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Synthesis, Crystal Structure and Antibacterial Activity of 7-Methoxy-2-oxo-2*H*-chromene-3-carboxylic acid ethyl ester

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Abstract - 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, ^{13}C-NMR. The structure was confirmed by single crystal X-ray diffraction technique. The compound crystallizes in monoclinic crystal system, *C2/c* space group, a = 25.885(3)Å, b = 6.8414(9)Å, c = 13.8147(16)Å, β = 104.878(4)° unit cell parameters, and Z = 8. In the crystal structure, the molecules are linked by intermolecular interactions of the type C—H…O. This newly synthesized compound was screened for antibacterial activity against two Gram positive and two Gram negative bacteria.

Keywords - FT-IR, ^{1}H-NMR, ^{13}C-NMR, X-ray diffraction technique, Antibacterial activity, *Bacillus Cereus*, *Staphylococcus Aureus*, *Pseudomonas Aeruginosa*, *Salmonella Typhimurium*

1. Introduction

The title compound is a coumarin derivative. The chemical structure of coumarin consists of a benzene ring, fused with a pyrone moiety. They are commonly found in plants. Coumarin derivative are found to posses various biological properties. Some of its derived compounds showed interesting antimicrobial activity against *Helicobacter pylori. H. pylori*, is a Gram-negative bacteria found in the stomach. Its infection is linked to the development of duodenal ulcers and stomach cancer [1]. The most promising lead compound [(3'R, 4'R)–3',4'–di–O-(-)-camphonyl-(+)-cis–khellactone] (DCK) which is a pyranocoumarin derivative, showed extremely high anti-HIV activity. Other series of DCK derivatives with various substitutions in the coumarin nucleus are investigated for various biological activities [2]. It is reported that the presence of a methyl group on the coumarin nucleus is extremely potent against HIV-1 replication in H9 lymphocyte cells [3]. In addition to this, some of the coumarin conjugates exhibit potent anti-hepatitis C virus activity [4].

With this background an attempt is made here to synthesis 7-methoxy-2-oxo-2*H*-chromene-3-carboxylic acid ethyl ester compound. Its structure is characterized using elemental analysis, NMR, FT-IR, XRD and screened for its antibacterial activity.

2. Experimental

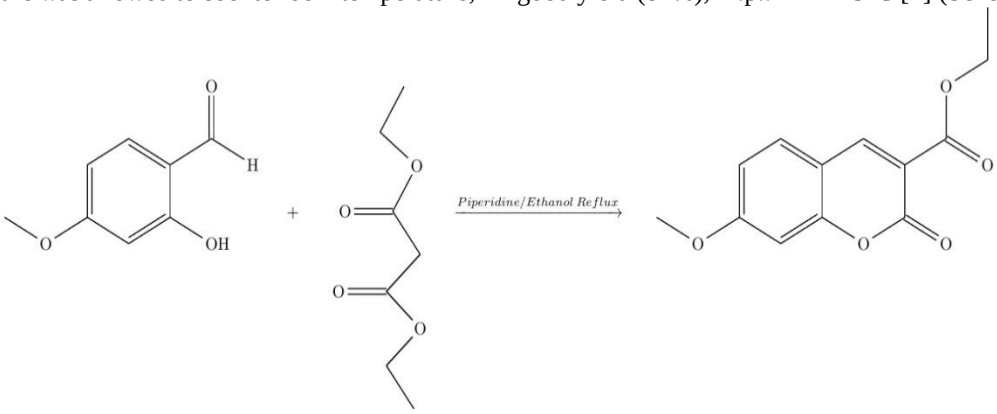
2.1. Materials and Methods

Chemicals were purchased from Sigma Aldrich Chemical Corporation. Thin Layer Chromatography (TLC) was performed on aluminum-backed silica plates and visualized by UV-light. Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. Infrared spectra were recorded on a Perkin Elmer spectrophotometer in the range 400-4000 cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker 400 MHz NMR spectrophotometer in $CDCl_3$ solvent and the chemical shifts were recorded in parts per million downfield from tetramethylsilane. Elemental analysis was obtained with a Perkin Elmer 2400 spectrophotometer and results of elemental analysis are within 0.4% of the calculated value.

2.2. Synthesis of 7-Methoxy-2-oxo-2H-chromene-3-carboxylic acid ethyl ester(3)

7-Methoxy-2-oxo-2H-chromene-3-carboxylic acid ethyl ester **(3)** was synthesized from 2-Hydroxy-4-methoxy-benzaldehyde (0.0037 mol) and diethyl malonate (0.0037 mol) in the presence of 0.1 mL of piperidine as a catalyst and ethanol as solvent. The reaction

mixture was refluxed for 8 hours and the reaction was monitored by TLC using benzene**:** ethyl acetate (4:1) as an eluent. The reaction mixture was allowed to cool to room temperature; the reaction mass was then quenched into ice cold water and the solid obtained was filtered to afford desired compound in good yield (87%), m.p.:124–125 ℃ [1] (Scheme- 1).



Scheme- 1

2.3. 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 ℃.

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 eighth, and tenth to twelfth test wells. 100 µL 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; twelfth 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 were 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, 0.39×10^{-2} mg/mL.

Inoculated plates were incubated at 37 ℃ for 24 hours. 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].

3. Results and Discussion

3.1. 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 $C_{13}H_{12}O_5$

Element	Element Experimental (%)	Calculated (%)
Carbon	62.90	62.92
Hydrogen	4.86	4.87

3.2. FT-IR Spectral Analysis

The FT-IR spectrum of the crystal structure is shown in Fig. 1. The peak at 3000 cm^{-1} is in correspondence to the C−H stretching of the aromatic protons. The peaks observed at 1745 cm^{-1} is assigned to the C=O of ethyl ester, and the peak at 1670 cm^{-1} is for C=O stretching vibration of coumarin. The peak at 1231 cm^{-1} is assigned for the C–O stretching.

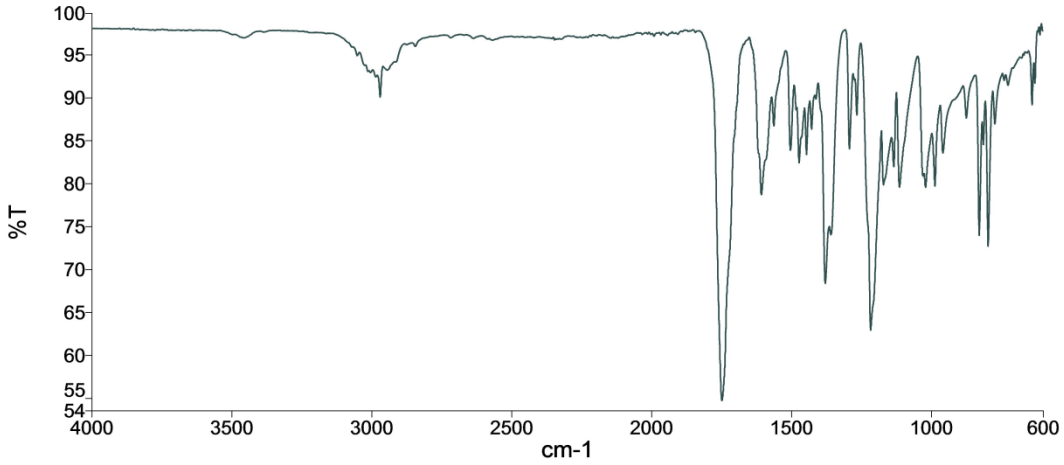


Figure 1. FTIR spectrum of title compound (3)

3.3. ^{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 1.29 (t, J = 6.6 Hz, 3H) is for three hydrogens in CH_3 of ester, the peak at δ 3.71 (s, 3H) is for three hydrogens of $–OCH_3$, peak at δ 4.42 (q, J = 5.9 Hz, 2H), is for two hydrogens of $-COOCH_2$ and the peaks at δ 7.52–7.29 (m, 4H), indicates the presence of four aromatic hydrogens of the compound. ^{13}C NMR 165.0, 162.0, 161.6, 152.6, 151.8, 127.6, 122.2, 120.1, 110.8, 106.9, 59, 56, 13. The data of the ^{13}C NMR exactly correspond to the carbon atoms of the compound.

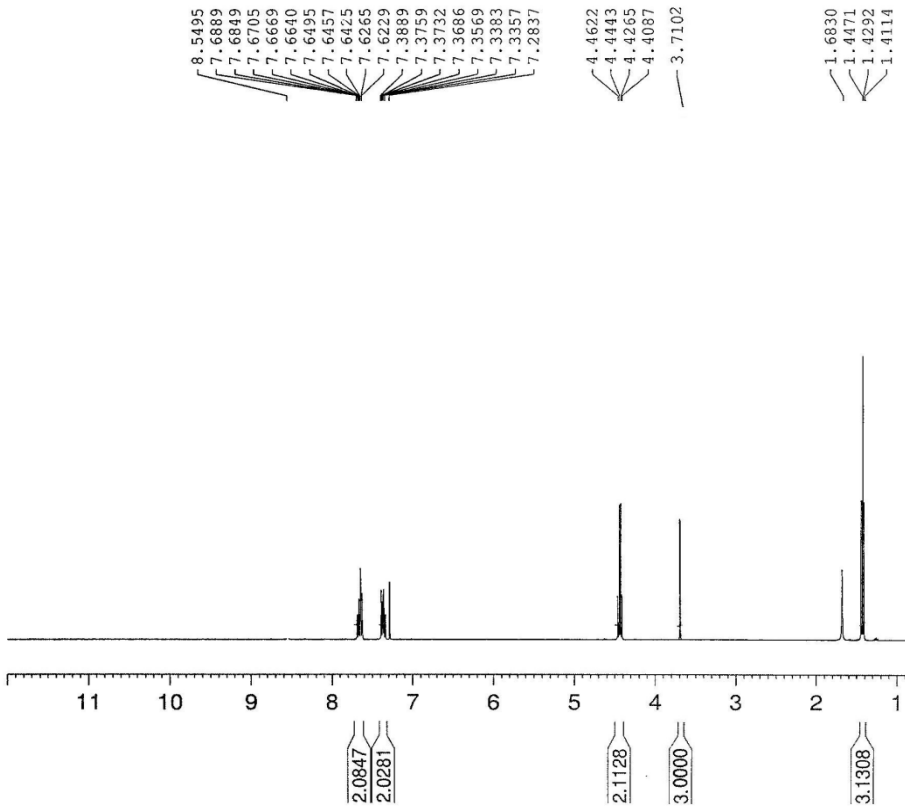


Figure 2. ^{1}H NMR Spectra of 2-oxo-2H-chromene-3-carboxylic acid ethyl ester

3.4. 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 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 against F^2 using *SHELXL-97* [8]. All non-hydrogen 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.

Table 2. The crystal data and structure refinement details

CCDC Deposit Number	994374
Empirical formula	$C_{13}H_{12}O_5$
Formula weight	248.23
Temperature	296(2) K
Wavelength	1.54178 Å
Crystal system	Monoclinic
Space group	*C2/c*
Cell dimensions	a = 25.885(3) Å α = 90.00 ° b = 6.8414(9) Å β = 104.878(4) ° c = 13.8147(16) Å γ = 90.00 °
Volume	2364.4(5) Å^3
Z	8
Density(calculated)	1.395 Mg m^{-3}
Absorption coefficient	0.912 mm^{-1}
F_{000}	1040
Crystal size	0.23 × 0.22 × 0.21 mm
θ range for data collection	6.63 ° to 64.51 °
Index ranges	$-29 \le h \le 30$ $-7 \le k \le 4$ $-16 \le l \le 14$
Reflections collected	9170
Independent reflections	1908 [R_{int} = 0.0225]
Refinement method	Full matrix least-squares on F ²
Data / restraints / parameters	1908 / 0 / 165
Goodness-of-fit on F ²	1.060
Final [I > 2σ(I)]	*R1* = 0.0465, *wR2* = 0.1259
R indices (all data)	*R1* = 0.0502, *wR2* = 0.1300
Largest diff. peak and hole	0.208 and −0.201 eÅ^{-3}

The geometrical calculations were carried out using the program *PLATON* [9]. The molecular and packing diagrams were generated using software *Mercury* [10]. Figure 3 shows the *ORTEP* diagram of the title compound with thermal ellipsoids drawn at 50% probability. Figures 4 and 5 show the packing of the molecules when viewed down *b*-axis, and *c*-axis respectively.

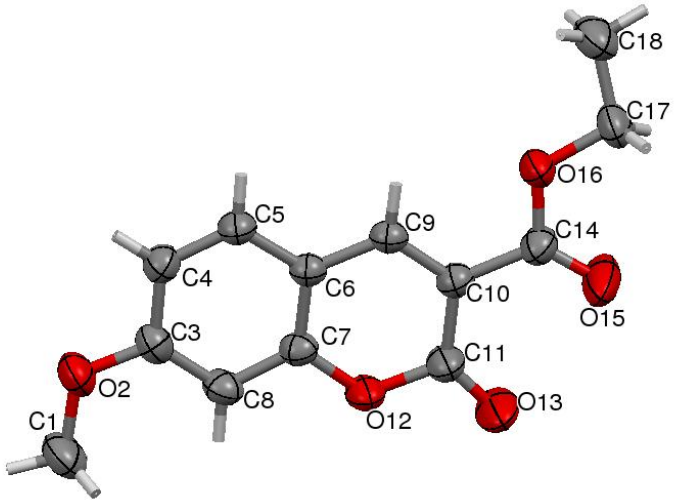


Figure 3. *ORTEP* diagram of the molecule with thermal ellipsoids drawn at 50% probability

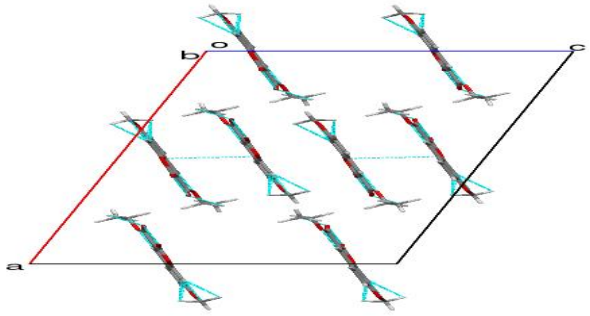


Figure 4. Packing of the molecule when viewed down *b*-axis

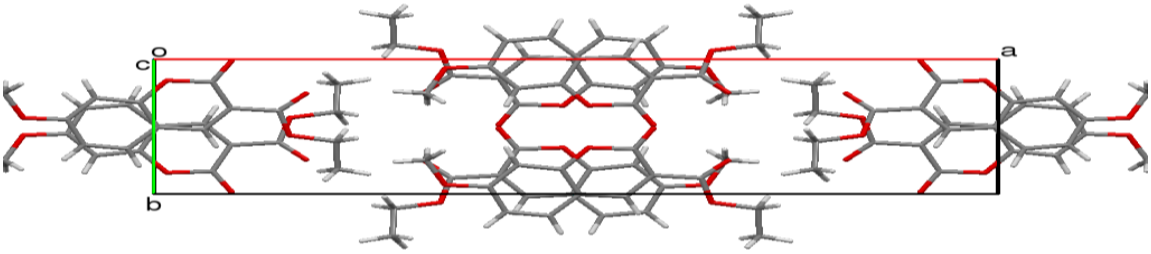


Figure 5. Packing of the molecule when viewed down *c*-axis

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.

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

C1-O2	1.427(2)	C9-C10	1.353(2)
O2-C3	1.3579(19)	C10-C11	1.460(2)
C3-C8	1.383(2)	C10-C14	1.483(2)
C3-C4	1.399(2)	C11-O13	1.194(2)
C4-C5	1.367(2)	C11-O12	1.395(2)
C5-C6	1.404(2)	C14-O15	1.190(2)
C6-C7	1.388(2)	C14-O16	1.319(2)
C6-C9	1.425(2)	O16-C17	1.4502(19)
C7-O12	1.370(2)	C17-C18	1.473(3)
C7-C8	1.387(2)	C3-O2-C1	118.29(15)
C10-C9-C6	121.80(14)	C9-C10-C11	120.18(14)
O2-C3-C8	124.31(15)	C9-C10-C14	121.27(15)
O2-C3-C4	114.87(15)	C11-C10-C14	118.55(14)
C8-C3-C4	120.82(14)	O13-C11-O12	115.14(16)
C5-C4-C3	120.03(15)	C7-C6-C5	117.68(14)
C4-C5-C6	120.77(15)	O12-C11-C10	116.21(14)
O13-C11-C10	128.65(16)	C7-C6-C9	118.07(14)
C7-O12-C11	123.08(13)	O15-C14-O16	122.46(16)
C5-C6-C9	124.25(14)	O12-C7-C8	116.55(14)

Table 4. Selected torsion angles (deg)

C1 − O2 − C3 − C4	179.87	C17 − O16 − C14 − C10	179.45
C1 − O2 − C3 − C8	0.48	C17 − O16 − C14 − O15	-0.25
C11 − O12 − C7 − C8	178.25	C14 − O16 − C17 − C18	-171.83
C7 − O12 − C11 − C10	1.42	C4 − C3 − C8 − C7	-0.55
C7 − O12 − C11 − O13	-178.42	O2 − C3 − C4 − C5	-178.68
C11 − O12 − C7 − C6	-1.45	O2 − C3 − C8 − C7	178.80

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

D—H…A	D—H	H…A	D…A	D—H…A
C(9)—H(9)…O(13)	0.93	2.57	3.464(2)	162
C(18)—H(18C)…O(15)	0.96	2.52	3.287(3)	136

Symmetry codes: (a) *x, -1+y, z*;

The phenyl ring (C3-C4-C5-C6-C7-C8) and the pyrone ring (O12-C7-C6-C9-C10-C11) are sp^2 hybridized and are nearly planar. The conformation of the pyrone moiety attached to the phenyl ring are well described by the torsion angle 0.78 ° and 0.50 ° respectively, which suggest that they adopt +synperiplanar conformations. The bond lengths and bond angles are in fairly good agreement with those of already reported coumarin derivatives. The bond length of the ester group (C14-O16) is 1.320(2) Å, which is 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 [11]. The value of C=O attached to pyrone moiety (C11-O13) is 1.194(3) Å, which is less when compared with the corresponding value of 1.203(2) Å reported for Cinnamyl 2-oxo-2*H*-chromene-3-carboxylate [12].

The bond length of 115.14(18) ° for O12-C11-O13 is smaller than 128.66(18) ° for O13-C11-C10, which can be ascribed to steric effect. The bond angles at the junctions of phenyl and pyrone rings of 2*H*-chromene are 116.52(14) ° for O-C-C and 124.25(13) ° for C-C-C. Generally these values are respectively smaller and greater than 120 ° for coumarin derivatives.

The pyrone ring is planar with a maximum deviation of 0.008(1) Å observed for the atom O12. The dihedral angle between pyrone and phenyl ring is 0.84(7) °, which indicates the planarity of the 2*H*-chromene (coumarin moiety). The molecule is planar. The structure exhibits intermolecular hydrogen bonds of the type C(9)—H(9)…O(13) and C(18)—H(18c)…O(15) whose symmetry code is *x, -1+y, z.*

3.5. In vitro antibacterial activity

The result of antibacterial activity of the title compound is as shown in the table 6. The antibacterial screening revealed that the compound shows lesser or average activity against different bacterial strains. The synthesized compound showed better inhibition against Gram negative bacteria *Salmonella typhimurium.*

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

Bacterial Strains	MIC (mg/mL)
Bacillus cereus	0.125
Staphylococcus aureus	0.0625
Salmonella typhimurium	0.03125
Pseudomonas aeruginosa	0.25

4. Conclusion

In view of biological importance of coumarin derivatives, we have synthesized one of the coumarin derivatives 7-Methoxy-2-oxo-2H-chromene-3-carboxylic acid ethyl ester and has been characterized by various techniques. Crystal structure of the title compound is determined by single crystal X-ray diffraction method and data compared with the reported compounds. The compound is also screened for its antibacterial activity. The compound showed significant activity when considering *Salmonella typhimurium.*

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References

[1] Chimenti, F., Bizzarri, B., Bolasco, A., Secci, D., Chimenti, P., Granese, A., Carradori, S., Rivanera, D., Zicari, A., Scaltrito, M.M., and Sisto, F. (2010). Synthesis, selective anti-*Helicobacter pylori* activity, and cytotoxicity of novel N-substituted-2-oxo-2H-1-benzopyran-3-carboxamides. *Bioorg. Med. Chem. Lett.*, 20, 4922-4926.

[2] Kuo-Hsiung, L., and Morris-Natschke, S. L. (1999). Recent advances in the discovery and development of plant-derived natural products and their analogs as anti-HIV agents. *Pure Appl. Chem.*, 71, 1045-1051.

[3] Lan, X., Yasuo, T., Mark Cosentino, L., and Kuo-Hsiung, L. (1998). Anti-AIDS agents 33.[1] synthesis and anti-HIV activity of mono-methyl substituted 3', 4'-di-o-(-)-camphanoyl-(+)-cis-khellactone (DCK) analogues. *Bioorg. Med. Chem. Lett.*, 8, 2151-2156.

[4] Johan, N., Erik De, C., Raghunath, S., Yung, H. C., Asish, R. Das, Subhasish, K. C., Shih, C. H., Shwu-Chen, T., Ming-Hua, H., and Jih, R. H. (2008). Structure-activity relationship of new anti-hepatitis C virus agents: heterobicycle-coumarin conjugates. *J. Med. Chem.*, 52, 1486-1490.

[5] Anja, K., Piskernik, S., Barbara, J., Sonja Smole, M. (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods*. 81, 121-126.

[6] Emad Abou, E., Hussein, F., and Usama abu, M. (2010). Antibacterial Activity and Phytochemical Analysis of Some Medicinal Plants from Gaza Strip-Palestine. *Journal of Al Azhar University–Gaza*, 12, 45-54.

[7] Bruker, (2001). APEX2, SAINT and SADABS, (2009). Bruker AXS Inc., Madison, Wisconsin, USA.

[8] Sheldrick, G. M. (2008). *Acta Cryst.*, A64, 112-122.

[9] Spek, A. L. (2003). A Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands. *J.Acta Cryst.*, 36, 7-13.

[10] Macrae, C. F., Bruno, I. J., Chisholm, J. A., Edgington, P. R., McCabe, P., Pidcock, E., Rodriguez-Monge, L., Taylor, R., Van de Streek, J., & Wood, P. A. (2008). *J. Appl. Cryst.*, 41, 466-470.

[11] Santos-Contreras, R. J., Martinez-Martinez, F. J., Garcia-Baez, E. V., Padilla-Martinez, I. I., Peraza, A. L., and Herbert, H. (2007). Carbonyl-carbonyl, carbonyl-π and carbonyl-halogen dipolar interactions as the directing motifs of the supramolecular structure of ethyl 6-chloro-2-oxo-2H-chromene-3-carboxylate and ethyl 6-bromo-2-oxo-2H-chromene-3-carboxylate. *Acta Cryst.* C63, o239-o242.

[12] Cui-Lian Xu, Nan Yang, Guo-Yu, Y., Su-Fang, F., and Cao-Yuan, N., (2009). Cinnamyl 2-oxo-2H-chromene-3-carboxylate. *Acta Cryst.*, *E*65, o2991.