Carbapenem chalcone derivatives: Synthesis, cytotoxicity and molecular docking studies

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Apparatus and analysis

All graded chemicals were used without further purification. ^{1}H and ^{13}C NMR spectra were on BruckerAvance instrument at 25^{o}C using field strength 400MHz and 100MHz respectively; TMS was used as internal standard. Chemical shifts were given in ppm. The Ir was recorded on a Perkin Elmer Precisely 100 FT-IR spectrometer in the 400-4000 cm^{-1} region. ESI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an ESI source. Elemental analyses were carried out using a Perkin-Elmer CHNS Elemental Analyzer model 2400. Melting points were recorded on a hot stage melting point apparatus Ernst LeitzWetzlar
, Germany and were uncorrected. All the reactions and the purity of products were monitored using thin layer chromatography (TLC) on aluminum-backed plates coated with Merck Kieselgel 60 F254 silica gel, visualizing the spots under ultraviolet light and iodine chamber.

Experimental Section:

TMS de protection

Mixture of TMS protected carbapenem, n-hexane (7ml), silica sulpuric acid (0.05 g), and wet Sio2 (0.2 g) was stirred at room temperature for 2 hrs. the reaction was monitored by TLC using 1:1 mixture of hexane and ethyl acetate. After completion of the reaction the mixture was filtered and the solid residue was washed with hexane. Upon evaporation gave pure alcohol [28].

Synthesis of Carbapenem chalcone derivatives

To a well-mixed solution of carbapenem (0.01 mol) and substituted aryl aldehydes (0.01 mol) in acetonitrile AAPTMS@MCM-41 catalyst was added and allowed it to stir foe few minutes, completion of the reaction was monitored by TLC. After the completion of the reaction it was worked up by diluting with water and acidifying it with 1M HCl to bring the PH to 3, latter it was extracted using ethyl acetate and the catalyst was filtered off. The resulted solid was purified using column chromatography to yield desired derivatives.

In vitro cytotoxic activity

The cell lines A-549 and MCF-7 used in the study were purchased from National Centre for Cell Sciences (NCCS), Pune, India. These cell lines were cultured in aseptic conditions on Dulbeccco's 70 modified eagles medium (DMEM), RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum and penicillin (100 units mL-1)/streptomycin (100 mg mL-1), pH-7.2 and 5% CO_2 humidified atmosphere at 37 ºC. The cells were tripisinizedwith 0.25 trypsin-EDTA after attaining 80% amalgam, and then diluted with media to a fixed number of cells.

Cell viability Assay for IC_{50}

The in vitro cytotoxic activity of the synthesized carbapenemchalcone derivatives (1-12) and carbapenem was evaluated by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay which was reported earlier[24], the cellular viability i.e., IC_{50} values were calculated in presence and absence of evaluating material. 100µl medium was inoculated into 96 well plate containing cells at density 10,000 cells/well, and these were incubated overnight in CO_2 incubator at 37^oC with 5% of humid conditions. Varying concentrations (0.1-10 µM) each compound is being treated with cell cultures and left it for one day in which four replicate wells were set up for each experiment condition. At the end of incubation was added 100µl of MTT reagent (5 mg of MTT/1 ml of PBS). The media was pippeted out and removed after three hours of incubation with MTT reagent, formozen crystals were dissolved in 150µl and was added to each well. The reduction of MTT was measured at 560 nm with ELISA reader, from the absorbance of formaozan crystals the percentage of viable cells in each well were calculated. All experiments were carried out quadrapulate along with a control (With solvent only) and a reference standard drug cisplatin (DDP) 100 is used. The growth inhibition is measured in presence and absence of test material and activity is expressed. The *in vitro* anti-cancer activity of the newly synthesized Carbapenem chalcone derivatives was evaluated against two human cancer cell lines using MTT essay with reference to a drug cisplatin (DDP). The results are presented in Table.3 and the values are reported in terms of IC_{50}.

Docking Method

3D co-ordinates of the ELK (pdb id: 2XP2) and ERβ (pdb id: 1UOM) were obtained from their crystal structures uploaded in the protein data bank (http://www.rcsb.org). The native ligands and water molecules of both proteins were not considered in the calculations, and were removed using the DS visualizer. The protonated states of both proteins were determined at physiological pH using the Prepare Protein algorithm in DS. The proteins were minimized using the conjugate gradient algorithm to remove the bad contacts in the DS. Different conformational isomers of representative compounds (CP, CPC-2 and CPC-4) compounds were obtained using the Generate Conformations module in DS. The lowest energy conformation of each RS was further geometrically optimized at DFT level using the combination of B3LYP functional and 6-31g [d,p] basis sets, in Gaussian 09 [29]. A binding sphere covering all the active site residues was generated using the Define and Edit Binding Site module, and docking was subsequently performed using the Flexible docking algorithm [27] considering the default parameters. Of the total poses identified, the best docked pose was selected on the basis of its scoring function (-CDOCKER energy), and processed further for the binding energy calculations**.**

Compound 2

3-(4-AminoPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 211-212^oC; ^1H NMR (400 MHz, DMSO) δ = 1.85 (3H,s), 2.00 (1H,s, OH), 3.74 (1H, q, *J* = 2.2 Hz), 4.02 (2H, s, NH_2), 4.52 (1H, d, *J* = 9.2 Hz), 6.00 (1H, d, *J* = 8.0 Hz), 6.35 (1H, d, *J* = 17.2 Hz), 6.39 (2H, d, *J* = 8.0 Hz), 7.03 (2H, d, *J* = 8.0 Hz), 7.60 (1H, d, *J* = 17.6 Hz) , 8.56 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO): 19.51, 52.93, 62.04, 71.02, 115.39, 117.36, 124.02, 127.02, 139.26, 145.22, 175.59, 180.42; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H), 3207 (NH_2);MS (ESI), *m*/*z* = 277 (M+1, 100%); Anal. Calcd ($C_{14}H_{16}N_2O_4$): C 60.86, H 5.84, N 10.14%. Found: C 60.87, H 5.87, N 10.16%.

Compound 3

3-(4-FluoroPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 213-214^{o}C; ^{1}H NMR (400 MHz, DMSO) δ = 1.85 (3H,s), 2.00 (1H,s, OH), 3.52 (1H, q, *J* = 2.4 Hz), 4.32 (1H, d, *J*=6.92Hz), 5.82 (1H, d, *J*=1.24Hz), 6.52 (1H, d, *J*=16.84Hz), 6.78 (1H, d, *J*=16.0Hz), 7.44-7.58 (4H, m) 8.56 (1H,s, NH); ^{13}C NMR (100 MHz, DMSO): δ= 19.50, 54.8, 62.4, 71.0, 117.6, 127.8, 130.5, 142.8, 161.3, 165.0, 179.9; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 280 (M+1, 100%); Anal. Calcd ($C_{14}H_{14}FNO_4$): C 60.21, H 5.05, N 5.02%. Found: C 60.22, H 5.07, N5.06%.

Compound 4
3-(4-ChloroPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 215-216^{o}C; ^{1}H NMR (400 MHz, DMSO) δ = 1.85 (3H,s), 2.00 (1H,s, OH), 3.52 (1H, q, *J* = 2.2 Hz), 4.32 (1H, d, *J*=9.2Hz), 5.82 (1H, d, *J*=1.24Hz), 6.34 (1H, d, *J*=16.8Hz), 7.20 (2H, d, *J*= 7.2Hz), 7.22 (2H, d,*J* =8.24Hz), 7.60 (1H, d, *J*=16.6Hz), 7.85 (1H,s, NH); ^{13}C NMR (100 MHz, DMSO): δ= 22.60, 63.28, 64.78, 79.20, 118.65, 128.45, 128.67, 133.54, 142.02, 161.44, 177.98; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 297 (M+1, 100%); Anal. Calcd ($C_{14}H_{14}ClNO_4$): C 56.86, H 4.77, N 4.74%. Found: C 56.88, H 4.80, N 4.78%.

Compound 5
3-(4-NitroPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester
Yellow solid: mp 220-221^{o}C; ^{1}H NMR (400 MHz, DMSO) δ = 1.65 (3H,s), 2.00 (1H,s, OH), 3.74 (1H, q, *J* = 2.28 Hz), 5.00 (1H, d, *J*= 9.2Hz), 5.98 (1H, d, *J*=8.0Hz), 6.64 (1H, d, *J*=17.2Hz), 7.74 (1H, d, *J*=17.6Hz), 8.07 (2H,d, *J*= 8.9Hz), 8.16, (2H, d, *J*=8.8Hz), 8.56 (1H,s, NH); ^{13}C NMR (100 MHz, DMSO): 19.51, 52.93, 62.04, 71.02, 115.39, 117.36, 123.26, 127.02, 141.02, 142.82, 147.36, 165.26, 179.43; IR (cm^{-1}): 1560 (Ar-NO_2), 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 307 (M+1, 100%); Anal. Calcd ($C_{14}H_{14}N_2O_6$): C 54.90, H 4.61, N 9.15%. Found: C 54.94, H 4.62, N 9.16%.

Compound 6
3-(4-MethoxyPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 218-219^{o}C; ^{1}H NMR (400 MHz, DMSO) δ = 1.85 (3H,s), 2.00 (s, 2H), 3.48 (s, 3H), 3.74 (1H, q, *J* = 2..4 Hz), 4.52 (1H, d, *J* = 9.2 Hz), 6.06 (1H, d, *J* = 7.2Hz), 6.35 (1H, d, *J*=17.2Hz), 6.39 (2H, *J*= 8.0Hz), 7.17 (1H, d, *J*= 8.0Hz), 7.60 (1H, *J*= 17.6Hz), 8.56 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO): 17.85, 55.49, 63.59, 64.35, 65.50, 114.27, 126.70, 129.86, 130.32, 131.91, 152.80, 164.57, 168.09, 190.78. IR (cm^{-1}): 1240-1200 (Ar-OCH_3), 1594-1681 (C=C), 1737 (C=O), 2782 (Lactam), 3011-3839 (Ar C-H); MS (ESI), *m*/*z* = 292 (M+1, 100%); Anal. Calcd ($C_{15}H_{17}NO5$): C 61.85, H 5.88, N 4.81%. Found: C 61.87, H 5.91, N 4.84%.

Compound 7

3-(4-BromoPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester
Yellow solid: mp 219-220^{o}C; ^{1}H NMR (400 MHz, DMSO) δ = 2.00 (3H,s), 2.49 (1H,s, OH), 4.24 (1H, q, *J* = 2.4 Hz), 4.61 (1H, d, *J*=4.32Hz), 5.55 (1H, d, *J*=1.64Hz), 6.61 (1H, d, *J*=14.56Hz), 6.90 (2H, d, *J*=8.96Hz), 7.25 (2H, d, *J*=8.8Hz), 7.83 (1H, d, *J*=18.08Hz), 9.27 (1H,s, NH); ^{13}C

NMR (100 MHz, DMSO): δ= 20.20, 54.82, 62.48, 71.0, 118.65, 122.82, 128.45, 131.77, 133.54 142.02, 167.44, 179.30; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 341 (M+1, 100%); Anal. Calcd ($C_{14}H_{14}BrNO_4$): C 49.43, H 4.15, N 4.12%. Found: C 49.46, H 4.17, N 4.18%.

Compound 8
3-(2, 4-DichloroPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 215-216°C; ^{1}H NMR (400 MHz, DMSO) δ = 1.21 (3H,s), 2.00 (1H,s, OH), 3.61 (1H, q, *J* = 2.2 Hz), 3.74 (1H, d, *J*=2.4Hz), 5.88 (1H, d, *J*=2.2Hz), 6.24 (1H, d, *J*=16.2Hz), 7.10-7.23 (3H, m,), 7.94 (1H, d, *J*= 17.02Hz), 9.20 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO): δ= 19.5, 54.8, 62.4, 71.0, 117.6, 126.9, 129.2, 132.9, 133.4, 142.8, 165.0, 179.0; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 331 (M+1, 100%); Anal. Calcd ($C_{14}H_{13}Cl_2NO_4$): C 50.93 H 3.97, N 4.24%. Found: C 50.98, H 3.98, N 4.26%.

Compound 9
3-(4-Hydroxy Phenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 221-222°C; ^{1}H NMR (400 MHz, DMSO) δ = 1.80 (3H,s), 2.54 (1H,s, OH), 3.44 (1H, q, *J* = 2.2 Hz), 4.25 (1H, d, *J*=1.4Hz), 4.53 (1H, s), 5.55 (1H, d, *J*=1.6Hz), 6.15 (2H, d, *J*=8.76Hz), 6.61 (1H, d, *J*=14.5Hz), 7.33 (2H, d, *J*=8.8Hz), 7.61 (1H, d, *J*=14.08Hz), 9.20 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO): δ= 26.43, 49.09, 62.44, 71.46, 120.11, 125.12, 129.32, 142.93, 156.86, 179.21, 186.54; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H), 3700-3500 (Ar-OH); MS (ESI), *m*/*z* = 278 (M+1, 100%); Anal. Calcd ($C_{14}H_{15}NO_5$): C 60.64, H 5.45, N 5.05%. Found: C 60.66, H 5.47, N 5.07%.

Compound 10
3-(2, 4-DihydroxyPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 209-210C; ^{1}H NMR (400 MHz, DMSO) δ = 1.85 (3H,s), 2.00 (1H,s, OH), 3.74 (1H, q, *J* = 2.28 Hz), 4.52 (1H, d, *J*=9.2Hz), 5.02 (2H, s), 5.83 (1H, d, *J*=8.0Hz), 6.22 (1H, d, *J*=17.6Hz), 6.44-6.08 (3H, m), 7.90 (1H, d, *J*=17.2Hz), 8.56 (1H,s, NH); ^{13}C NMR (100 MHz, DMSO): δ= 22.49, 63.63, 64.30, 65.64, 102.52, 108.05, 115.64, 117.90, 129.24, 142.89, 156.41, 157.90, 167.90, 177.98; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H), 3700-3500 (Ar-OH); MS (ESI), *m*/*z* = 294 (M+1, 100%); Anal. Calcd ($C_{14}H_{15}NO_6$): C 57.34, H 5.16, N 4.78%. Found: C 57.36, H 5.18, N 4.81%.

Compound 11

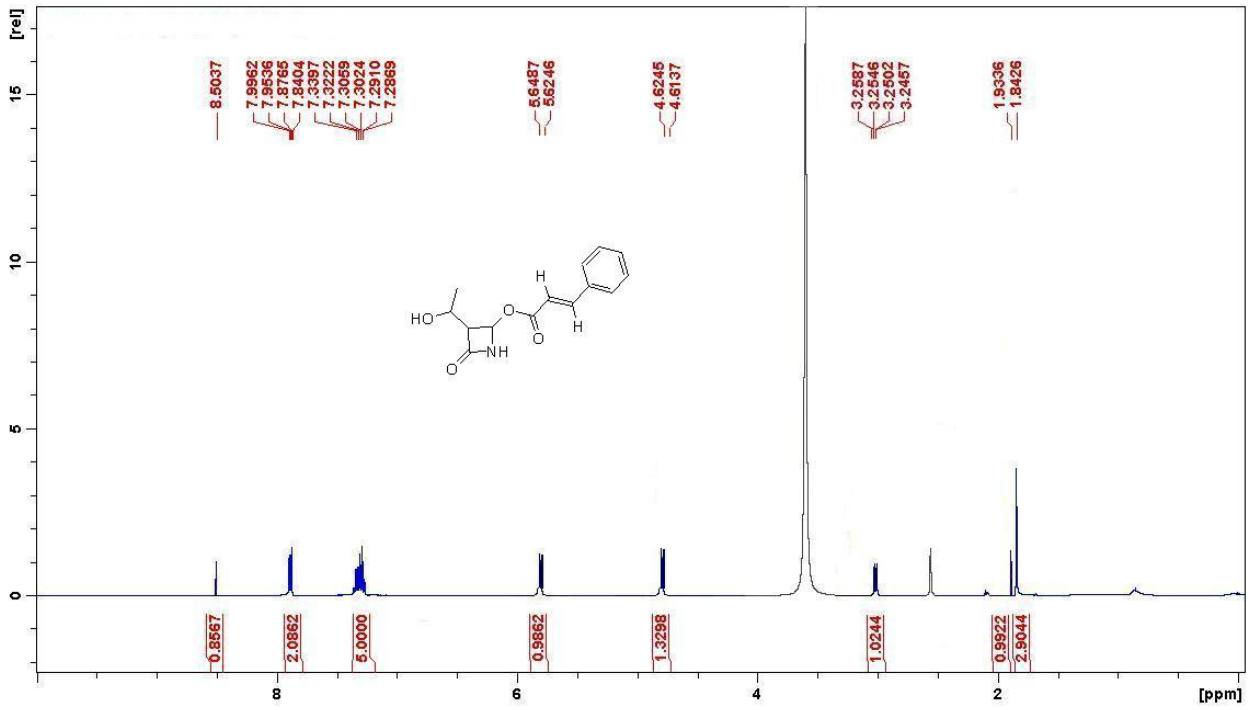
3-(2, 4-DimethoxyPhenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

Yellow solid: mp 215-216°C; ^{1}H NMR (400 MHz, DMSO) δ = 1.21 (3H,s), 2.00 (1H,s, OH), 3.61 (1H, q, *J* = 2.2 Hz), 3.73 (6H, s), 3.74 (1H, d, *J*=2.4Hz), 5.88 (1H, d, *J*=2.2Hz), 6.22 (1H, d, *J*=16.2Hz), 6.34-7.08 (3H, m,), 7.91 (1H, d, *J*= 17.02Hz), 9.20 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO): δ= 22.50, 55.49, 63.62, 64.31, 65.64, 99.63, 106.53, 112.86, 117.64, 129.24, 142.19, 160.91, 162.52, 167.78, 179.79; IR (cm^{-1}): 1240-1200 (Ar-OCH_3)1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 322 (M+1, 100%); Anal. Calcd ($C_{14}H_{14}FNO_4$): C 59.81 H 5.96, N 4.35%. Found: C 59.85, H 5.61, N 4.37%.

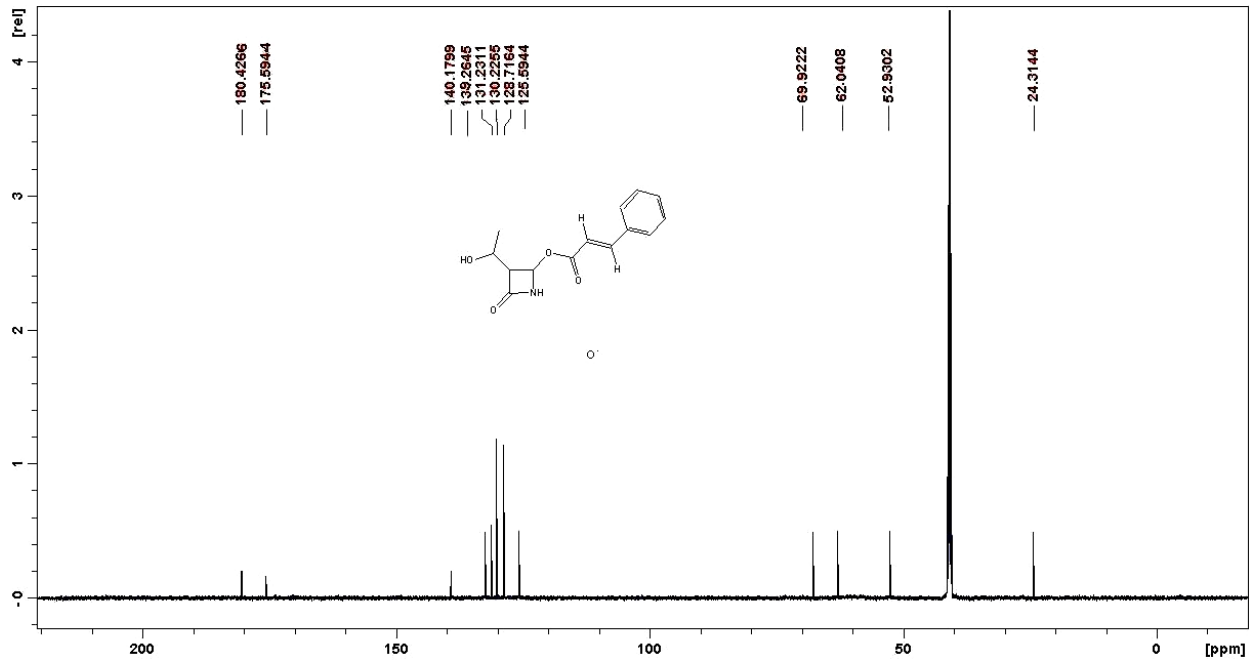
Compound 12

3-(4-N, N Dimethyl Phenyl)-acrylic acid 3-(1-hydroxy-ethyl)-4-oxo-azetidin-2yl ester

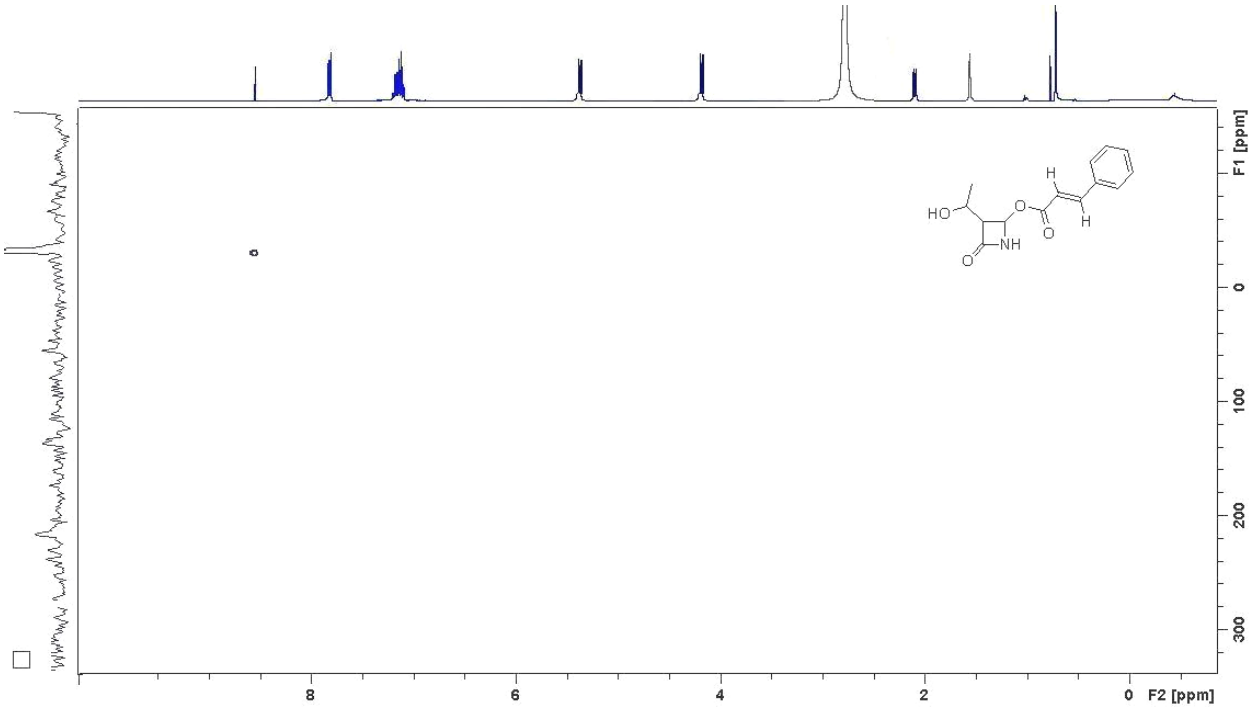
Yellow solid: mp 217-218^{o}C; ^{1}H NMR (400 MHz, DMSO) δ = 1.21 (3H,s), 2.00 (1H,s, OH), 2.85 (6H,s), 3.61 (1H, q, *J* = 2.2 Hz), 3.74 (1H, d, *J*=2.4Hz), 5.88 (1H, d, *J*=2.2Hz), 6.39 (1H, d, *J*=16.2), 6.54 (2H, d *J*=8.2Hz), 7.12 (2H, d *J*=8.4Hz), 7.64 (1H, d, *J*=17.2Hz), 9.20 (1H, s, NH); ^{13}C NMR (100 MHz, DMSO): δ= 19.5, 54.8, 62.4, 71.0, 117.6, 126.9, 129.2, 132.9, 133.4, 142.8, 165.0, 179.0; IR (cm^{-1}): 1660-1610 (C=C), 1720 (C=O), 1760-1730 (Lactam), 1720 (C=O), 3100-3000 (Ar C-H); MS (ESI), *m*/*z* = 331 (M+1, 100%); Anal. Calcd ($C_{16}H_{20}N_2O_4$): C 63.14 H 6.62, N 9.20%. Found: C 63.16, H 6.64, N 9.24%.



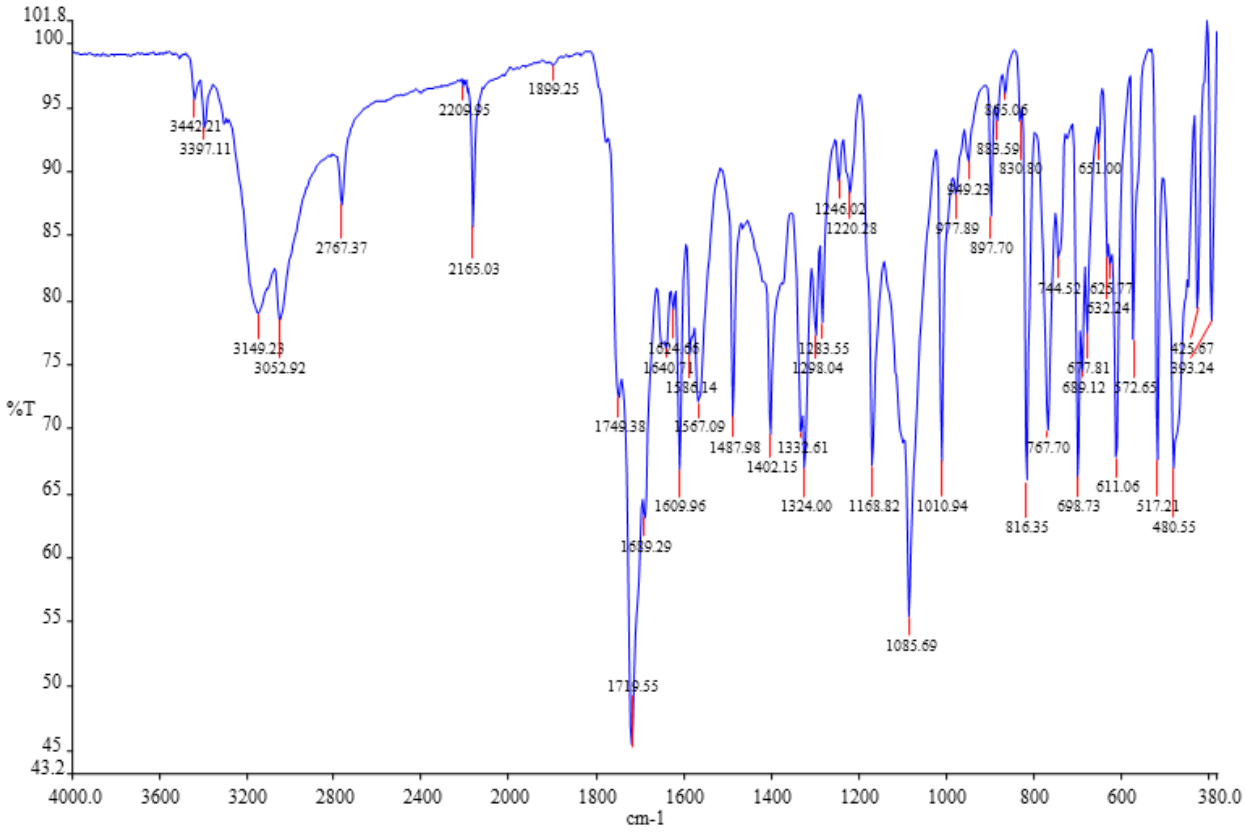
1H Spectrum of CPC 1



^{13}C Spectrum of CPC 1



^{15}N GHSQC spectrum of CPC 1



IR Spectrum of compound 1