Synthesis of New Coumarin and 2-quinolone Derivatives with Expected Biological Activities

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Abstract

A series of new coumarin and N-amino-2-quinolone derivatives have been synthesized. The reaction of coumarin (1) with excess of Hydrazine hydrate 98% yielded 1-amino-2-quinolone (2), Compound (2) was reacted with different Sulfonyl chloride to yield Sulfonamides [N-(2-oxoquinolin-1(2H)-yl) methane sulfonamide (3), N-(2-oxoquinolin-1(2H)-yl) Benzene sulfonamide (4) and 4-methyl-N-(2-oxoquinolin-1(2H)-yl) benzene sulfonamide (5)], while reaction of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid (8) with different amines yielded compounds [2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(2-oxoquinolin-1(2H)-yl) acetamide (9) and N-(5-methyl-1,3,4-thiadiazol-2-yl)-2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetamide (10)] through amide linkage.The reactions and purity of the products were checked by TLC. The structures of the final compounds and their intermediates were confirmed by their melting points, IR spectroscopy, and elemental microanalysis.The coumarin and N-amino-2-quinolone derivatives were evaluated for their anti bacterial and antifungal activity.

Key words: coumarin , 1-amino-2-quinolone, sulfonamide, amide, biological activity.

تخليق مشتقات كومارين و ٢ – كوينولون جديدة ذات فعالية بايولوجية متوقعة

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الخلاصة

مجموعة جديدة لمشتقات الكومارين و ن- امينو -٢- كوينولون تم تصنيعها. تفاعل الكومارين (١) مع زيادة من الهيدرازين المائي ٩٨% ينتج ١- امينو -٢- كوينولون (٢)، المركب (٢) يتفاعل مع كلوريدات سلفونيل مختلفة لينتج سلفون امايد (٣،٥،٤)، بينما تفاعل 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid (٨) مع امينات مختلفة انتج المركبين (٩،١٠) خلال رابطة الامايد. تم مراقبة جميع التفاعلات والتأكد من نقاوة المركبات بواسطة كروماتوغرافيا الطبقة الرقيقة، كما تم متابعة المركبات الوسطية والمركبات النهائية وتمييزها من خلال قياس درجات الانصهار والتحليل الطيفي للاشعة تحت الحمراء، والتحليل الدقيق للعناصر. مشتقات الكومارين و ن- امينو -٢- كوينولون تم تقيم فعاليتها ضد الجراثيم و الفطريات.

الكلمات المفتاحية: كومارين ، ٢- كوينولون ، سلفون آمايد ،آمايد ، الفعالية البايولوجية .

Introduction

Heterocyclic chemistry is one of the largest areas of research in organic chemistry and it is growing rapidly. Of all published organic chemistry literature, papers on heterocyclic synthesis accounted for around 60 % in 1998, but nowadays the fraction is much larger considering that novel heterocyclic compounds are published in different fields such as biochemistry, pharmaceuticals, materials and others [(1)]. A similar trend is seen for coumarin, a heterocyclic system with a very large number of different derivatives[(2)]. Coumarin (also known as 1,2-benzopyrone or less commonly, as *o*-hydroxycinnamic acid-8-lactone), itself is a natural heterocyclic organic aromatic compound, present in a wide variety of microorganisms and higher plants [(3-5)], It was first isolated by Vogel in 1820 by extraction from tonka beans (*Dipteryx odorata*) specie previously known as *Coumarona odorata*, hence the term coumarin. It was subsequently identified in a large number of plants belonging to many different families. The diverse biological activities of natural and synthetic coumarin derivatives as anticoagulants and antithrombotics are well known, so that; they are effective for the prevention and treatment of venous and arterial thrombosis [(6)], Some of the coumarin derivatives are also reported as antifungal and antibacterial agents [(7)], antiviral and antitumor agents [(8)], lipid-lowering agents[(9)], anti-HIV agents [(10)],

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antioxidants and lipoxygenase inhibitor [11], They have also been found to possess antiproliferative, vasorelaxing activities [12], anti-inflammatory activity[13], anthelmintic, hypnotic, insecticidal activities, and diuretic properties [14].Quinolone is one of the most popular N-heteroaromatic compounds incorporated into the structures of many pharmaceuticals. Many quinolone-containing compounds exhibit a wide spectrum of pharmacological activities, such as antibacterial, antimalarial, antidepressant, anticancer and antioxidant activity [15]. Many sulfonamide derivatives were synthesized, characterized and tested for antibacterial[16], anti-carbonic anhydrase[17], mycobacterium tuberculosis[18], anti-inflammatory[19], anti-tumour[20] ,diuretic[21], and hypoglycemic properties[22].

Experimental Section

Materials and Methods

All the chemicals used in the synthesis were of analytical grade. The Melting points of the compounds and their intermediates were determined (uncorrected). Thin layer chromatography was performed and R_f values of the intermediates and final products which showed single round spots appeared after exposing the chromatograms to iodine vapor indicating the purity and the completion of the reactions. Determinations of infrared spectra were performed in KBr disc using FTIR spectrophotometer Shimadzu in the, College of Pharmacy, University of Baghdad and College of Science, University of Al-Mustanseriya. The elemental microanalysis of the synthesized final products was done in Cleveland clinical foundation learner research institute-France, by using Carlo Erba elemental microanalyzer. Thomas Hoover Electronic Melting Point Apparatus was used to determine all melting points reported in this work. The antimicrobial study of the synthesized final products was done in Al-Kindy college of Medicine / University of Baghdad.

Synthesis of 1-amino-2-quinolone (2) [23]

A solution of (1.46g, 0.01 mol) coumarin and excess hydrazine hydrate (98%) (5g, 0.1 mol) in absolute ethanol (25 mL) was refluxed for 24 h, the solvent was concentrated and the separated solid product was filtered and washed with cold ethanol, and recrystallized from chloroform, to give yellow crystals. The physical appearance, percentage yield, melting point and R_f values were listed in table 1, IR characteristics absorption bands were listed in Table 3.

Synthesis of N-(2-oxoquinolin-1(2H)-yl) methane sulfonamide (3) [24]

Compound (2) (0.65 g, 0.004mol) in dichloromethane (20mL) was stirred overnight at room temperature with methanesulfonyl chloride (0.48g, 0.004mol) in the presence of triethylamine (1.4mL, 0.01mol). The mixture was poured into a separatory funnel and washed with 100mL distilled water. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed with a rotary evaporator. The residue was purified by flash chromatography to give brown oily product, the physical appearance, percentage yield, and R_f values were listed in table 1, IR characteristics absorption bands were listed in table 3.

Synthesis of N-(2-oxoquinolin-1(2H)-yl) Benzene sulfonamide (4) and 4-methyl-N-(2- oxoquinolin-1(2H)-yl) benzene sulfonamide (5) [25]

To the mixure of compound (2) (0.65g, 0.004mol) in pyridine (10 mL) benzenesulfonyl chloride (0.7g, 0.004mol) for compound (4) and *p*- toluenesulfonyl chloride (0.76g, 0.004mol) for compound (5) were added drop wise at 0 ◦C. The resulting solution was stirred at room temperature for 5 h. At the end of this period, the reaction solution was poured into mixture of ice and concentrated hydrochloride acid and water. The precipitate was filtered, dried, and recrystallized from the ethanol: water to give pale yellow crystals, The physical appearance, percentage yield, melting point and R_f values were listed in table 1, IR characteristics absorption bands were listed in table 3.

Synthesis of ethyl-2-[(4-methyl-2-oxo-2H-chromen-7-yl)-oxy] acetate (7) [26]

Mixture of 7-hydroxy-4-methycoumarin (6) (1,76g, 0.01mol), ethyl bromoacetate (2.5g, 0.015 mol) and potassium carbonate (2.07g, 0.015mol) in dry acetone was refluxed for about 16 h. The mixture was filtered and solvent was removed under reduced pressure. The resulting solid was washed with excess of water. The crude product was purified by crystallization from ethanol to give off-white crystals. The physical appearance, percentage yield, melting point and R_f values were listed in table 1, IR characteristics absorption bands were listed in table 3.

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid (8) [26]

A solution of compound (7) (1.57g, 0.006mol) in ethanol (35mL) and 5% Sodium

hydroxide (6mL) was heated under reflux for 2 h. After cooling, the solution was evaporated to dryness and the residue was dissolved in water and acidified with diluted hydrochloric acid (PH 5-6). The white precipitate was filtered, dried and crystallized from ethanol to give off-white crystals. The physical appearance, percentage yield, melting point and R_f values were listed in table 1, IR characteristics absorption bands were listed in table 3.

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(2-oxoquinolin-1(2H)-yl) acetamide (9) [27]

To a stirred solution of compound (8) (0.7g, 0.003mol) in (20ml) of N,N-Dimethyl formamide (DMF), (0.48g, 0.003mol) of compound (2) was added, the mixture was cooled down to (-10°C) then (0.81g ,0.006mmol) of 1-hydroxy benzotriazole (HOBt) and (0.62g ,0.003mol) of N,N' Dicyclohexyl carbodiimide (DCC), were added with stirring, which was continued for 2days at 0°C and then at room temperature for 5days.The reaction mixture evaporated to exclude DMF and re dissolved in chloroform from which the N,N–Dicyclohexyl urea (DCU) was filtered off. The clear filterate washed twice with 5% sodium bicarbonate solution, 0.1N hydrochloric acid, once with water, and with saturated sodium chloride solution. The chloroform layer was dried with anhydrous magnesium sulphate and evaporated under vacuum; the resulted product was collected, recrystallized from (chloroform:ether) (1:1) , to give beige crystals, The physical appearance, percentage yield, melting point and R_f values were listed in table 1, IR characteristics absorption bands were listed in table 3.

Synthesis of N-(5-methyl-1,3,4-thiadiazol-2-yl)-2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetamide [10]

To a stirred solution of compound (8) (0.7g, 0.003mol) in (20ml) of N,N-Dimethyl formamide (DMF),(0.4g, 0.003mol) of 5-methyl-1,3,4-thiadiazole-2-thiol was added, the mixture was cooled down to (-10°C) then (0.81g, 0.006mmol) of1-hydroxy benzotriazole (HOBt) and (0.62g, 0.003mol) of N,N' Dicyclohexyl carbodiimide (DCC), were added with stirring, which was continued for 2days at 0°C and then at room temperature for 5days.Then complete the procedure as mentioned in the synthesis of compound **9**. A yellow crystal was obtained. The physical appearance, percentage yield, melting point and R_f values were listed in table 1, IR characteristics absorption bands were listed in table 3.

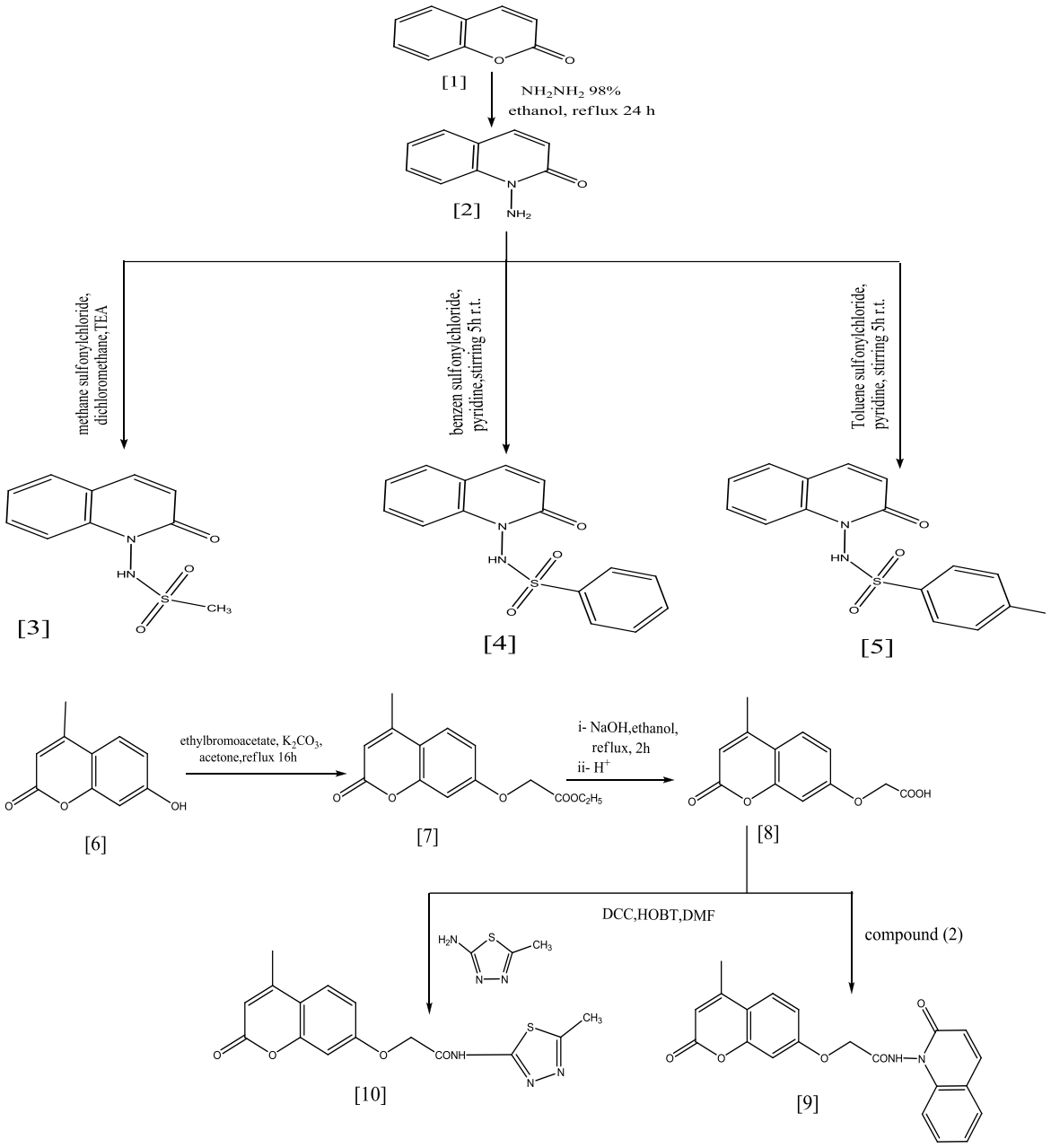
Antimicrobial activity [28]

The synthesized compounds were screened for their antibacterial activity against three strains of bacteria i.e. *Staphylococcus aureus*, beta-hemolytic-*Streptococcus pyogenes*, *proteus spp.* and two species of fungi i.e. *Aspergillus niger* and *Candida albicans* by disc diffusion method. Nutrient agar was used as culture medium for bacteria; blood agar was used for *Streptococcus pyogenes*, while Sabouraud dextrose was used for the fungal growth agar medium. Compounds were dissolved in DMSO at concentration 20µg/ml, 50µg/ml, 120µg/ml. Ofloxacin and ketoconozole was used as reference antibiotic and DMSO as control. The zones of inhibition were determined at the end of an incubation period of 24 hr at 35° C for bacteria and 5 days at 28° C for Fungi. Inhibition zone were measured.

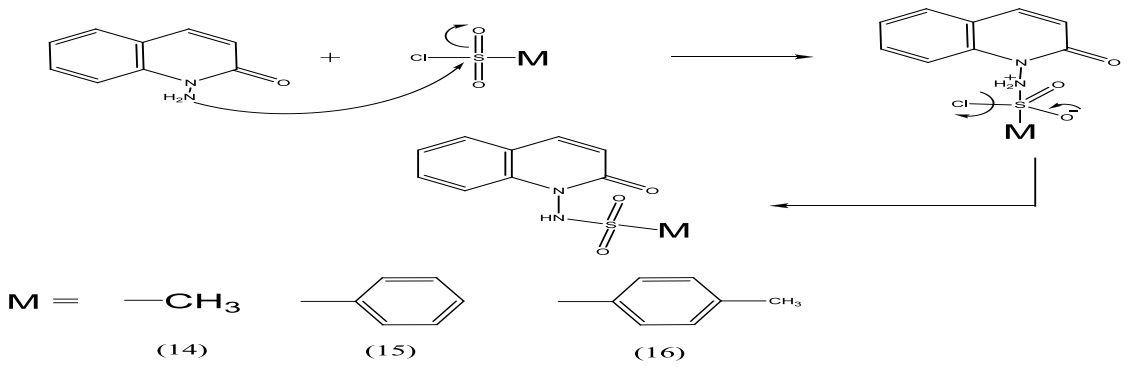
Results and Discussion

Synthesis of compounds (3,4 and 5)

Sulfonamides (compounds 3, 4 and 5) have been synthesized by reaction of compound (2) with methanesulfonyl chloride, benzenesulfonyl chloride and *p*-toluenesulfonyl chloride respectively, in dichloromethane and triethylamine as a base in case of compound (3), and in pyridine in case of compounds (4) and (5) . The reaction proceeds via nucleophilic attack of the amine on sulfur atom of the sulfonylchloride with liberation of HCl, as shown in the (Scheme 2).The structures of these compounds have been characterized by disappearance of symmetric and asymmetric absorption bands for (NH_2) of compound (2) and appearance of new absorption band in the synthesized compounds between (3242-3260) cm^{-1} belong to (NH) group. Other IR characteristics absorption bands were listed in Table (3), Melting points and R_f values were listed in Table (1), Elemental microanalysis was listed in Table (2).



Scheme 1:general scheme of the synthesized compounds.

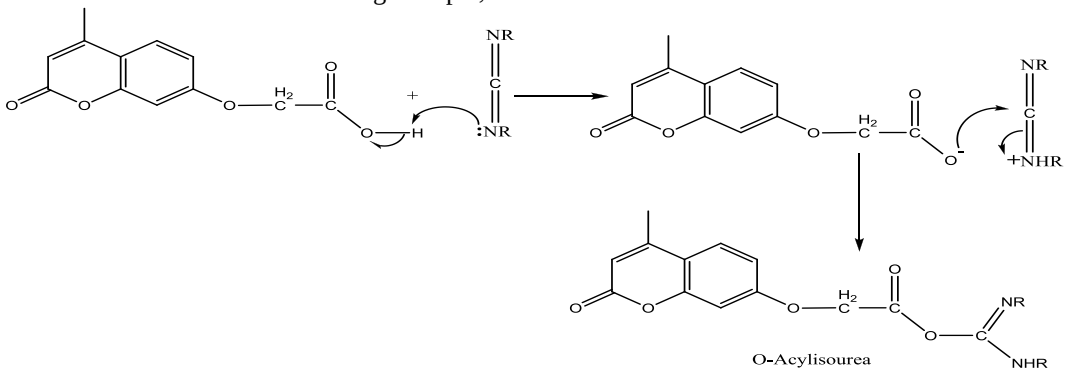


Scheme 2 :Mechanism of sulfonamide formation

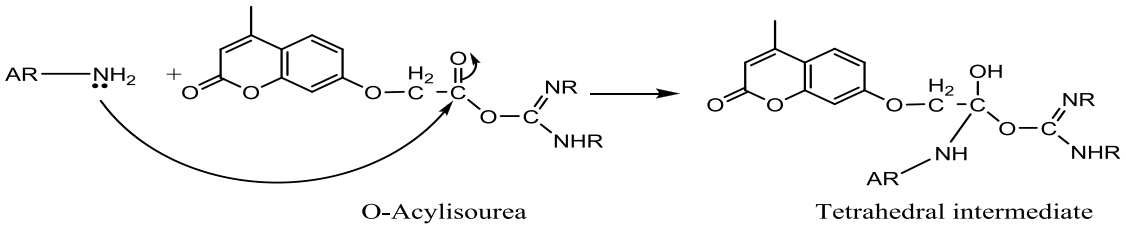
Synthesis of compounds [9 - 10]

In order to form amide bond between compound (8) and an appropriate amino compounds, the carboxyl group of compound (8) must be activated. Many different ways have been accomplished for this purpose. In this work the method used was the direct coupling with DCC/HOBt method. This method is characterized as being simple, efficient, and leading to a good yield at R.T.[29].The mechanism of amide bond formation by DCC promoted condensation of carboxylic acid and amine.

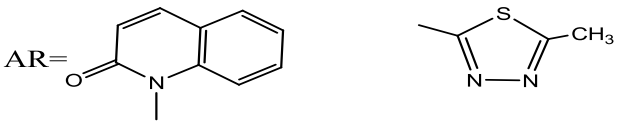
Step 1: in the first stage of the reaction, the carboxylic acid adds to one of the double bonds of DCC to give an O–acylisourea



Step 2: Structurally, O–acylisoureas resemble carboxylic acid anhydrides and are powerful acylating agents. In this stage the amine adds to the carbonyl group of the O–acylisourea to give a tetrahedral intermediate.



Step 3: The tetrahedral intermediate dissociates to amide and N, N–dicyclohexyl urea (DCU).



(9) (10)

Scheme 3: mechanism of amide formation.

The structures of these compounds have been characterized by disappearance of absorption bands for (C=O) and (OH) of –COOH of compound (8) and appearance of new absorption band in synthesized compounds between (3227-3229) cm^{-1} belong to (NH) group of 2^o amide. Other IR characteristics absorption bands were listed in table (3), melting points and R_f values were listed in table (1), elemental microanalysis was listed in table (2). The IR spectra of the synthesized compounds showed a characteristic bands of absorption which were in consistence with the chemical structures of the proposed compounds. All new compounds were analyzed for C, H, N, O and S and the results are in acceptable range. Uncorrected melting points of the compounds (3-5), (9 and 10) and their intermediates were determined and were found to be different from melting points of their starting materials. As shown in table (1).

Table 1: Physical appearance, percentage yield, melting points and R_f values of intermediates and compounds.

Compound No.	Physical appearance	% Yield	Melting point ^oC	R_f Value
2	yellow crystal	75	130-132	0.20 A
3	Brown oily product	45	-	0.89 B
4	Pale Yellow powder	58	151-153	0.84 B
5	Pale Yellow powder	55	158-160	0.79 B
7	Off-white crystal	81	93-95	0.8 A
8	Off-white crystal	67	204-206	0.70 D
9	Beige crystal	75	188-189	0.87 C
10	Yellow crystal	66	192-193	0.33 A

A: (Chloroform 9:1 Methanol) B: (Water 1:1 Methanol) C: (Chloroform 1:1 Methanol)
D: (Chloroform 3: Methanol 3: Ether 4)

Table 2: Elemental analysis % of the final products

Cpd No.	Molecular weight	Chemical Formula	Calculated/Found				
			C	H	N	O	S
4	**300.33**	$C_{15}H_{12}N_2O_3S$	59.99 60.20	4.03 4.18	9.33 9.57	15.98 15.47	10.68 11.18
5	**314.36**	$C_{16}H_{14}N_2O_3S$	61.13 60.06	4.49 4.61	8.91 8.69	15.27 15.42	10.20 10.78
9	**376.36**	$C_{21}H_{16}N_2O_5$	67.02 66.37	4.28 4.39	7.44 7.25	21.26 21.62	
10	**331.35**	$C_{15}H_{13}N_3O_4S$	54.37 54.81	3.95 4.03	12.68 12.72	19.31 19.05	9.68 9.30

Table 3: The characteristic IR bands of synthesized compounds.

Compound No.	Characteristic IR bands Cm^{-1}
2	(3299, 3200 NH_2 Str.) , (1643 C=O Str.), (1595, 1452 C=C_{ar}. Str.), (3045 C-H_{ar}.Str.) (1242 C-N Str.).
3	(3260 N-H Str.), (3024 CH_{aro} Str.), (2935$_{assy}$, 2865$_{sy}$ C-$H_{aliph.}$ Str.), (1681 C=O Str. quinolone), (1614 - 1454 C=C_{ar} Str.), (1352$_{assy}$, 1161$_{sy}$ S=O Str.), (792,769 CH_{aro} out of plane).
4	(3242 N-H Str.), (3099, 3066 CH_{aro} Str.), (1685 C=O Str. quinolone), (1616 - 1450 C=C_{ar} Str.), (1340$_{assy}$, 1166$_{sy}$ S=O Str.), (756-688 CH_{ar} out of plane).
5	(3254 N-H Str.), (3024 CH_{aro} Str.), (2935$_{assy}$, 2865$_{sy}$ C-$H_{aliph.}$ Str.), (1699 C=O Str. quinolone), (1597 - 1456 C=C_{ar} Str.), (1340$_{assy}$, 1165$_{sy}$ S=O Str.), (813,758 CH_{ar} out of plane).
7	(3076 CH_{aro} Str.), (2980$_{assy}$, 2872$_{sy}$ C-$H_{aliph.}$ Str.), (1759 C=O Str. ester), (1708 C=O Str. coumarine), (1606 - 1508 C=C_{ar} Str.), (1197 C-O Str. ester), (1220$_{assy}$, 1062$_{sy}$ Ar-O-C str.).
8	(3300-2500 OH str. Of COOH), (3068 CH_{aro} Str.),(2987$_{assy}$, 2916$_{sy}$ C-$H_{aliph.}$ Str.), (1732 C=O Str. coumarin), (1717 C=O Str. COOH), (1618 - 1510 C=C_{ar} Str.), (1253 C-O Str. ester), (1207$_{assy}$, 1080$_{sy}$ Ar-O-C str.).
9	(3329 NH str.), (1714 C=O coumarin), (3040 CH_{ar} str.), (2929 $_{assy.}$, 2852 $_{sy}$ $CH_{aliph.}$ Str.), (1693 C=O amide), (1627-1514 C=C str.), (1573 NH bend. amide II), (1153 C-O str.), (754CH_{ar} out of plane).
10	(3329 NH str.), (1724 C=O coumarin), (3060 CH_{ar} str.), (2928 $_{assy.}$, 2852 $_{sy}$ $CH_{aliph.}$ Str.), (1696 C=O amide), (1626 C=N str.), (1573 NH bend. amide II), (1149 C-O str.), (719 CH_{ar} out of plane).

(Str. = stretching vibration , ar = aromatic , aliph.= aliphatic, bend. = bending vibration.)

Antimicrobial Activity

The newly synthesized compounds were screened for their antimicrobial activity. From the result in Table 4, Compounds 10 and 4 showed good activity against *Staphylococcus aureus* while compounds 5, and 10 show moderate activity against *streptococcus pyogenes* when 2µg/ml conc. was used. At conc. 50 µg/ml compound 10 showed significant activity against *Staphylococcus aureus.* while compounds 4 and 9 showed moderate activity. At conc. 120 µg/ml compound 10 demonstrated good activity against *Staphylococcus aureus.* While compounds 9 and 4 showed moderate activity against *Staphylococcus aureus* while all tested compounds show low to no activity against *streptococcus and proteus spp.* when compared to Oflxacin. While compound 10 showed good activity against *Aspergillus niger* and compounds 4, 5, and 9 showed moderate activity against *Aspergillus niger*. All remaining compounds demonstrated moderate to low activity against *Candida albicans* when compared to Ketoconazole.

Table 4: Antimicrobial screening data (zone of inhibition in mm) for final compounds

Compound No.		Zone of Inhibition in mm				
		Staphy. aureus	**Strept. pyogenes**	**Proteus spp.**	**Aspergillus niger**	**Candidia albicans**
4	2µg/ml	9	7	7	/	/
	50µg/ml	12	11	7	/	/
	120µg/ml	15	13	15	18	15
5	2µg/ml	8	9	No activity	/	/
	50µg/ml	12	11	9	/	/
	120µg/ml	14	14	12	17	18
9	2µg/ml	7	7	No activity	/	/
	50µg/ml	13	12	8	/	/
	120µg/ml	16	14	16	18	16
10	2µg/ml	10	9	No activity	/	/
	50µg/ml	18	12	8	/	/
	120µg/ml	19	17	16	20	12
Ofloxacin	2µg/ml	11	12	11		
	50µg/ml	16	18	16		
	120µg/ml	22	23	22		
Ketoconazole	120µg/ml				26	36

Conclusion

The synthesis of these proposed compounds was successfully achieved by following the stated procedures as previously described. The results obtained from this investigation indicated that the strategy adapted for the synthesis of the designed derivatives was successful, since the conformity of synthesized compounds was achieved according to the data shown by the physical and chemical analysis including (TLC, melting point, FT-IR and Elemental analysis (CHNSO). Most of these compounds show good antimicrobial activity comparable with marketable compounds.

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