Potential antibacterial activity of coumarin and coumarin-3-acetic acid derivatives

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Abstract: Coumarin and coumarin-3-acetic acid derivatives were synthesized by reacting phenols with malic acid, ethyl acetoacetate and ethyl acetylsuccinate in appropriate reaction conditions. All synthesized compounds were subjected to test for their antimicrobial activities against variety of gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative bacterial stains (*Shigella sonnei, Escherichia coli*) by agar dilution method. Several of them exhibited appreciable good antibacterial activity against the different strains of gram positive and gram negative bacteria. These findings suggest a great potential of these compounds for screening and use as antibacterial agents for further studies with a battery of bacteria.

Keywords: Coumarin-3-acetic acid, Coumarins, antibacterials, Minimum inhibition concentration.

INTRODUCTION

Coumarins belong to an important class of compounds found in plants and are responsible for the characteristic odour. Their fame is mainly because of their anti-thrombotic, anti-inflammatory and vasodilatory, activities (Thastrup *et al*., 1985; Piller, 1975; Namba *et al*., 1988). Among them warfarin is a well-known coumarin which is used as an oral anticoagulant and, particularly as a rodenticide (Keating and O'Kennedy, 1977). Coumarins are highly toxic in rodents but it is observed that such toxic coumarin derivatives may be excreted without any harm in the urine of humans (Weinmann, 1997).

Coumarins have an indirect negative effect on infections by stimulation of macrophages. Hydroxylated derivatives of coumarin are called phytoalexins, mainly produced in carrots in response to fungal infection and can be presumed to have antifungal activity (Hoult and Paya, 1996). Coumarin and coumarin-related compounds proved to have significant therapeutic potential. They have been extracted from a wide variety of natural source particularly from plants. A vast number of coumarin derivatives have been isolated from the plants of Umbelliferae, Rutaceae and Compositae families. Their vital role in plants and animal biology has not been fully exploited. Coumarins possess diverse biological properties along with effects on the different cellular systems (Nofal *et al*., 2000; Murray *et al*., 1982).

Coumarins possess wide spread applications like antibacterial, antifungal, insecticidal, antiviral, anti-leishmaniasis, immuno-modulatory and antitumor activities (Brooker *et al*., 2000; Lee *et al*., 2007; Napolitano *et al*., 2004; Xu *et al*., 2001). The hydroxycoumarins act as potent metal chelators, free radical scavengers and also as powerful antioxidants by chain-breaking process (Jurd *et al*., 1971). The present studies were conducted to synthesize various derivatives and monitor the antibacterial activity of these molecules.

MATERIALS AND METHODS

All chemicals were purchased from Sigma Aldrich (United Kingdom) and Merck (Pakistan). Some inorganic chemicals were purchased from Scharlau (France). The solvents were purchased from Fluka (Germany). Household microwave oven (Dawlance DW-162, 1000 W, 2450 MHz) was used for microwave irradiations.

Synthesis

Coumarins

Coumarins were synthesized by the reported method (Chattha *et al*. 2008, Frere *et al*., 2001). A mixture of a phenol, malic acid/ethyl acetoacetate was heated under microwave irradiation for appropriate time (Table 1). After cooling the reaction contents were poured over crushed ice. The precipitates were filtered and recrystallised from ethanol

Coumarin-3-acetic acids

The method of Dey and Sankaranarayanan was used for the synthesis of coumarin-3-acetic acid (10). Salicylaldehyde was reacted with succinic anhydride in the presence of dried sodium succinate to form coumarin-3-acetic acid. Coumarin-3-acetic acid was nitrated to form 6-nitrocoumarin-3-acetic acid (11), then reduced to form 6-aminocoumarin-3-acetic acid (12, table 2).

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4-Methylcoumarin-3-acetic acids

Different derivatives (table 2, 13-19) were prepared by the method of Dey and Sankaranarayanan by reacting phenols with diethyl acetylsuccinate. Diethyl acetylsuccinate was mixed with different phenols, and reaction contents were poured into the cold concentrated sulfuric acid in the form of thin stream. On cooling and acidifying, the precipitates were filtered and crystallized from ethanol.

Antibacterial activity

Antibacterial activity was performed in sterile 96-wells microplates under aseptic environments**.** The method is based on the principle that microbial cell number increases as the microbial growth proceeds in the log phase of growth which results in increased absorbance of broth medium (Patel *et al*. 2009; Kaspady *et al*. 2009). Two gram-negative (*Shigella sonnei*, *Escherichia coli*) and two gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) were included in the study. The organisms were grown on stock culture agar. The test samples (20 µg/well) with suitable solvent and dilution were pipetted into wells. Overnight-maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm ($1x10^{-5}$ CFU). The total volume in each well was kept to 200 µL. The incubation was done at 37°C for 16-24 hours with lid on the microplate. After shaking, the absorbance was measured at 540 nm using Synergy HT BioTek® USA microplate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula: Inhibition (%) = 100 (X-Y) /X where X is absorbance in control with bacterial culture and Y is

Table 1: Optimization of reaction conditions and physical properties of coumarins

No.	Compound	Phenol	Reactant	Yield (%)	M. P. (°C)	M. P.$^{lit.}$ (°C)
1	7-methoxy coumarin	*m*-methoxy phenol	malic acid	71	116	117-8
2	6-hydroxy coumarin	hydroquinone	malic acid	72	248-250	250
3	7-hydroxy coumarin	resorcinol	malic acid	64	228	228-230
4	7-methyl coumarin	*m*-cresol	malic acid	96	128	128-130
5	6-methyl coumarin	*p*-cresol	malic acid	60	74	73-78
6	4-methyl coumarin	phenol	diethyl acetoacetate	50	83	83-85
7	4,6-dimethyl coumarin	*p*-cresol	diethyl acetoacetate	54	151-152	150
8	7-methoxy-4-methyl coumarin	*m*-methoxy phenol	diethyl acetoacetate	67	156-157	156-158
9	6-hydroxy-4-methyl coumarin	hydroquinone	diethyl acetoacetate	56	244	245

Table 2: Optimization of reaction conditions and physical properties of coumarin-3-acetic acids

No.	Compound	Reactant 1	Reactant 2	Yield (%)	M. P. (°C)	M. P. $^{lit.}$ (°C)
10	coumarin-3-acetic acid	salicylaldehyde	Succinic anhydride	68	154	157-158
11	6-nitrocoumarin-3-acetic acid	coumarin-3-acetic acid	HNO_3/H_2SO_4	92	208-210	-
12	6-aminocoumarin-3-acetic acid	6-nitrocoumarin-3-acetic acid	Fe/HCl	40	196-198	-
13	7-hydroxy-4-methylcoumarin -3-acetic acid	resorcinol	diethyl acetylsuccinate	56	263-265	268-269
14	5-hydroxy-4,7-dimethylcoumarin-3-acetic acid	orcinol	diethyl acetylsuccinate	63	257-8	259
15	4,6-dimethylcoumarin-3-acetic acid	*p*-cresol	diethyl acetylsuccinate	25	177-178	180
16	4,7-dimethylcoumarin-3-acetic acid	*m*-cresol	diethyl acetylsuccinate	46	202	203
17	7-methoxy-4-methylcoumarin-3-acetic acid	*m*-methoxy phenol	diethyl acetylsuccinate	46	195-196	199
18	7-chloro-4-methylcoumarin-3-acetic acid	*m*-chloro phenol	diethyl acetylsuccinate	22	>300	-
19	7-bromo-4-methylcoumarin-3-acetic acid	*m*-bromo phenol	diethyl acetylsuccinate	26	Dec.287	-

absorbance in test sample. Results are mean of triplicate (n=3, mean ± sem). Ciprofloxacin and moxifloxacin were taken as standard. Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5-30µg/ well) and results were calculated using EZ-Fit5 Perrella Scientific Inc. Amherst USA software and data expressed as MIC_{50} and $MIC_{90.}$

RESULTS

All synthesized coumarin and coumarin-3-acetic acid derivatives were tested by agar dilution method for their minimal inhibitory concentration MIC_{50} and MIC_{90}. Antimicrobial activity of the of compounds along with standard drugs (ciprofloxacin, moxifloxacin) is given in table 3 and table 4 with variety of gram positive (*Bacillus subtilis, Staphylococcus aureus*) and gram negative bacterial strains (*Shigella sonnei, Escherichia coli*) .

Minimal inhibitory concentration values shows that experimental compounds were found less active in comparison to standard drug ciprofloxacin but showed parallel activity to moxifloxacin. From table 3, it can be concluded that all the compounds have displayed good activity against *B. subtilis*. Compounds were enough active against this bacterial strain especially 7-Methoxy coumarin (1), 7-Methyl coumarin (4), 6-methylcoumarin (5), 4,6-Dimethylcoumarin (7), 7-methoxy-4-methylcoumarin (8) and 7-methoxy-4-methylcoumarin-3-acetic acid (17). 6-Methylcoumarin (5), 7-methoxy-4-methylcoumarin (8) and 6-aminocoumarin-3-acetic acid (12) showed excellent activities against *B.* subtilis even better than standard drug amoxifloxacin.

6-Hydroxycoumarin (2), 6-nitrocoumarin-3-acetic acid (11) and 7-methoxy-4-methylcoumarin-3-acetic acid (17) also showed very good activity against *S. sonnei*, 4,6-Dimethylcoumarin (7), 6-hydroxy-4-methylcoumarin (9) and 17 are highly active against *E. coli* while 6-methylcoumarin (5), coumarin-3-acetic acid (10), 6-nitrocoumarin-3-acetic acid (11), 6-aminocoumarin-3-acetic acid (12) and 7-hydroxy-4-methylcoumarin-3-acetic acid (13) are comparable to standard drug as shown in table 4. 7-Hydroxycoumarin (3), 7-methoxy-4-methylcoumarin (8), coumarin-3-acetic acid (10) and 6-nitrocoumarin-3-acetic acid (11) possessed good activity against *S. aureus*.

In comparison to standard drug compounds 7-Hydroxy coumarin (3), 4-Methylcoumarin (6), 7-Hydroxy-4-methylcoumarin -3-acetic acid (13), 5-Hydroxy-4,7-dimethylcoumarin-3-acetic acid (14), 4,7-Dimethylcoumarin-3-acetic acid (16) and 7-Bromo-4-methylcoumarin-3-acetic acid (19) showed little or no appreciable activity against any of the gram positive and gram negative bacteria strain. Particularly compound 6 and 16 were highly inactive against all bacterial strains.

DISCUSSION

Antimicrobial activities of coumarins have been presented by several workers. Ojala *et al*. described that coumarin as phytoalexins compounds produced in plants as defense on attack by other organisms or when it gets wounded. They prepared methanol extracts from number of plants and studied antimicrobial effects of pure compounds and were merely observed against plant pathogens and proved the role of coumarins and furanocoumarins as defensive compounds. Similarly, Widelski *et al*. analyzed plants extracts and isolated constituents from *A. lucida* and evaluated their antimicrobial activity against six gram positive and negative bacteria, two oral pathogens and three human pathogenic fungi. Data exhibited an interesting antimicrobial profile. All compound showed

MIC values higher than the standard drug amoxicillin used in the studies.

From table 3, it is shown that all the compounds have displayed maximum activity against *B. subtilis*. Similarly, other compounds have shown antibacterial activity against two or more pathogens except 16 which exhibited no activity against any of the bacteria used in the study. All compound showed MIC value higher than the standard drug amoxicillin. According to our experiment, 6-methylcoumarin (5) and 7-methoxy-4-methylcoumarin (8) showed MIC value slightly higher than ciprofloxacin drug, hence proved to be good antimicrobials. It is seen that less polar group on coumarin exerts better antimicrobial effect as compared to the coumarin groups with polar substituents.

Coumarins and coumarin-3-acetic acids antimicrobial activity are comparable to standard drug ciprofloxacin the compounds are less active, while other drugs moxifloxacin have parallel activity. From table 3, it can be concluded that all the compounds have displayed maximum activity against *B. subtilis*. The compounds 6-hydroxycoumarin (2), 6-nitrocoumarin-3-acetic acid (11) compounds and 17 also showed very good activity against *S. sonnei*, while 6-methylcoumarin (5), 7-methoxy-4-methylcoumarin (8) 6-aminocoumarin-3-acetic acid (12) showed good activities against *B. subtilis*. The compounds 4,6-dimethylcoumarin (7), 6-hydroxy-4-methylcoumarin (9) and 7-methoxy-4-methylcoumarin-3-acetic acid (17) are highly active against *E. coli* while 5, coumarin-3-acetic acid (10), 6-nitrocoumarin-3-acetic acid (11), 6-aminocoumarin-3-acetic acid (12) and 7-hydroxy-4-methylcoumarin-3-acetic acid (13) are comparable to standard drug as shown in table 4. 4-Methylcoumarin (6), coumarin-3-acetic acid (10), 6-nitrocoumarin-3-acetic acid (11) and 6-aminocoumarin-3-acetic acid (12) possessed good activity against *S. aureus*.

Table 3: Measurement of antibacterial activity of coumarin and coumarin-3-acetic acid derivatives for *B. subtilis* and *S. sonnei* at MIC_{50} and MIC_{90} (µg/mL). Data is n = 3, mean ± sem.

Compound	*B. subtilis*		*S. sonnei*	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
1	12.76±0.10	34.13±0.15	17.43±0.24	28.36±0.23
2	17.40±0.29	29.79±0.28	10.78±0.09	26.81±0.16
3	13.70±0.12	34.13±0.11	17.40±0.28	29.79±0.19
4	12.78±0.11	30.31±0.27	17.10±0.17	28.21±0.23
5	10.53±0.10	28.42±0.10	15.52±0.11	28.69±0.16
6	-	-	-	-
7	14.57±0.31	31.95±0.45	12.52±0.79	29.12±0.54
8	11.76±0.11	28.36±0.23	13.47±0.02	27.79±0.28
9	-	-	-	-
10	13.57±0.27	34.13±0.15	12.52±0.07	29.12±0.54
11	13.47±0.21	27.79±0.24	11.46±0.28	27.25±0.11
12	11.76±0.16	28.02±0.10	14.28±0.24	34.61±0.27
13	13.08±0.11	28.62±0.19	15.33±0.27	28.32±0.23
14	13.19±0.28	31.40±0.19	15.33±0.21	38.11±0.03
15	13.92±0.19	27.52±0.18	12.51±0.27	29.26±0.27
16	-	-	-	-
17	12.11±0.27	32.85±0.29	10.10±0.27	34.19±0.27
18	16.23±0.14	25.61±0.37	12.11±0.27	36.27±0.27
19	18.00±0.16	29.03±0.06	-	-
Ciprofloxacin	8.36±0.12	20.07±0.24	7.31±0.08	24.87±0.26
Moxifloxacin	13.66±0.04	34.98±0.26	11.98±0.11	34.34±0.24

Table 4: Measurements of antibacterial activity of coumarin and coumarin-3-acetic acid derivatives for *E. coli* and *S. aureus*, at MIC_{50} and MIC_{90} (µg/mL). Data is n = 3, mean ± sem.

Compound	*E. coli*		*S. aureus*	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
1	14.28±0.15	34.61±0.21	17.40±0.21	28.74±0.26
2	13.57±0.21	34.13±0.16	15.52±0.19	28.67±0.18
3	14.08±0.24	31.95±0.18	10.78±0.46	20.04±0.39
4	11.50±0.29	28.62±0.14	17.43±0.13	39.11±0.13
5	14.57±0.26	30.67±0.15	13.47±0.52	35.69±0.49
6	-	-	-	-
7	11.31±0.27	26.55±0.16	12.52±0.11	32.04±0.12
8	12.76±0.22	27.23±0.62	11.36±0.20	27.70±0.11
9	17.21±0.22	38.84±0.48	-	-
10	13.19±0.25	28.02±0.42	11.19±0.42	27.58±0.31
11	12.52±0.32	29.12±0.36	10.81±0.17	34.96±0.18
12	13.08±0.14	28.38±0.14	15.33±0.32	29.15±0.28
13	15.52±0.16	28.69±0.15	20.57±0.18	59.13±0.17
14	12.51±0.15	29.26±0.24	19.10±0.11	47.37±0.21
15	13.72±0.17	27.52±0.12	12.51±0.31	38.03±0.26
16	-	-	-	-
17	15.33±0.14	28.32±0.25	-	-
18	-	-	15.20±0.12	30.08±0.18
19	-	-	-	-
Ciprofloxacin	8.21±0.03	23.77±0.03	9.42±0.037	26.24±0.03
Moxifloxacin	11.32±0.22	31.09±0.22	10.65±0.25	34.61±0.35

CONCLUSIONS

In the present studies, 6-methylcoumarin and 7-methoxy-4-methylcoumarin showed MIC value even less than moxifloxacin but slightly higher than ciprofloxacin. It is seen that less polar groups on coumarin exert better antimicrobial effect as compared to the coumarin groups with polar substituents. Coumarin and coumarin-3-acetic acids antimicrobial activities are comparable to standard drug.

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