INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

New Methodology for Synthesis of Coumarin Derivatives as Potent Antimicrobial Agents

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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** and **5a,b**) 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 were screened for the antimicrobial activity against gram positive and gram negative bacteria. Molecular docking studies were performed to explore the possible interactions of these compounds with GlcN-6-P synthase enzyme.

Keywords: Coumarinylchalcones, microwave-assisted synthesis, antimicrobial activity, Docking.

1. INTRODUCTION

Microwave heating offers several advantages over conventional techniques.[1-10] 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[11,12] 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.[13-16] There is great interest in the development of new Coumarin derivatives because of their interesting biological and pharmacological activities.[17,18] Coumarins have been used in preclinical studies for several therapeutic indications including anticoagulant, antibacterial, anti-inflammatory, antioxidant, anthelmintic, anti- HIV and anticancer activities.[19-23] Chalcones are α, β-unsaturated ketones containing a reactive ketoethylenic group, –CO-CH=CH. The presence of α, β- unsaturated carbonyl system are an important group of natural or synthetic flavonoids that are known to exhibit an impressive array of biological properties.[24,25] Encouraged from these findings, coumarinylchalcones were synthesized using both microwave irradiation method as a greener approach and screened them against pathogenic microbes.

2. EXPERIMENTAL SECTION

2. 1. Chemistry

2.1.a 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°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-d_6; 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.

i- General procedure for the synthesis of 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives ($3_{a,b}$ and $5_{a,b}$).

Microwave method (Method A):

A mixture of equimolar amounts of 3-acetyl coumarin (**1**) and the corresponding aromatic aldehydes (**2**$_{a,b}$ and **4**$_{a,b}$) (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 (**3**$_{a,b}$ and **5**$_{a,b}$).

Conventional method (Method B):

3-Acetylcoumarin (1.88 g, 0.01 M) and the substituted aromatic aldehydes (**2**$_{a,b}$ and **4**$_{a,b}$) (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 (**3**$_{a,b}$ and **5**$_{a,b}$).

3-(3-(furan-2-yl)acryloyl)-2H-chromen-2-one (3_a): 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-d_6) 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-d_6) 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 (3_b): 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-d_6) 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-d_6) 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 (5_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-d_6) 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-d_6) 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-(2-nitrophenyl)acryloyl)-2H-chromen-2-one (5_b): 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-d_6) 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%.

2.2. Molecular modeling

Molecular modeling studies were performed using Molecular Operating Environment (MOE, 10.2008) software on an Intel Core i5 processor 2.53 GHz, 4 GB memory with Windows XP 32-bit operating system. Energy minimizations were performed with RMSD gradient of 0.05 kcal/ mol and MMFF94X forcefield using MOE and partial charges were automatically calculated. The X-ray crystallographic structure of glucosamine-6-phosphate (ligand) co-crystallized with GlcN-6-P synthase enzyme was obtained from Protein Data Bank , the PDB file is 2VF5. The target enzyme was prepared for docking by: (i) Removing the ligand from the active site of the enzyme. (ii) Addition of hydrogen atoms to the structure with their standard geometry. (iii) Detecting the active site in the enzyme by MOE Alpha Site Finder. (iv) The obtained pocket was saved as moe to be used in predicting the ligand enzyme interactions at the active site and docking of the compounds.

3. MATERIALS AND METHODS:

3.1Biological Testing.

Antimicrobial Testing

The newly synthesized compounds **($3_{a,b}$ and $5_{a,b}$)** 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.[27-29] The minimal inhibitory concentration (MIC) for the most active compound **5_b** 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.[30]

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 5.0 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, 5_b** were found to be the most active against both of Gram-positive bacteria (Bacillus subtilis) and Gram-negative bacteria **(**Escherichia coli). (Table 2)

The minimal inhibitory concentration (MIC) for the most active compounds **3_b, 5_b** against the same microorganism compared to standard Amikacin antibacterial agent was observed as outlined in table 3 and figure. Compound **5_b** 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)

4. RESULTS AND DISCUSSION

4. 1. Chemistry

A variety of different modified 3-Aryl-1-(3′-coumarinyl) propen-1-one derivatives (**$3_{a,b}$** and **$5_{a,b}$**) have been previously synthesized using the classical approaches in solution, which are expensive and time-consuming.[26,27] In this report we describe new simple, efficient procedures for the synthesis of the target coumarinylchalcones (**$3_{a,b}$** and **$5_{a,b}$**) . Microwave irradiation (method A) was used to obtain the desired products (**$3_{a,b}$** and **$5_{a,b}$**) 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 (**$2_{a,b}$** and **$4_{a,b}$**) in in an excellent yield (83-90%) (Table 1).

The structure of the obtained products (**$3_{a,b}$** and **$5_{a,b}$**) 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 **3_b** revealed a molecular formula $C_{16}H_{10}O_3S$. LC-MS (ionization method): m/z 266 [M]. IR showed signals at υ 1722 and 1655 cm^{-1} assigned for the presence of (C=O) group. Another band appeared at υ 1662 cm^{-1} corresponding to the (C=O, α, β-unsaturated cyclic)

group. ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of compound **3_b** showed a dsinglet at δ = 7.76 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-d_6) 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.

4.2. Molecular docking studies:

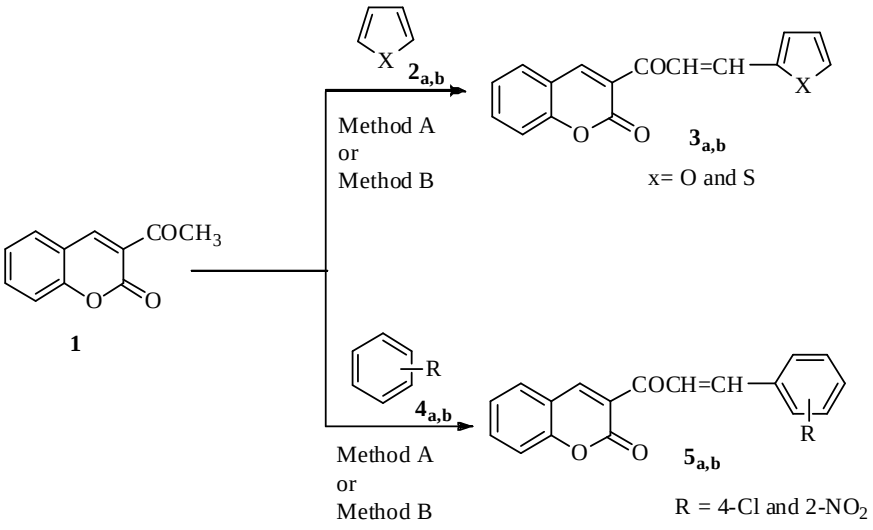
Molecular docking was performed on the active site of GlcN-6-P synthase as the target receptor. glucosamine-6-phosphate (ligand) co-crystallized with GlcN-6-P synthase was obtained from Protein Data Bank (http:// www.pdb.org/pdb/home/home.do), the PDB file is 2VF5. Validation process was performed by redocking glucosamine-6-phosphate into the active site of the receptor at root mean standard deviation (rmsd) = 1.25, the observed docking score energy was -17.13 kcal/mol and amino acid interactions as shown in Fig.1.All compounds **$3_{a,b}$** and **$5_{a,b}$** were docked into the active site of GlcN-6-P synthase enzyme. Docking score energies, amino acid interactions were summarized in Table 1. The best score energy was shown by compounds 5b (-17.72 kcal/mol) and **3_b** (-13.90 kcal/mol), in addition both compounds revealed an amino acid interaction with Ala 602. Compound **3_a** had amino acid interactions with Val 605 and Thr 352, whoever compound **5_a** showed interactions with Gln 348 and Ser 308. These observed results help explaining the observed antibacterial activity as the best fitting energy was observed for the most active compounds **3_b** and **5_b**. On the other hand both compounds revealed interaction with Ala 602 amino acid and this encouraged us to expect that the ability of the compound to interact with Ala 602 amino acid in GlcN-6-P synthase may help in increasing its activity as antibacterial drug.

5. CONCLUSION

1. Compounds **3_b** and **5_b** showed a broad spectrum activity against all the tested microorganisms.
2. In addition compound 5b revealed to be more active than amikacin antibiotic against Escherichia coli, their MIC was 98 and 121 ***μg/ml*** respectively.
3. Compound 5b followed by compound 3b were the most active against all the tested microorganisms except BS, where compound 3b was found to be more active than 5b.
4. Compounds **3_b** and **5_a** showed inhibitory activity against BS and EC microorganisms and showed no activity against SA and PV.

6. ACKNOWLEDGEMENT

We thank Dr. Amani belal who performed docking study.



Method A: Pip-acetate/EtOH/Reflux
Method B: KOH/EtOH/MWI

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 ($3_{a,b}$ and $5_{a,b}$).

Entry	*X*	*R*	*Conventional*		*Microwave (MW)*	
			Time	*Yield %*	*Time*	*Yield %*
3_a	O		6h	76	5 min	88
3_b	S		7h	78	4 min	90
5_a		4-Cl-C_6H_4	6h	73	5 min	86
5_b		2-NO_2-C_6H_4	8h	63	6 min	83

Table 2
Antimicrobial activity of compounds ($3_{a,b}$ and $5_{a,b}$).

Compd. No	*Inhibition (%)*			
	BS	*SA*	*EC*	*PV*
3_a	19	0.0	21	0.0
3_b	31	18	32	15
5_a	22	0.0	28	0.0
5_b	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

Table 3
MIC of compounds 3_b, 5_b

	MIC (μg/ml)			
Sample	***BS***	***SA***	***EC***	***PV***
3_b	123	170	192	161
5_b	134	150	98	152
Amikacin	98	142	121	133

Table 4
Docking score energy and amino acid interactions for the docked compounds and the ligand.

Compound No.	**Score energy**	**Amino acid interaction**
3_a	-11.23	Val 605, Thr 352.
3_b	-13.90	Ala 602.
5_a	-10.34	Gln 348, Ser 303.
5_b	-17.72	Ala 602.
Glucosamine-6-phosphate	-17.13	Ala 602, Val 399, Thr 302, Ser 303, Gln 348, Ser 347, Ser 349, Thr 352.

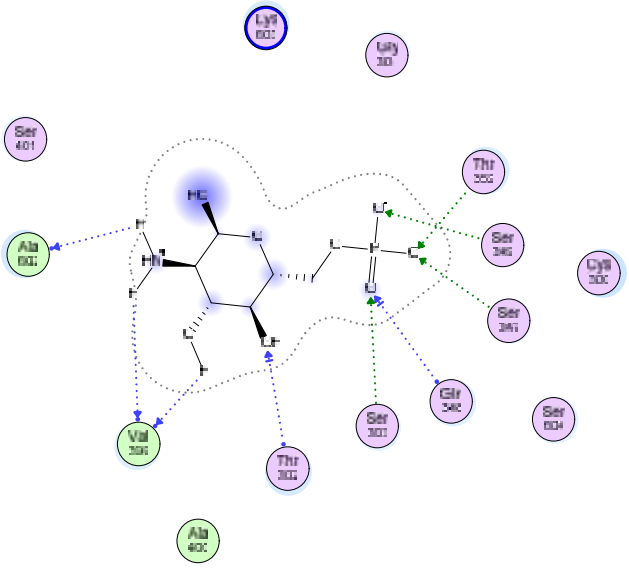
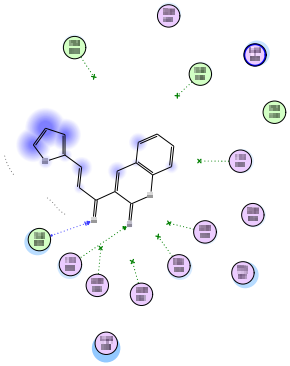
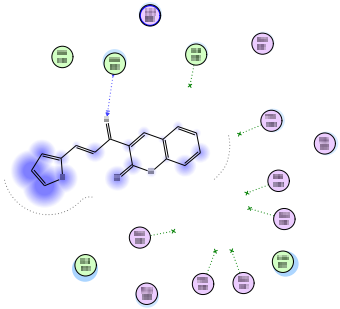


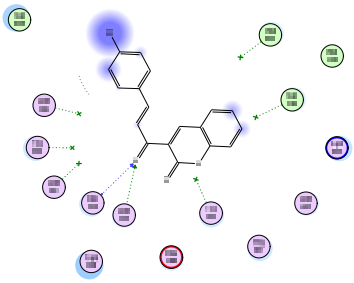
Fig. 1
Cocrystallized ligand with the active site of GlcN-6-P synthase. (S=- 17.13 Kcal/mol).



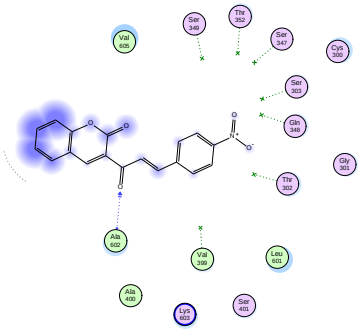
Compound 3_a (S= -11.23Kcal/mol)



Compound 3_b (S=-13.90 Kcal/mol)



Compound 5_a (S = -10.34 Kcal/mol)



Compound 5_b (S=-17.72 Kcal/mol)

Fig.2
Compounds 3a,b and 5a,b in the active site of GlcN-6-P synthase.

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