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Synthesis, Characterization, and Antibacterial Activity of 7-Fluoro-2-oxo-2*H*-chromene-3-carboxylic Acid Ethyl Ester

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The title compound was synthesized by reacting 2-hydroxy-4-methoxy-benzaldehyde with diethyl malonate in the presence of catalyst piperidine. The compound was characterized by elemental analysis, FT-IR, ^{1}H-NMR, and ^{13}C-NMR. The structure was confirmed by single crystal X-ray diffraction technique. The compound crystallizes in the monoclinic crystal system, $P2_1/n$ space group with unit cell parameters $a = 7.8824(7)$ Å, $b = 13.5854(10)$ Å, $c = 20.6072(16)$ Å, $\beta = 98.786(3)^\circ$, and $Z = 8$. The molecular and crystal structure of the title compound are stabilized by inter- and intramolecular interactions of the type C—H . . . O. This newly synthesized compound was screened for antibacterial activity with two Gram positive and three Gram negative bacteria.

Keywords Bacillus cereus; coumarin; Escherichia coli; pseudomonas aeruginosa; salmonella typhimurium; staphylococcus aureus

Introduction

The title compound $C_{12}H_9FO_4$ is a coumarin derivative. Coumarin belongs to the class of benzopyrones, which consists of a benzene ring fused to a pyrone nucleus. Coumarin and its derivatives are found to be a group of promising bioactive heterocyclic compounds. A few of its derived compounds showed extremely high anti-HIV activity [1]; a few are potent against hepatitis C viral activities [2]. Its derivatives are used as antibiotics, fungicides, antiviral, bactericidal, anticoagulant, and antitumor agents [3].

Halogen plays an important role in natural systems. Halogen containing coumarin derivative ($C_{12}H_8ClNO_4$) possesses anti-inflammatory as well as antibacterial activities [4]. Introduction of the fluorine moiety into the coumarin chain helps to enhance the

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biological activity [5]. Hence, it was thought worthwhile to synthesize the compound 7-fluoro-2-oxo-2*H*-chromene-3-carboxylic acid ethyl ester. In the present work, we discuss synthesis, characterization, and crystal structure of the title compound. The newly synthesized compound was screened for antibacterial activity.

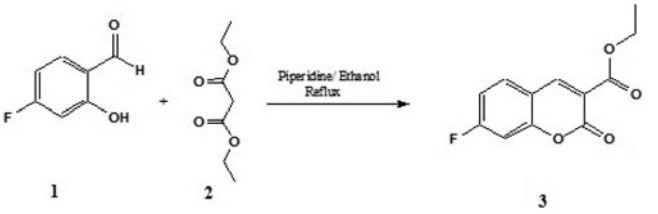
Experimental

Materials and Methods

Chemicals were purchased from Sigma Aldrich Chemical Corporation. Thin-layer chromatography (TLC) was performed on aluminum-backed silica plates from Merck & Co., and visualized under UV-light. Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. IR spectra were recorded on Perkin-Elmer spectrophotometer in the range 400–4000 cm^{-1}. ^1H NMR spectra were recorded on a Bruker 400 MHz NMR spectrophotometer in DMSO-d_6 solvent and the chemical shifts were recorded in δ (ppm) downfield from tetramethylsilane. Mass spectra were obtained with a VG70-70H spectrophotometer. Elemental analysis was done by Perkin-Elmer 2400 elemental analyzer, and results are within 0.4% of the calculated value.

Synthesis of 7-fluoro-2-oxo-2H-chromene-3-carboxylic Acid ethyl Ester (3)

7-Methoxy-2-oxo-2H-chromene-3-carboxylic acid ethyl ester (3) was synthesized form 2-hydroxy-4-methoxy-benzaldehyde **(1)** (0.0042 mol) and diethyl malonate **(2)** (0.0042 mol) in the presence of 0.1 mL of piperidine as a catalyst and ethanol as solvent. The reaction mixture was refluxed for 6 hr and the reaction was monitored TLC by using benzene**:** ethyl acetate (4:1) as an eluent. The reaction mixture was allowed to cool at room temperature, then the reaction mass was quenched in to ice cold water and the solid obtained is filtered to afford desired compound **(3)** in good yield (85%), m.p. 116–118°C (Scheme 1).



Scheme 1. Scheme **of 7-f**luoro-2-oxo-2*H*-chromene-3-carboxylic acid ethyl ester.

In Vitro Antibacterial Activity

In view of the biological importance of different series of coumarin derivatives, the synthesized title compound was screened for its antibacterial activity.

Antibacterial assays were carried out at Department of Studies in Microbiology, University of Mysore, Mysore. The compound was screened for antibacterial activity against two Gram-positive bacteria namely *Bacillus cereus* (MTCC (Microbial Type Culture Collection) No. 1272), *Staphylococcus aureus* (MTCC No. 7443), and two Gram-negative bacteria namely *Pseudomonas aeruginos*a (MTCC No. 7093), and *Salmonella typhimurium*

(MTCC No. 733). The bacterial strains were inoculated in nutrient broth, and kept for overnight culture at 37°C.

The MIC is defined as the minimum inhibitory concentration able to inhibit any visible bacterial growth. Antibacterial activity was determined by broth microdilution method performed in 96 well microtiter plate, using 2,3,5-triphenyl tetrazolium chloride (TTC) as an indicator for bacterial growth [5], by dissolving 5 mg of sample in 1 mL of ethanol solvent.

For susceptibility testing, 100 μL of nutrient broth was distributed from first to 8th, and 10th to 12th test wells. One hundred microliters of compound initially dissolved in ethanol was distributed to first well, from which 100 μL was taken and transferred till the concentration reaches 0.39×10^{-2} mg/mL. Tenth and eleventh wells served as negative and positive (gentamicin) controls, respectively; 12th well was a sterility control. Later 50 μL of the final bacterial inoculum was added to the appropriate wells.

The concentration of the prepared solutions was as follows: 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.625×10^{-1} mg/mL. 0.3125×10^{-1} mg/mL, 0.156×10^{-1} mg/mL, 0.78×10^{-2} mg/mL, and 0.39×10^{-2} mg/mL.

Inoculated plates were incubated at 37°C for 24 hr. One hour before the end of incubation 10 μL of TTC was added to the wells and the plates were incubated for another hour. The lowest concentration of each well showing no visible growth was recorded as the MIC [6]. The optical density of the plate was measured at 600 nm, on ELISA reader of Thermo Scientific.

Results and Discussions

Elemental Analysis

In order to confirm the chemical composition of the synthesized compound, carbon (C) and hydrogen (H) analysis was carried out. The experimental and calculated percentages of C and H are given in Table 1. The differences between experimental and calculated percentages of C and H are very small and are within the experimental errors. This confirmed the formation of the product in the stoichiometric proportion.

Table 1. Elemental analysis for $C_{12}H_9FO_4$

Element	Element experimental (%)	Calculated (%)
Carbon	61.02	62.04
Hydrogen	3.84	3.82

FT-IR Spectral Analysis

The FT-IR spectrum of the crystal structure is shown in Fig. 1. The peak at 3080 cm^{-1} is in correspondence to the C—H stretching of the aromatic protons. The peaks observed at 1740 cm^{-1} are assigned to the C=O of ethyl ester, and the peak at 1619 cm^{-1} is for C=O stretching vibration of coumarin. The peak at 1255 cm^{-1} is assigned for the C—O stretching.

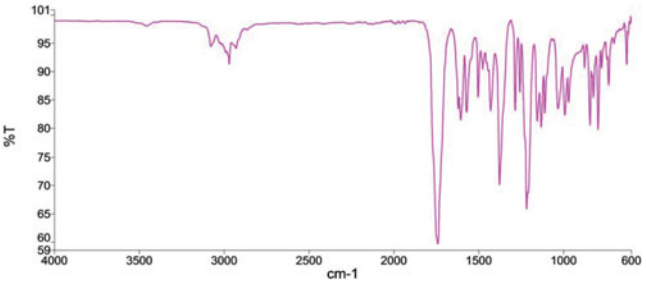


Figure 1. FTIR spectrum of title compound.

^{1}H NMR ^{13}C NMR Spectral Analysis

The spectrum ^{1}H NMR of the crystal structure is shown in Fig. 2. The NMR peak at δ 1.4 (t, J = 6.6 Hz, 3H) is for three hydrogen atoms in CH_3 of ester, the peak at δ 4.4 (q, J = 5.9 Hz, 2H), is for two hydrogen atoms of $^{-}COOCH_2$ and the peaks at δ 7.3–7.7 (m, 4H) clearly indicates the four aromatic hydrogen atoms of the compound. ^{13}C NMR 165.0, 162.0, 161.7, 152.6, 152.4, 128.2, 123.4, 122.2, 112.2, 108.3, 59.6, and 13.7.

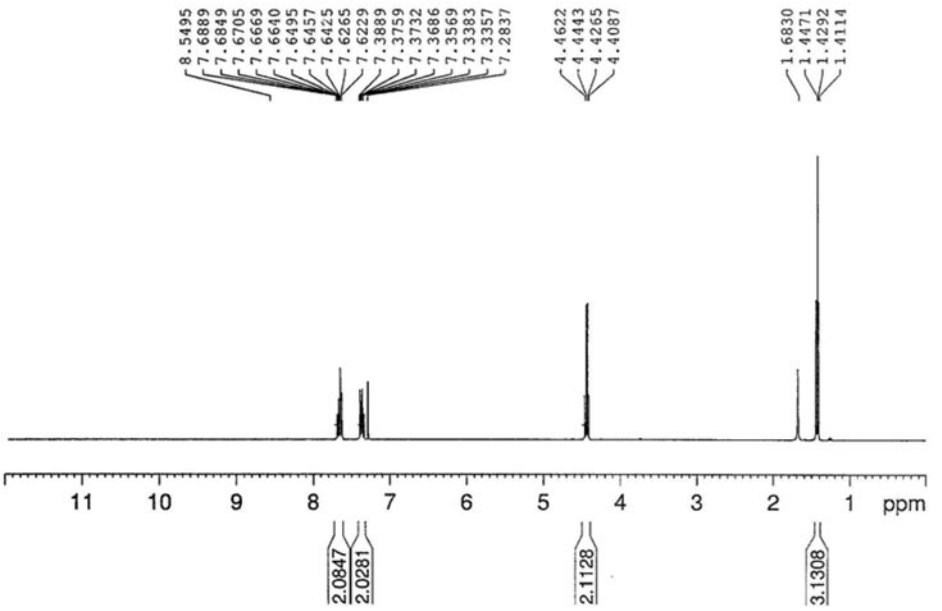


Figure 2. ^{1}H NMR spectra of the title compound.

X-ray Crystal Structure Determination

Single crystal suitable for a structural analysis using X-ray diffraction technique was obtained by slow evaporation method using ethanol as solvent. A yellow-colored single crystal of the title compound with approximate dimensions 0.23 × 0.22 × 0.21 mm

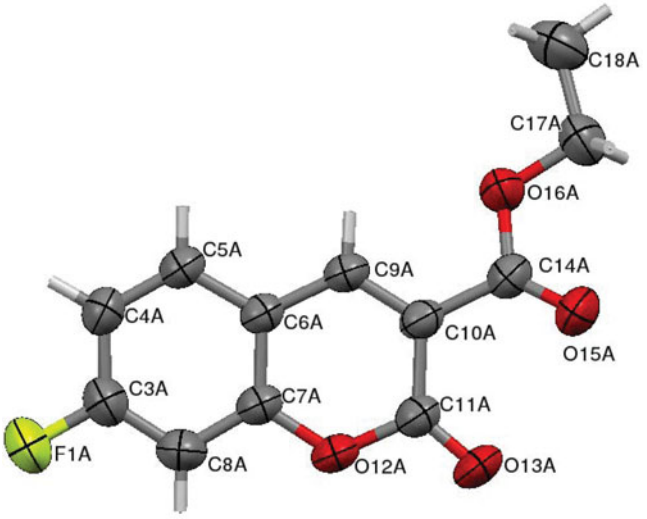


Figure 3. The *ORTEP* diagram of molecule A of the title compound with 50% probability.

Table 2. The crystal data and structure refinement details

CCDC deposit number	1007752
Empirical formula	$C_{12}H_9FO_4$
Formula weight	236.19
Temperature	296 K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	$P2_1/n$
Cell dimensions	a = 7.8824(7) Å, c = 20.6072(16) Å b = 13.5854(10) Å, β = 98.786(3)°
Volume	2180.8(3) Å^3
Z	8
Density (calculated)	1.439 Mg m^{-3}
Absorption coefficient	1.027 mm^{-1}
F_{000}	976
Crystal size	0.23 × 0.22 × 0.21 mm
θ range for data collection	5.8–64.6
Index ranges	$-8 \le h \le 9$ $-15 \le k \le 15$ $-24 \le l \le 22$
Reflections collected	11,564
Independent reflections	3,540
Refinement method	Full matrix least-squares on F^2
Data/restraints/parameters	3,540/0/309
Goodness-of-fit on F^2	1.07
Final [$I > 2\sigma(I)$]	$R1 = 0.0558$, $wR2 = 0.1833$
Largest diff. peak and hole	0.38 and –0.21 eÅ^{-3}

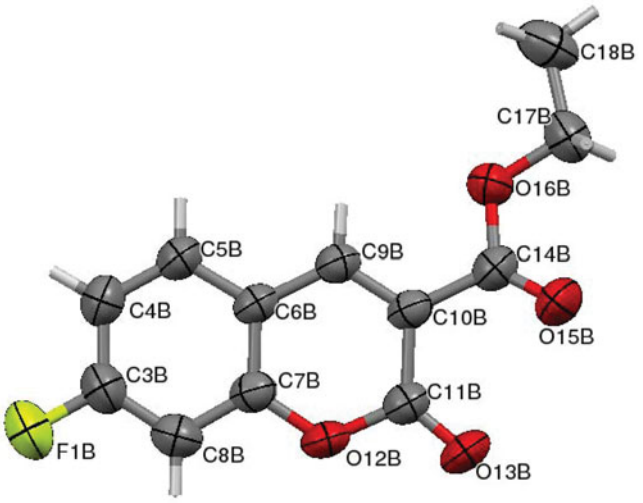


Figure 4. The *ORTEP* diagram of molecule B of the title compound with 50% probability.

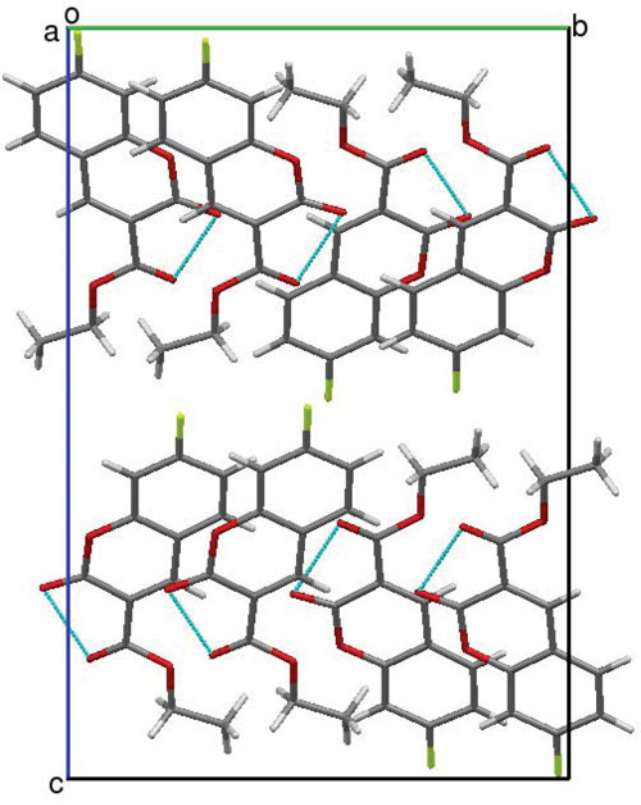


Figure 5. Packing of molecules when viewed along *a*-axis.

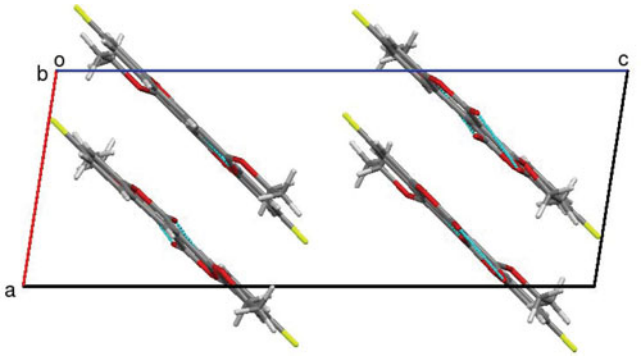


Figure 6. Packing of molecules when viewed along *b*-axis.

was used for X-ray diffraction study. Data were collected on a Bruker CCD diffractometer equipped with Cu K_α radiation. Data reduction of all the measured reflections and absorption corrections were carried out using the *APEX 2* package [7]. Crystal structure was solved by direct methods using *SHELXS-97* and refined by full-matrix least squares refinement

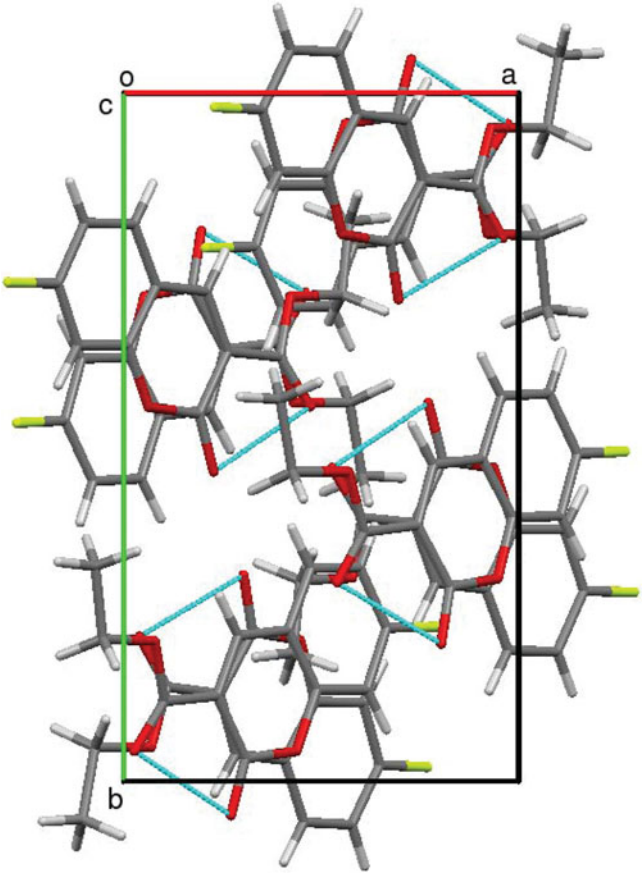


Figure 7. Packing of molecules when viewed along *c*-axis.

Table 3. Selected bond lengths and bond angles (Å, deg.)

F1A-C3A	1.358(3)	F1B-C3B	1.348(3)
C6A-C7A	1.387(2)	C3B-C4B	1.375(3)
C6A-C9A	1.427(2)	C3B-C8B	1.371(3)
O12A-C7A	1.367(2)	C4B-C5B	1.376(3)
C7A-C8A	1.376(3)	C17B-C18B	1.495(4)
C9A-C10A	1.342(2)	C10B-C14B	1.489(3)
C10A-C11A	1.467(2)	C10B-C11B	1.468(3)
C10A-C14A	1.488(2)	C9B-C10B	1.339(2)
C17A-C18A	1.496(4)	C6B-C9B	1.436(2)
C7A-O12A-C11A	122.82(14)	C7B-O12B-C11B	122.46(14)
C14A-O16A-C17A	115.96(16)	C14B-O16B-C17B	115.45(17)
F1A-C3A-C4A	118.21(18)	C9B-C10B-C14B	121.56(15)
C4A-C5A-C6A	120.70(17)	C6B-C7B-C8B	121.59(17)
C5A-C6A-C7A	118.44(15)	C3B-C8B-C7B	116.99(19)
O12A-C7A-C6A	121.07(15)	O12B-C11B-C10B	116.24(15)
O12A-C7A-C8A	116.91(16)	O15B-C14B-C10B	126.31(18)
C6A-C9A-C10A	122.32(15)	O16B-C14B-C10B	110.90(16)
C9A-C10A-C14A	121.92(15)	O16B-C17B-C18B	107.23(19)

against F^2 using *SHELXL-97* [8]. All nonhydrogen atoms were refined anisotropically and hydrogen atoms were placed in chemically acceptable positions. The crystal data and structure refinement details are given in Table 2.

The geometrical calculations were carried out using the program *PLATON* [9]. The molecular and packing diagrams were generated using software *Mercury* [10].

Bond lengths and bond angles are listed in Table 3. Torsion angles are given in Table 4. Hydrogen-bond geometry is given in Table 5.

Two molecules are present in the asymmetric unit. It contains of carboxylic acid ethyl ester substituted to pyrone ring, which is fused to a benzene ring with a fluorine attached to it. The pyrone ring is highly planar (r.m.s. deviation 0.022(2) Å), with a maximum deviation of 0.024(2) Å for C(11A) in molecule A and (r.m.s. deviation 0.006(2) Å), with maximum deviation of 0.008(1) Å for O(12B) in molecule B.

The bond lengths and bond angles are in fairly good agreement with those of already reported coumarin derivatives. The bond lengths of ester group (C14-O16) of molecules A and B are 1.335(2) Å and 1.338(2) Å, respectively, which are greater than the corresponding values of 1.200(3) Å and 1.198(4) Å reported for $C_{12}H_9ClO_4$, and $C_{12}H_9BrO_4$, respectively

Table 4. Selected torsion angles (deg.)

C11A-O12A-C7A-C8A	−178.54(17)	F1B-C3B-C4B-C5B	−179.0(2)
C7A-O12A-C11A-C10A	−4.1(2)	C8B-C3B-C4B-C5B	−0.3(4)
C7A-O12A-C11A-O13A	176.91(16)	C9B-C6B-C7B-C8B	−179.30(17)
C17A-O16A-C14A-O15A	−1.9(3)	C7B-C6B-C9B-C10B	−0.4(3)
C14A-C10A-C11A-O13A	1.9(3)	C5B-C6B-C7B-C8B	−1.1(3)
C9A-C10A-C11A-O13A	−177.84(18)	O12B-C7B-C8B-C3B	−178.23(18)

Table 5. Hydrogen-bond geometry (Å, deg.)

D—H . . . A	D—H	H . . . A	D . . . A	D—H . . . A
C(5A) –H(5A) ..O(13A)[(b)]	0.93	2.46	3.286(2)	148
C(5A) –H(5A) ..O(15A)[(b)]	0.93	2.42	3.193(2)	141′
C(5B) –H(5B) ..O(13B)[(a)]	0.93	2.59	3.410(3)	148
C(5B) –H(5B) ..O(15B)[(a)]	0.93	2.38	3.157(2)	142′
C(9A) –H(9A) ..O(16A)*	0.93	2.34	2.678(2)	101
C(9A) –H(9A) ..O(13A)[(b)]	0.93	2.47	3.292(2)	148′
C(9B) –H(9B) ..O(16B)*	0.93	2.35	2.686(2)	101
C(9B) –H(9B) ..O(13B)[(a)]	0.93	2.50	3.346(2)	151′

*Intramolecular hydrogen bond interactions.
Symmetry codes: (a) $3/2 - x, -1/2 + y, 1/2 - z$.
(b) $5/2\text{-}x, -1/2+y, 1/2\text{-}z$.

[12]. The value of C=O bond length attached to pyrone moiety (C11-O13) in molecules A and B are 1.195(2) Å and 1.198(2) Å, respectively, which are less when compared with the corresponding value of 1.203(2) Å reported for Cinnamyl 2-oxo-2H-chromene-3-carboxylate [12].

The pyrone rings (O12-C7-C6-C9-C10-C11) of molecules A and B are *sp*2 hybridized and are planar. They are well described by the torsion angles 2.57° and 0.71° respectively, which suggest that they adopt +syn-periplanar conformations.

The bond angles (O12-C11-O13) in molecules A and B are 115.53(15)° and 116.24(17)°, respectively, which are lesser than 128.02(17)° and 127.5(2)°, respectively, for (O13-C11-C10) of molecules A and B. This can be ascribed to the steric effect. The bond angles at the junctions of phenyl and pyrone rings of 2*H*-chromene are 116.91(16)° and 116.69(16)° for (O12-C7-C8), 124.11(15)°, and 123.12(16)° for (C5-C6-C9) of molecules A and B, respectively. Generally, these values are respectively smaller and greater than 120° for coumarin derivatives. All these features are common in coumarin derivatives, and are also present in the title compound.

The torsion angle of molecules A and B (C11-C10-C14-C16) are, respectively, 167.17(15)° and 168.18(17)°, which indicates no significant change with the substitution of carboxylic group at C10 position. This is comparable to the substitutions of carboxylic group at same positions reported in Refs. [11] and [12]. The inter and intramolecular hydrogen bonding of type C—H . . . O involved in the stabilization of the crystal structure with bond distance and bond angles are shown in Table 5.

In Vitro Antimicrobial Activity

The results of antibacterial activity of the title compound are as shown in the Table 6. The antibacterial screening revealed that the compound shows smaller or average activity against various bacterial strains. The synthesized compound showed good inhibition against antibacterial activity against *Salmonella typhimurium.*

Table 6. MIC of the titled compound against various bacterial strains

Bacterial strains	MIC (mg/mL)
Bacillus cereus	0.25
Staphylococcus aureus	0.125
Salmonella typhimurium	0.3125×10^{-1}
*Pseudomonas aeruginos*a	0.25

Acknowledgments

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