-assisted synthesis, antimicrobial activity.

INTRODUCTION:

The chemistry of hetaryl-substituted coumarins has developed intensively in the last decades because of their interesting biological and pharmacological activities.[17,18] Coumarins have been used in preclinical studies for several therapeutic indications including anticoagulant, antibacterial, antiinflammatory, antioxidant, anthelmintic, anti- HIV and anticancer activities.[3-9.] Encouraged from these findings, coumarinylchalcones were synthesized using both microwave irradiation method as a greener approach and screened them against pathogenic microbes. Microwave heating offers several advantages over conventional techniques.[10-19] These include the dramatic reduction time and the efficient internal heating of reaction mixtures, which can induce the completion of chemical transformations in a few minutes or seconds, while several hours or days are required under conventional conditions. Microwave heating for chemical synthesis also increases product yields and enhances product purities by reducing unwanted side reactions. Recently, microwave-assisted technique for organic synthesis is synonymous with green chemistry. Indeed, solvent-free methods[20,21] is especially adapted to organic synthesis, resulting in very efficient and clean procedures. It is evident that this eco-friendly approach has not eluded the chemists during the last decade.[22-25]

An important feature of chalcones is their ability to act as an intermediate for the synthesis of biologically active heterocyclic compounds such as cyclohexenone, isoxazoline, pyrazoline, pyridine and pyrimidine, derivatives.[26,27] Pyrimidines and related pyrimidines are classes of fused heterocycles that are of considerable interest because of the wide range of their biological properties[28], herein we reported the synthesis of some new of 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-ones (**5a,b** and **8a-c**).

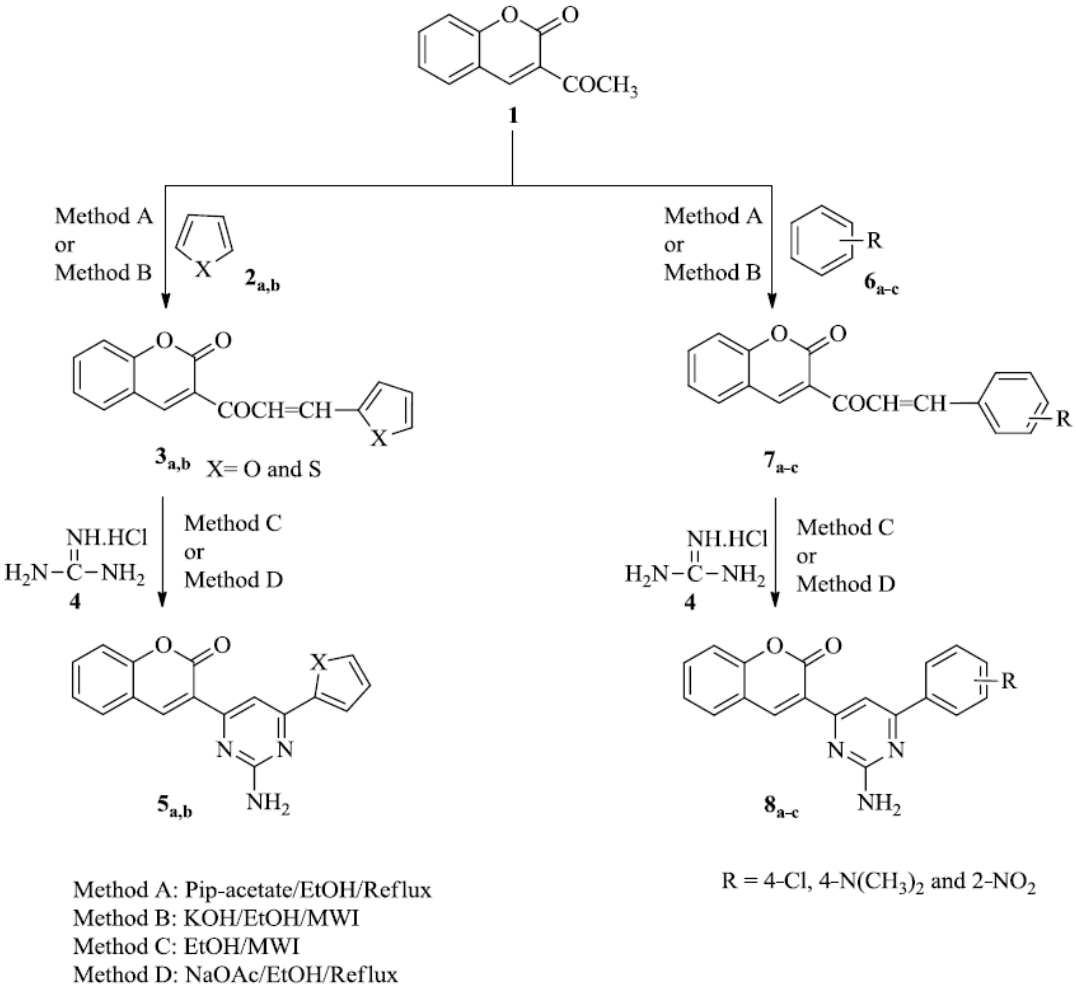
RESULTS AND DISCUSSION

A variety of different modified 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives (**3a,b** and **5a,b**) have been previously synthesized using the classical approaches in solution, which are expensive and time-consuming.[29,30] In this report we describe new simple, efficient procedures for the synthesis of the target coumarinylchalcones (**3a,b** and **7a-c**). Microwave irradiation (method A) was used to obtain the desired products (**3a,b** and **7a-c**) in short time (4-6 min) under solvent- free conditions. piperidine was used as a catalyst to facilitate the reaction between 3-acetyl coumarin (**1**) and various substituted aromatic aldehydes (**2a,b** and **6a-c**) in in an excellent yield (83-90%) (Table 1). The structure of the obtained products (**3a,b** and **5a,b**) was confirmed on the bases of their elemental analyses and spectral data (LC-MS/MS, IR, UV, 1D- and 2D-NMR). Thus, the analytical data for **3b** revealed a molecular formula $C_{16}H_{10}O_3S$. LC-MS (ionization method): m/z 266 [M]. IR showed signals at υ 1722 cm^{-1} assigned for the presence of

(C=O) group. Another band appeared at υ 1665 cm^{-1} corresponding to the (C=O, *α,β*-unsaturated cyclic) group. ^{1}H- NMR (400 MHz, DMSO-*d6*) spectrum of compound **3b** showed a singlet at δ = 7.16 ppm corresponding to the proton at C-4 of the coumarine ring. Another signals appeared at δ = 7.40- 7.46 ppm as multiplet corresponding to the thienyl protons. ^{13}C-NMR (100 MHz, DMSO-*d6*) gave signal at δ = 159.0 assign for the the coumarine C-2. The acetoxy carbon of *α,β*-unsaturated cyclic (C-1""); appeared at δ = 186.8 ppm.

Condensation of 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives (**3a,b** and **5a,b**) with guanidine hydrochloride **(4)** in dry ethanol in the presence of anhydrous sodium acetate under efflux for 8-10 hours yielded a novel series of 2-amino-4-(coumarin-3-yl)-6-substituted-3-(2-amino-6-substituted-pyrimidin-4-yl)-2H-chromen-2-ones (**5a,b** and **8a-c**). The same target compounds (**5a,b** and **8a-c**) were synthesized using the microwave irradiation in short time with an excellent yield. (Table1)

The structure of the final compounds was assigned based on their elemental analysis and spectral data. For example IR spectrum of the compound **(**3-(2-amino-6-(4-(dimethylamino)phenyl)pyrimidin-4-yl)-2H-chromen-2-one **(8b)** revealed the broad band at υ =3149 cm^{-1} due to amino group. In addition to this ^{1}H-NMR (400 MHz, DMSO-*d6*) spectrum showed the singlet signal at δ = 6.70 ppm due to the amino group. Another signal appeared at δ = 6.94 ppm as singlet corresponding to the pyrimidine-H. ^{13}C-NMR (100 MHz, DMSO-*d6*) showed signal at δ = 40.1 ppm assign for the two methyl groups. However, the coumarine C-2 appeared at δ = 161.8 ppm. Moreover, mass spectrum and elemental analysis data also supported the structure assigned to the final compound as the analytical data revealed a molecular formula $C_{21}H_{18}N_4O_2$. LC-MS (ionization method): m/z 358.0 [M].



Scheme 1: Synthetic protocols of target compounds (5a,b and 8a-c).

Table 1: The differences in yield and time between the microwave and classical method in synthesis of modified 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives (**3a,b** and **7a-c**) and 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-ones **(5a,b** and **8a-c).**

Entry	*X*	*R*	*Conventional*		*Microwave (MW)*	
			Time	*Yield %*	*Time*	*Yield %*
3a	O		6h	76	5 min	88
3b	S		7h	78	4 min	90
5a	O		9h	61	15 min	85
5b	S		9h	64	15 min	87
7a		4-Cl-C_6H_4	6h	73	5 min	86
7b		4-N(CH_3)2	7h	71	5min	92
7c		2-NO_2-C_6H_4	8h	63	5 min	83
8a		4-Cl-C_6H_4	8h	65	14 min	88
8b		4-N(CH_3)2	8h	63	14min	91
8c		2-NO_2-C_6H_4	10h	60	16 min	80

EXPERIMENTAL SECTION GENERAL

Microwave synthesis was performed using CEM Microwave system. Melting points were determined on (Pyrex capillary) Gallenkamp apparatus. Infrared spectra were recorded with a Thermo Nicolet Nexus 470 FT-IR spectrometer in the range 4000-400 cm^{-1} using potassium bromide disks. The ultraviolet absorption spectra, in the region 200–600 nm were recorded using a Secoman Anthelie 2 Advanced spectrophotometer in 1.00 cm cells at $25^{o}C$. The spectra were run in spectraquality methanol using concentration of $5x10^{-5}$ M.^{1}H-NMR spectra, APT, DEPT, ^{13}C-NMR spectra were obtained on Varian Gemini 400 and 200 MHz FT NMR spectrometer in $CDCl_3$ and DMSO-*d6*; chemical shifts were recorded in □□(ppm) units, relative to Me_4Si as an internal standard. The mass spectra were recorded on Shimadzu LCMS-QP 800 LC-MS and AB- 4000 Q-trap LC-MS/MS. Analytical data were obtained using PerkinElmer 2400 II series CHN Analyzer. Thin-layer chromatography (TLC) was carried out on precoated Merck silica gel F_{254} plates and UV light was used for visualization. Column chromatography was performed on a Merck silica gel. The reagents were purchased from Aldrich and used without further purification.

General procedure for the synthesis of 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives (3a,b and 7a-c).

Microwave method (Method A):

A mixture of equimolar amounts of 3-acetyl coumarin (**1**) and the corresponding aromatic aldehydes (**2a,b** and **6a-c**) (5 mmol) were dissolved in 10 mL of ethanol containing catalytic amount of potassium hydroxide was irradiated for an appropriate time (Table 1) in a 10 ml closed vial using CEM Microwave system. After completion of the reaction, as indicated by TLC, the obtained products were purified by crystallization from EtOH-DMF to afford 3-Aryl-1- (3′-coumarinyl) propen-1-one derivatives (**3a,b** and **7a-c**).

Conventional method (Method B):

3-Acetylcoumarin (1.88 g, 0.01 M) and the substituted aromatic aldehydes (**2a,b** and **6a-c**) (0.02 M) were dissolved in 10 mL of ethanol by heating. Piperidine (0.4 mL) was added to this reaction mixture, followed by the addition of glacial acetic acid (0.3 mL). The reaction was carried out under reflux till its completion, as indicated by TLC. Ethanol was recovered by vacuum distillation after the completion of a reaction and the residue was triturated with 1 mL of methanol. The reaction mixture was filtered off and the crude product was recrystallized using an appropriate solvent to afford Coumarinylchalcones (**3a,b** and **7a-c**).

3-(3-(furan-2-yl)acryloyl)-2H-chromen-2-one (3a):

mp 102 °C; IR (KBr, cm^{-1}) υ 1728 (C=O) and 1662 (C=O ,α, β-unsaturated cyclic) ; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 6.78 (d, 1H, Coumarin), 7.28-7.40 (m, 3H, furyl), 7.55 (s, 1H, Coumarin), 7.61-7.67 (m, 3H, Coumarin), 7.78 (d, 1H, =C-H), 8.54 (d, 1H, =C-H)); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 112.8 (C-4"), 116.7 (C-3"), 118.6 (C-7), 121.6 (C-5), 124.9 (C-6), 125.3 (C-9), 129.9 (C-10), 130.3 (C-2""), 134.2 (C-3""), 134.7 (C-8), 137.5 (C-5"), 145.5 (C-4), 147.8 (C-3), 151.7 (C-2"), 155.2 (C-2), 186.1 (C-1""); LC-MS (ionization method): m/z 266 [M]; Anal. Calcd for $C_{16}H_{10}O4$: C, 72.18; H, 3.79%. Found: C, 72.01; H, 3.88%.

3-(3-(thiophen-2-yl)acryloyl)-2H-chromen-2-one (3b):

mp 221 °C; IR (KBr, cm^{-1}) υ 1722(C=O) and 1665 (C=O , *α,β*-unsaturated cyclic) ; ^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 7.16 (d, 1H, Coumarin), 7.40-7.46 (m, 3H, thienyl), 7.70 (s, 1H, Coumarin), 7.72-7.77 (m, 3H,Coumarin), 7.92 (d, 1H, =C-H), 8.57 (d, 1H, =C-H)); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 116.6 (C-4"), 118.9 (C-3"), 123.5 (C-7), 125.4 (C-5), 125.6 (C-6), 129.4 (C-9), 130.9 (C-10), 131.2 (C-2""), 134.2 (C-3""), 134.7 (C-8), 137.5 (C-5"), 140.1 (C-4), 147.5 (C-3), 155.0 (C-2"),159.0 (C-2), 186.8 (C-1""); LC-MS (ionization method): m/z 266 [M]; Anal. Calcd for $C_{16}H_{10}O_3S$: C, 68.07; H, 3.57%. Found: C, 68.1; H, 3.59%.

3-(3-(4-chlorophenyl)acryloyl)-2H-chromen-2-one (7_a):

mp 189 °C; IR (KBr, cm^{-1}) υ 1719 (C=O) and 1666 (C=O , *α,β*-unsaturated cyclic); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 7.13 (d, 1H, Coumarin), 7.26 -7.41 (m, 4H, Ar-H), 7.59-7.83 (m, 4H, Coumarin), 7.97 (d, 1H, =C-H), 8.60 (d, 1H, =C-H),); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 116.5 (C-4"), 118.4 (C-3"), 124.6 (C-7), 124.9 (C-5), 125.2 (C-6), 130.3 (C-9), 130.4 (C-10), 131.1 (C-2""), 132.1 (C-3""), 133.7 (C-8), 134.5 (C-6"), 137.5 (C-5"), 140.1 (C-4), 147.5 (C-3), 155.2 (C-2"), 158.8 (C-2), 186.4 (C-1""); LC-MS (ionization method): m/z 311 [M+1]; Anal. Calcd for $C_{18}H_{11}ClO_3$: C, 69.58; H, 3.57%. Found: C, 69.45; H, 3.59%.

3-(3-(4-(dimethylamino)phenyl)acryloyl)-2H-chromen-2-one (7_b):

mp 111 °C; IR (KBr, cm-1) υ 1730 (C=O) and 1651 (C=O , *α,β*-unsaturated cyclic); 1H-NMR (400 MHz, DMSO-d_6) δ = 3.29 (s, 6H, $2CH_3$), 6.94 (d, 1H, Coumarin), 7.19 - 7.85 (m, 4H, Ar-H), 7.61-7.68 (m, 4H, Coumarin), 8.01 (d, 1H, =C-H), 8.62 (d, 1H, =C-H),); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 38.6 (2CH3); 116.8 (C-4"), 118.6 (C-3"), 124.6 (C-7), 125.1 (C-5), 125.5 (C-6), 129.9 (C-9), 130.2 (C-10), 130.9 (C-2""), 132.1 (C-3""), 133.9 (C-8), 134.6 (C-6"), 137.6 (C-5"), 141.2 (C-4), 147.2 (C-3), 155.2 (C-2"), 158.6 (C-2), 186.1 (C-1""); LC-MS (ionization method): m/z 319 [M]; Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; H, 3.57; N, 4.39%. Found: C, 75.15; H, 3.61; N, 4.49%.

3-(3-(2-nitrophenyl)acryloyl)-2H-chromen-2-one (7_c):

mp 193 °C; IR (KBr, cm^{-1}) υ 1719 (C=O) and 1666 (C=O, *α,β*-unsaturated cyclic); 1H-NMR (400 MHz, DMSO-d_6) δ = 7.17 (d, 1H, Coumarin), 7.28 -7.43 (m, 4H, Ar-H), 7.59-7.83 (m, 4H, Coumarin), 7.97 (d, 1H, =C-H), 8.58 (d, 1H, =C-H),); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 116.3 (C-4"), 118.3 (C-3"), 124.8 (C-7), 125.0 (C-5), 125.4 (C-6), 130.4 (C-9), 130.6 (C-10), 131.1 (C-2""), 131.9 (C-3""), 133.6 (C-8), 134.7 (C-6"), 137.5 (C-5"), 140.2 (C-4), 147.5 (C-3), 155.2 (C-2"), 158.9 (C-2), 186.2 (C-1""); LC-MS (ionization method): m/z 322 [M+1]; Anal. Calcd for $C_{18}H_{11}NO_5$: C, 67.29; H, 3.45; N, 4.36%. Found: C, 67.25; H, 3.55; N, 4.31%.

General procedures for the synthesis of 3-(2-amino-6-substituted pyrimidin-4-yl)-2H- chromen-2-one ($5_{a,b}$ and 8_{a-c}).

Microwave method (Method C):

A mixture of 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives **(3a,b and 7a-c)** (0.01mol), and guanidine hydrochloride **(4)** (0.01 mol) was irradiated for an appropriate time (Table 1) in a 10 ml closed vial using CEM Microwave system. After completion of the reaction, as indicated by TLC, the obtained products were purified by crystallization from the proper solvent to afford 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-one **(5a,b and 8a-c).**

Conventional method (Method D):

A solution of 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives **($3_{a,b}$ and 7_{a-c})** (0.01mol), sodium acetate (0.1 g) in 30 ml ethanol, guanidine hydrochloride **(4)** (0.01 mol) was added. The reaction mixture was refluxed for 8-10 h. The solid formed was collected by filtration, dried and crystallized from the proper solvent to give the 3-(2-amino-6-substituted pyrimidin-4-yl)-2H-chromen-2-one (**$5_{a,b}$** and **8_{a-c}**).

3-(2-amino-6-(furan-2-yl)pyrimidin-4-yl)-2H-chromen-2-one (5_a):

mp 139 °C; IR (KBr, cm^{-1}) υ 3153 (NH) and 1741 (C=O); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 6.65 (s, 2H, NH_2), 6.77 (d, 1H, Coumarin), 6.90 (d, 1H, pyrimidine), 6.98-7.30 (m, 3H, furyl), 7.45 (s, 1H, Coumarin), 7.51- 7.65 (m, 3H, Coumarin); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 113.5 (C-3""), 116.4 (C-5"), 117.6 (4""), 119.7 (C-9), 121.7 (C-5), 123.5 (C-7), 124.8 (C-6), 126.7 (C-8), 129.0 (C-4"), 132.2 (C-5""), 135.4 (C-4), 138.6 (C-10), 142.8 (C-2""), 146.5 (C-3), 151.4 (C-2"), 153.7 (C-6") 159.7 (C-2); LC-MS (ionization method): m/z 305 [M]; Anal. Calcd for $C_{17}H_{11}N_3O_3$: C, 66.88; H, 3.63; N, 13.76%. Found: C, 67.01; H, 3.58; N, 13.79%.

3-(2-amino-6-(thiophen-2-yl)pyrimidin-4-yl)-2H-chromen-2-one (5_b):

mp 171 °C; IR (KBr, cm^{-1}) υ 3149 (NH) and 1746 (C=O); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 6.58 (s, 2H, NH_2), 6.79 (d, 1H, Coumarin), 6.91 (d, 1H, pyrimidine), 6.97-7.31 (m, 3H, furyl), 7.44 (s, 1H, Coumarin), 7.49-7.64 (m, 3H, Coumarin); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 113.8 (C-3""), 116.5 (C-5"), 117.8 (4""), 119.5 (C-9), 121.8 (C-5), 123.5 (C-7), 124.6 (C-6), 126.8 (C-8), 128.8 (C-4"), 132.4 (C-5""), 135.5 (C-4), 138.9 (C-10), 142.5 (C-2""), 146.6 (C-3), 151.2 (C-2"), 153.8 (C-6") 160.0 (C-2); LC-MS (ionization method): m/z 322 [M+1]; Anal. Calcd for $C_{17}H_{11}N_3O_2S$: C, 63.54; H, 3.45; N, 13.08%. Found: C, 63.51; H, 3.47; N, 13.11%.

3-(2-amino-6-(4-chlorophenyl)pyrimidin-4-yl)-2H-chromen-2-one (8_a):

mp 166 °C; IR (KBr, cm^{-1}) υ 3160 (NH) and 1750 (C=O);

^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 6.72 (s, 2H, NH_2), 6.81 (d, 1H, Coumarin), 6.96 (d, 1H, pyrimidine), 7.19 -7.35 (m, 4H, Ar-H), 7.39-7.60 (m, 4H, Coumarin); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 113.4 (C-3""), 113.9 (C-5"), 116.0 (C-5"), 119.1 (C-5), 119.5 (C-9), 121.8 (C-7), 125.6 (C-8), 128.5 (C-6), 130.9 (C-2""), 131.2 (C-6""), 137.8 (C-4), 141.1 (C-3), 144.1 (C-4""), 145.8 (C-4"), 153.6 (C-10), 155.7 (C-1""), 157.9 (C- 2"), 158.5 (C-6"), 162.2 (C-2); LC-MS (ionization method): m/z 349.0 [M]; Anal. Calcd for $C_{19}H_{12}ClN_3O_2$: C, 65.24; H, 3.46; N, 12.01%. Found: C, 64.99; H, 3.49; N, 11.88%.

3-(2-amino-6-(4-(dimethylamino)phenyl)pyrimidin-4-yl)-2H-chromen-2-one (8_b):

mp 97 °C; IR (KBr, cm^{-1}) υ 3149 (NH) and 1744 (C=O); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 3.13 (s, 6H, $2CH_3$), 6.70 (s, 2H, NH_2), 6.84 (d, 1H, Coumarin), 6.94 (d, 1H, pyrimidine), 7.16 -7.30 (m, 4H, Ar-H), 7.42-7.63 (m, 4H, Coumarin); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 40.1 ($2CH_3$), 112.3 (C-3""and C-5""), 116.7 (C-5"), 119.6 (C-5), 119.7 (C-9), 122.0 (C-7), 125.4 (C-8), 128.8 (C-6),130.7 (C-2""and C-6""), 137.3 (C-4), 141.3 (C-3), 143.9 (C-4""), 145.3 (C-4"), 153.3 (C-10),155.3 (C-1""), 157.7 (C-2"), 158.7 (C-6"), 161.8 (C-2); LC-MS (ionization method): m/z 358.0 [M]; Anal. Calcd for $C_{21}H_{18}N_4O_2$: C, 70.38; H, 5.06; N, 15.63%. Found: C, 70.55; H, 4.91; N, 15.78%.

3-(2-amino-6-(2-nitrophenyl)pyrimidin-4-yl)-2H-chromen-2-one (8_c): mp 159 °C; IR (KBr, cm^{-1}) υ 3157 (NH) and 1753 (C=O); ^{1}H-NMR (400 MHz, DMSO-*d6*) δ = 6.77 (s, 2H, NH_2), 6.89 (d, 1H, Coumarin), 6.99 (d, 1H, pyrimidine), 7.16 -7.37 (m, 4H, Ar-H), 7.42-7.61 (m, 4H, Coumarin); ^{13}C-NMR (100 MHz, DMSO-*d6*) δ = 113.7 (C-5""), 114.3 (C-5"), 116.2 (C-4"), 119.4 (C-5), 119.7 (C-9), 121.3 (C-7), 125.8 (C-8), 128.9 (C-6), 131.7 (C-2""), 131.9 (C-6""), 138.4 (C-4), 141.3 (C-3), 145.7 (C-3""), 146.5 (C-4"), 154.1 (C-10), 156.9 (C-1""), 158.0 (C- 2"), 158.8 (C-6"), 163.1 (C-2); LC-MS (ionization method): m/z 359.0 [M-1]; Anal. Calcd for $C_{19}H_{12}N_4O_4$: C, 63.33; H, 3.36; N, 15.55%. Found: C, 63.45; H, 3.51; N, 15.78%.

BIOLOGICAL TESTING. ANTIMICROBIAL TESTING MATERIALS AND METHODS:

The newly synthesized compounds **($3_{a,b}$ and $7_{a,c}$)** were tested for their in vitro growth inhibitory activity against a panel of standard strains of the Institute of fermentation of Osaka (IFO) namely; the Gram-positive bacteria (Staphylococcus aureus IFO 3060 and Bacillus subtilis IFO 3007), the Gram-negative bacteria (Escherichia coli IFO 3301 and Proteus vulgaris IFO 3851. The primary screening was carried out using the agar disc-diffusion method using Müller- Hinton agar medium.[31,32] The minimal inhibitory concentration (MIC) for the most active compound **7_c** against the same microorganism used in the primary screening was carried out using the microdilution susceptibility method in Müller-Hinton Broth and Sabouraud Liquid Medium.[33]

Agar disc-diffusion method

Sterile filter paper discs (5 mm diameter) were moistened with the compound solution in dimethylsulphoxide of specific concentration (300 µg/disc) were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37°C for 24 hours, and the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism (% inhibition = sample inhibition zone (cm)/plate diameter× 100). All measurements were done in DMSO as a solvent which has zero inhibition activity. The obtained results were compared with some reference antibiotics.

Determination of Minimal inhibitory concentration (MIC)

Stationary-phase cultures of bacteria were prepared at 37°C and used to inoculate fresh ml culture to an OD600 of 0.05. The 5.0 ml cultures were then incubated at 37°C until an OD600 of 0.10 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6×10^{-5} colony forming units (CFUs)/ml. Serial dilutions from the treatments (0- 320 µg/ml) were prepared and mixed with 5.0 ml of the standardized bacteria suspension and then added to the plates and incubated for 24 h at 37°C. The turbidity produced in each tube was recorded by using a UV–visible spectrometer.

In general, our synthesized coumarin derivatives showed activity against the tested Gram- positive bacteria and the Gram-negative bacteria. Compound, **3_b, 7_c** were found to be the most active against both of Gram-positive bacteria (Bacillus subtilis) and Gram-negative bacteria **(**Escherichia coli). (Table 2)

Table 2: Antimicrobial activity of compounds **($3_{a,b}$ and $7_{a,c}$)**.

Compd. No	*Inhibition (%)*			
	BS	***SA***	***EC***	***PV***
3_a	19	0.0	21	0.0
3_b	31	18	32	15
7_a	22	0.0	28	0.0
7_c	28	23	41	24
Control: DMSO	0.0	0.0	0.0	0.0
Penicillin	0.0	14	0.0	20
Amikacin	39	27	37	30

The minimal inhibitory concentration (MIC) for the most active compounds **3_b, 7_c** against the same microorganism compared to standard Amikacin antibacterial agent was observed as outlined in table 3 and figure. Compound **7_c** showed higher activity than Amikacin against Bacillus with lower minimum inhibition concentration. On the other hand, compound **3_b** showed good activity against the bacillus subtilis as gram-positive bacteria. (Table 3)

Table 3: MIC of compounds **3_b, 7_c**.

	MIC (μg/ml)			
Sample	***BS***	***SA***	***EC***	***PV***
3_b	123	170	192	161
7_c	134	150	98	152
Amikacin	98	142	121	133

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