Synthesis, Characterization and Antibacterial Activity of Novel Coumarin

TIAN-LIN ZHANG^{1,*}, HUI-MIN MA^{1,2}, CAI-YAN ZHU^{1,2}, JIA-JIA WANG^{1,2} and BING-BING WANG³

¹Department of Chemical Engineering, Huaihai Institute of Technology, Lianyungang 222005, P.R. China

²School of Chemical Engineering & Technology, China University of Mining and Technology, Xuzhou 221116, P.R. China

³College of Environment and Spatial Informatics, China University of Mining and Technology, Xuzhou 221116, P.R. China

*Corresponding author: E-mail: mhmwbb@163.com

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The novel coumarin derivative based quaternary ammonium salt had been designed and synthesized in two steps starting from 5-chloromethylsalicylaldehyde. 5-Chloromethylsalicylaldehyde first reacted with tertiary amine to yield the quaternary ammonium salt, then this compound reacted with the diethyl malonate on the appropriate condition to yield the corresponding coumarin derivative. The chemical structure had been elucidated by infrared spectra, ¹H NMR spectra, LC-MS and melting point analysis. The novel coumarin had been evaluated for its antibacterial activities *in vitro* against four gram-negative bacteria (*Edwardsiella tarda*, *Aeromonas hydrophila*, *Vibrio anguillarum* and *Vibrio harveyi*). The bioactive assay showed that the title compound displayed comparable antibacterial efficacy in comparison with some of other reference drugs.

Key Words: Coumarin, 5-Chloromethylsalicylaldehyde, Quaternary ammonium salt, Antibacterial activity.

INTRODUCTION

Coumarin and its derivatives are an important class of compounds, widely present in nature. They have drawn much attention due to their optical property and bioactivity, such as antibacterial^{1,2}, antifungal, antituberculosis³, antioxidant⁴ and anticancer activities⁵. Therefore, they are widely used in dyes and drugs^{6,7}. Hitherto, numerous coumarin derivatives and their applications have been reported. Liu *et al.*⁸ have synthesized a series of coumarin derivatives successfully and some of the synthesized compounds exhibited significant inhibitory effect on the diphenolase activity of mushroom tyrosinase. The metal complexes of coumarin also showed good antibacterial activity⁹⁻¹¹ on gram-negative and gram-positive bacterial strains.

It's well known that most bacterial cell walls are negatively charged, containing phosphatidylethanolamine (70 %) as the major component, hence most antimicrobial agents are positively charged. As early as the 1930s quaternary ammonium compounds were found to have antimicrobial activities, they have been developed as the antimicrobial agents¹²⁻¹⁵. In this regard agents with quaternary ammonium may enhance the coumarin's antibacterial potency. We have synthesized the novel coumarin with a quaternary ammonium group at the 7 position in the coumarin ring (Fig. 1) and the results of antibacterial activity have been discussed in this paper.

Fig. 1. Novel coumarin containing quaternary ammonium salt

EXPERIMENTAL

5-Chloromethylsalicylaldehyde (5-CS) was synthesized according to our previous work¹⁶. All reagents for synthesis obtained commercially without further purification.

Preparation of N,N,N-triethyl-N (3-hydroxy-4-formyl) benzyl ammonium chloride (1): Triethylamine (10.1 g) was dropwise added into the ethyl acetate solution (50 mL) containing 5-chloromethylsalicylaldehyde (5-CS) (17.05 g) in a 3-necked flask, then was stirred at the room temperature for 12 h. The white powder product was filtered off. After being dried under vacuum at 50 °C for 48 h, 24.15 g of product containing the quaternary ammonium and salicylaldehyde units was obtained. Then the crude was recrystallized from ethanol to yield needle product.

Synthesis of novel coumarin: The title compound was synthesized from **1** and diethyl malonate. To a 4-necked flask, **1** (15 g) was dissolved in the heated ethanol (50 mL), diethyl malonate (8.5 g) and 1 mL of piperidine and acetic acid was

dropwise added into the solution. And then the mixture was reflux for 5 h. The white needle crystal was filtrated off. Then dried under vacuum at 50 °C for 48 h, 3.54 g of product was obtained (**Scheme-I**).

Scheme-I: Routes to synthesize the the novel coumarin

^1H NMR was recorded with Bruker AV-400MHz spectrometer ($\text{C}_2\text{D}_6\text{SO}$), chemical shifts (δ values) are given in parts per million with tetramethylsilane as an internal standard. Fourier transform infrared spectra were recorded on a Nicolet MAGNA-IR 550 (II) spectrophotometer using KBr pellets. Mass spectra were carried on Agilent MassHunter. The melting point was carried on SGW X-4 microscopic melting point meter.

RESULTS AND DISCUSSION

Structural characterization: Parameters of the novel coumarin: m.p. 199-200 °C; ^1H NMR (D_2O , 400 MHz) δ : 1.18-0.92 (m, 12H), 3.05(q, $J = 7.3$ Hz, 6H), 4.70 (s, 4H), 6.9-7.3 (m, 4H); IR (KBr, ν_{max} , cm^{-1}) 2995, 2950, 2850, 2750, 1750, 1595, 1495, 1490, 1400, 1250; MS (175.0 eV) m/z (%): 391.2844 ($\text{M} + \text{Na}^+$, 7.92); 318.3004 (100); 274.2742 (90); 149.0232 (48.96).

This coumarin derivative is white crystal (m.p. 199-200 °C). As seen from Fig. 2, sharp peaks at 2995, 2950, 2850, 2750 cm^{-1} are due to C-H stretch vibration. The strong band at 1750 cm^{-1} is assigned to the C=O stretch vibration. The peaks at 1595, 1495, 1490 and 1400 cm^{-1} were due to the C=C stretch vibration in the benzene ring. The sharp peak at 1250 cm^{-1} is assigned to C-O (in lactone) stretch vibration.

Fig. 2. IR spectra of the title compound

The chemical shifts at δ 6.9-7.3 (m, 4H) were assigned to the H in the benzene ring and the heterocyclic. Then the chemical shifts at 4.70 (s, 4H) were due to the CH_2 which are connected with the N or O atom while the other three $-\text{CH}_2-$ connected with $-\text{CH}_3$ were at 3.05 (q, $J = 7.3$ Hz, 6H). And these H on the $-\text{CH}_3$ were at 1.18-0.92 (m, 12H).

As seen from Table-1, the ion at m/z 391.2844 is diagnostic for the presence of a Na^+ in the molecular. The ion at m/z 318.3004 is due to the loss of the Cl and a CH_3 , while the ion at m/z 274.2742 was originated from the loss of a methyl and an ethyl radical furthermore.

TABLE-1
MASS SPECTRA DATA OF THE TITLE COMPOUND

Peak number	Abundance	Abundance (%)	m/z
1	89326.8	10.81	108.0576
2	404745.3	48.96	149.0232
3	76526.1	9.26	205.0858
4	69777.6	8.44	279.159
5	255481	30.91	301.1411
6	65485.2	7.92	391.2845
7	744981.3	90.12	274.2742
8	826650.6	100	318.3006
9	155593.7	18.82	319.3038
10	291184.8	35.22	362.3267

Antibacterial activity

Biological screening: The coumarin was screened for its ability to inhibit the growth of a number of gram-negative bacterial strains *in vitro*. The gram-negative strains studied were *Edwardsiella tarda*, *Aeromonas hydrophila*, *Vibrio anguillarum* and *Vibrio harveyi*. Whilst a number of coumarin-based compounds have previously shown good antibacterial activities¹⁷⁻²¹, non of current coumarin-based compounds containing quaternary ammonium salt antibacterial activity had been checked.

Minimum inhibitory concentration: Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents²². A lower MIC is an indication of a better antimicrobial agent. A MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. The minimum inhibitory concentration (MIC) of this coumarin to 4 selected strains tested with cuvette double dilution method, and then concentration of this coumarin was range from 5-0.15 mg/mL. The MICs of the title compound against bacterium are: 0.31 mg/mL for *Edwardsiella tarda*, 0.625 mg/mL for *Aeromonas hydrophila*, 0.15 mg/mL for *Vibrio anguillarum* and 0.31 mg/mL for *Vibrio harveyi*.

Conclusion

The novel coumarin containing quaternary ammonium salt had been successfully synthesized. The yield coumarin derivative had been characterized by ^1H NMR spectroscopy, Fourier transform infrared spectra, LC-MS and melting point analysis. The antibacterial activity results showed that the

coumarin had excellent antibacterial properties for *Dwardsiella tarda*, *Aeromonas hydrophila*, *Vibrio anguillarum* and *Vibrio harveyi*. The MICs against them are 0.31, 0.625, 0.15 and 0.31 mg/mL, respectively. In conclusion, maybe this novel coumarin can be used as the antimicrobial agent for aquatic product.

REFERENCES

1. T. Yukiko, S. Yasuhiro, T. Yoshihisa, S. Hirofumi and H. Tomihiko, *Phytochemistry*, **59**, 649 (2002).
2. S.A. Alaa, M.M. Hany, M. Shawkat, Z. Shamsulhaq, A. Athar, H.B. Ahmed, M.E. Ahmed and D.H. Pierre, *J. Heterocycl. Chem.*, **44**, 1287 (2007).
3. A. Afsheen, O. Hasnah, C.B. Mark, K.L. Chan, M. Suriyati and S.M.Z. Anis, *Eur. J. Med. Chem.*, **46**, 3788 (2011).
4. H. Naceur, B. Feten, R. Antonio and B. Rached, *C.R. Chim.*, **13**, 1261 (2010).
5. T.K. Joanna, H. Elzbieta, K. Hanna and W.D.M. Irena, *Eur. J. Med. Chem.*, **46**, 2252 (2010).
6. Z.Y. Lu, T.S. Yuan, Y.L. Chen, X.Q. Wei, W.G. Zhu and M.G. Xie, *Chin. Chem. Lett.*, **13**, 674 (2002).
7. L. Geoffrey, US005286803A (1994).
8. J.B. Liu, F.Y. Wu, L.J. Chen, L.Z. Zhao, Z.B. Zhao, M. Wang and S.L. Lei, *Food Chem.*, **135**, 2872 (2012).
9. S.C. Bernadette, A.E. Denise, K. Dariusz, K. Kevin, M. Malachy, M. Mary, N. Andy, T. Bhumika and W. Maureen, *J. Inorg. Biochem.*, **101**, 1108 (2007).
10. S.C. Bernadette, A.E. Denise, K. Dariusz, K. Kevin, M. Malachy, M. Mary, N. Andy, T. Bhumika and W. Maureen, *Inorg. Chim. Acta*, **359**, 3976 (2006).
11. A.P. Sangamesh, N.U. Shrishila, D.K. Ajaykumar, H.N. Vinod, S.B. Prema, *J. Mol. Strut.*, **985**, 330 (2011).
12. P. Juliusz and C. Przemyslaw, *Eur. J. Med. Chem.*, **38**, 1035 (2003).
13. T. Maximilian, P. Gabriele, O. Knut and H. Ulrike, *Chem. Med. Chem.*, **7**, 22 (2012).
14. A.G. Harold *et al.*, US44790 (1984).
15. M.B. Alexandra and F.G. Marta, *Prog. Polym. Sci.*, **37**, 281 (2012).
16. J.J. Wang, H.M. Ma, J.T. Ni and T.L. Zhang, *Fine Chem*, **29**, 471 (2012) (in Chinese).
17. B. Sandhya, D. Giles, M. Vinod, B. Guru and A. Rekha, *Eur. J. Med. Chem.*, **46**, 4696 (2011).
18. G.K. Rajesh, M.K. Geeta, M.K. Chandrappa and N. Premkumar, *Eur. J. Med. Chem.*, **43**, 2178 (2008).
19. A. Afsheen, O. Hasnah, C. Mark, K.L. Chan, M. Suriyati, S.M.Z. Anis, *Eur. J. Med. Chem.*, **46**, 3788 (2011).
20. P. Manojkumar, T.K. Ravi and S. Gopalakrishnan, *Eur. J. Med. Chem.*, **44**, 4690 (2009).
21. Y. Shi and C.H. Zhou, *Bioorg. Med. Chem. Lett.*, **21**, 956 (2011).
22. J.M. Andrews, *J. Antimicrob. Chemother.*, **49**, 1049 (2002).