RESEARCH ARTICLE

Study the Biological Activity for Shiff Base and B–Lactam Compounds that Synthesis and Identification from Pyrimidine Derivatives

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ABSTRACT

In This study We are synthesis and characterization of some Schiff base and β- lactam derivatives) by three steps. The First react 2-amino-4-Chloro-6-methyl pyrimidine with 4-amino acetophenone in an acid medium to get shiff base derivative(E)-4-(1-((4-Chloro-6-methyl pyridine-2-yl)imino)ethyl)aniline (1), the second step (1) react with (3,4-dimethoxybenzal dehyde,4-methyl benzaldehyde,4-dimethylamino benzaldehyde,4-bromo benzaldehyde,4–hydroxy benzaldehyde, 4-Nitro benzaldehyde) to get Schiff base derivatives (2-7), the last step (2-7) derivatives react with Chloro acetyl chloride to get –β-lactam derivatives.(8-13) All these compounds are characterization by Fourier Transform Infrared Spectroscopy (FTIR), (^{1} H-NMR),(^{13}C- NMR). After that study, the biological activity for all these derivatives to word two kinds of bacteria study the Enzymatic and Cancer Cell.

Keywords: Bacteria, Enzymate, Biological activity, Cancer cell, Schiff base, β-lactam.

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INTRODUCTION

In this research, we prepare as a medium derivative to Prepareβ-Lactam Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antibacterial, antifungal, and antitumor activity.[1] Schiff bases of aliphatic aldehydes are relatively unstable and are readily polymerizable while those of aromatic aldehydes, having an effective conjugation system are more stable.[2] According to the first synthesis of compounds by the scientist, Staudinger in 1907[3] beta-lactam compounds consist of a four-ring connected with six-membered of dihydrothiazin ring called cephalosporins, but if it is connected with five-membered thiazolidine ring called penicillins,[4] β-lactamases are a family of bacterial enzymes that cleave penicillins and cephalosporins with high catalytic efficiency and render these bacteria resistant to β-lactam antibiotics.[5,6] Also, the 2-aze tidinone (ß-lactam) is the essential feature of a large number of biologically active compounds, namely penicillin's, cephalosporin's, carmona, thienamycine, and the norcardicins.[7,8] Enzyme function prediction is a very challenging task in Bioinformatics. It is the most esse ntial mole cule in our life. Enzyme is responsible for catalysis of biochemical reaction for metabolism, structuring the organs and for maintenance of cellular component.[9,10] Cancer is an important reason causing death in many countries. So cancer studies become as interest ing research field. Many research groups approached cancer treatment with some different strategies.[11,12] Primary liver cancers include hepatocellular carcinomas (HCCs), cholangiocarcinomas (CCAS) combined HCC-CCAs, hepatoblastomas, and fibrolamellar hepatocellular carcinomas (FL- HCCS).[13]

MATERIALS AND METHODS

2-Amino-4-Chloro-6-Methyl Pyrimidine, 4-amino acetophenone, glacial Acetic acid, Ethanol, 3, 4-DiMethoxy Benzaldehyde, 4-Methyl Benzaldehyde, 4-di Methyl amino benzaldehyde, 4-Bromo benzaldehyde, 4-Hydroxybenzaldehyde, 4nitrobenzaldehyde, Chloro acetyl Chloride, Triethylamine, 1-4Dioxan. The Materials and all solvents used from (BDR, FLHC, Aldrich.GCC, CDH) (FTIR) recorded on the FIMIR SHIMADZU FTIR-8400S. ^{13}C-NMR and ^{1}HNMR were recorded on the Fourier Transformer spectrometer (500MHZ)with measurements (DMSO-ds) in the Chemistry Department, University of Tehran, Iran.

SynthesisSchiffbases (1)E-4- ((1- ((4-Chloro-6-methylpyrimidin-2-yl)imino)ethyl) aniline.

A mixture of equimolar quantities of the amino compound of (2-amino-4-Chloro-6- methyl pyrimidine) (0, 94 g, 0.0069 mol) and (4-aminoacetophenone (1g, 0069 mol).

Were added ethanol (25mL) heat Mix with refluxed and added drop from glacial acetic acid and for (4hrs) at (78°C) The mixture was cooled and kept for (24 hours), the crystals

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found were filtered, dried and recrystallization from ethanol alcohol and then calculates the weight of the output and prove the physical properties.[14]

Synthesis Derivatives Schiff bases (2, 3, 4, 5, 6, 7)

A mixture of equimolar quantities (1) (1g, 0.0038 mol) with benzyldehyde derivatives (3, 4-Di Methoxy benzaldehyde (0.63 g, 0.0038 mol) (2) (4-methyl benzaldehyde (0.45ml, 0.46g) (3) (4-Dimethylaminobenzaldehyde (0.57g, 0.0038mol) (4) (4-Bromo benzaldehyde (0.71g, 0.0038mol) (5) (4-Hydroxybenzaldehyde (0.46g, 0.0038mol) (6) (4-nitro benzaldehyde (0.58g, 0.0038mol).[7] were refluxed for (5-6 h) at (78 ^{0}C)in (25 mL)of ethanol alcohol, then it was added (3) drops of glacial acetic acid as catalyst. The mixture was cooled and kept for (24-hour), the crystals found were filtered, dried and recrystallized from ethanol alcohol and then calculates the weight of the output and prove the physical properties.[15]

Synthesis Derivatives β–lactama (8, 9, 10, 11, 12, 13)

The mixed of compounds (2, 3, 4, 5, 6, 7) (0.01mol)with (0.006mol)from tri ethyl amine (0.20 mL)of (25 mL 1-4Dioxan) added to Mixed cooled with stirring (0.0024 mol) in the from of solution drops chloro acetyl chloride (0.04 ml) at 10°C for (8-9 hours) precipitates the filtered, dried and recrystallization from ethanol alcohol and then calculates the weight of the output and prove the physical properties [(16)] .

Preparation of Microbiology Culture Media

38g of nutrient agar is dissolved in (1L)of distillation water, then put in an autoclave for (15) minutes at (121°C) for sterilization pouring the media after becoming at (37C) in Petri dishes, made ready for streaking by bacteria. It was getting (Escherichia Coli)and (Staphylococcus aureus)isolated bacteria from hospital.it was cultured and these plates were incubated at (37°C) for (24 hours) for both bacteria, DMSO was used as a solvent to prepare solutions of the various compounds in 5 mL DMSO after that the inhibition zones were examined for all the compounds under test.[17]

Preparation of Solution

Buffer phosphate

The dissolved solution (phosphate buffer, PH = 7.3, 0.2M) was prepared by dissolving 2.8 g of Na_2HPO_4 M.W = 141.69) in 100 ml of distilled water. Then modify the PH by adding drops of hydrochloric acid HCl and used directly.

Detector (DTNB)

This reagent - 5, 5Dithiobis-2-nitro benzoic acid was prepared at a concentration of 0.001 M by dissolving (1 0.0 g) of DTNB (M.W = 396.36) in 25 mL of distilled water with continuous stirring, then kept in a freezer in a vial Opaque, because it is light-sensitive material. This solution was prepared twice a week.

Acetyl Thiocholine Iodide Solution

The base solution was obtained by dissolving (0.017 g) of M.W = 289.18 ACSChl in (1) mL of distilled water. This solution is prepared daily and used directly.

Determination of the Effectiveness of Cholinesterase Enzyme in Human Serum Using the "WHO" Modified MethodAgencies

Place a 2.25 mL of solution solution (PH = 7.3) in a test tube and add 50 µL of the DTNB and 10 µL of the serum solution.

2- The first mixture (2 ml) was withdrawn and placed into a measuring cell (3 mL), then added to the base material (Acetyl thiocholin iodide) (34 µL) at a concentration (0.06 M) and was read The amount of change in the absorption strength of the enzyme before and after the addition of the base material to the length Waveform (412 nm), for every three minutes of enzyme reaction and base material.[18-22]

Survey of new prepared ComPound against Liver Cancer Initialize Cancer Cell Line

The line of liver cancer cells (Hep-G.2) was processed and cultured and the following tissue culture steps were performed:

The tissue culture vessel containing the cancer cells from the agricultural medium was discharged RPMI Media 1640 Wash by neutral phosphate solution and remove immediately and then add 1.5 Ml of trypsin solution- EDTA Place the container in the incubator at 30 ° C for 5–2 minutes, remove the container from the incubator and perform light strokes on the side to facilitate the dissolution of cells into cells individually And then added to the agricultural medium containing 10% of the vaccine and 20 million liters with the events of simple rotational movement to remove the largest amount of cells in the container and then poured in a sterile glass container and homogenized cells by pulling them and quietly discarded by a sterile pipette The button dishes are equipped Microtitter plates Sterilized and container on 96 hole Before the transplantation process, the cells were separated by withdrawing 200 microliters from the cells homogenized in the container by microbiate and placed in each hole of the tissue culture dish with three replicates for each concentration. After the implantation, return the plastic cover to the plate and then put in the incubator at a temperature 37°C until the growth and adhesion of cells in the bottom of the hole forming a single layer of cells with a day to two days and by cell type.[23]

Treatment of cancer cells with β- lactam

After the cancer cells were cultured in the tissue culture dishes, diluted solutions of the beta-lactam complex were obtained. 1 mg of the beta lactam was dissolved in 50 µl of solution Dimethyl Sulfoxide And then complete the volume to 1000 µL in combination with the agricultural medium free of serum pigment and brought from the compound a series of diluted solutions starting at 320, 160, 80, 40, 20, 10 microgram / ml. After the preparation of the solutions, extract the agricultural dish containing the cancer cells planted in the drill and examined the vitality of the cells using the inverted optical microscope to make sure that these cells are ready for treatment and in sterile conditions has been lifted the old agricultural center and washed the agricultural drill once with the solution of phosphate neutral to remove the remnants of

the old agricultural medium with Dead and post cells Add 200 µL of prepared concentrations in each hole (at three replicates per concentration) .

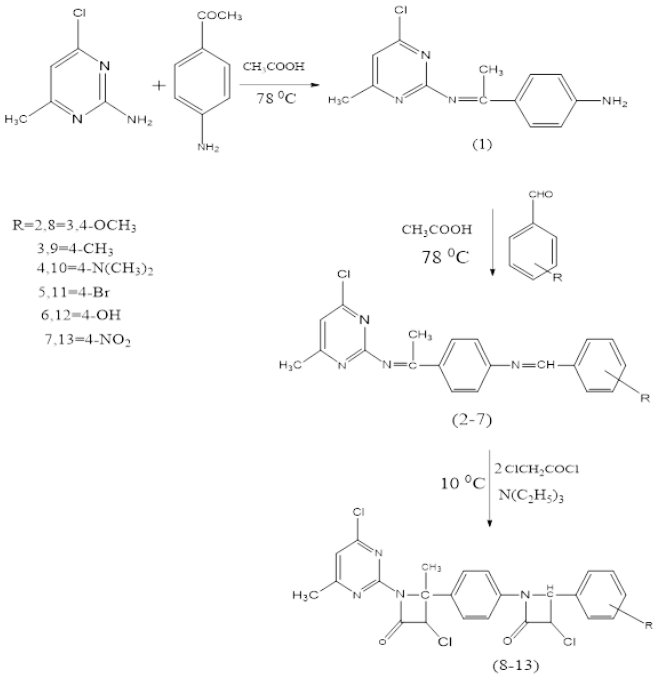
Cellular toxicity study by Violet crystal dye

After lifting the agricultural medium in the boreholes, the drill holes used in the experiment were washed with 200 mL of neutral phosphate precipitation solution and then added 100 µl of dye Stain Violet Crystal The dish was left with the dye for 20 minutes in the room atmosphere. The dye was then removed and discarded and the dish was washed a few times in a bowl in a bowl containing tap water at room temperature until the excess Violet was removed. And then left the agricultural dishes to dry in the atmosphere of the room after drying the plate 100 µL of methyl alcohol 99% purity of each hole of the experiment drilling in the calibration plate for the purpose of dissolving the color of the violet and homogeneous and then read the density of color to dig the plate using the ELISA reader and along the wavelength of 570 nanometers Gel effect of a compound on the vitality of calculating cells β-lactam antibiotics ratio and measure half of the effective influence of the substance in the two lines of liver cancer cells Hep-2.G Using statistical methods by the statistical program.

RESULTS AND DISCUSSION

Compound (1) : (E) -4- (1- ((4- Chloro -6- methyl pyrimidin-2-yl) imino) ethyl) aniline.

The FT-IR data of comp. 1 showed band at (3317)$cm^{-1}$for (NH_2), (3008)cm^{-1} for (Ar-H), (1589)cm^{-1} (C=N)inside Pyrimidin ring, (1658)$cm^{-1}$for (C=N)Shiff base, (2931)$cm^{-1}$for (C-H) for (CH_3), (802) $cm^{-1}$for (C-Cl)and (1512)cm $^{-1}$due to aromatic (C=C).



Scheme 1: Synthesis of Some heterocyclic Compounds derivatives

Compound (2): (1E)-N- (4-Chloro-6-methyl pyrimidin-2-yl)-1- (4- ((3, 4-di methoxy benzylidene)amino)phenyl) ethan-1-imine.

The FT-IR data of comp. 2 showed band at 1272 cm $^{-1}$ for (OCH_3), 3008 Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=N) Shiff base, 2939 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 802 cm^{-1} for (C-Cl).

Compound (3) : (1E)-N- (4-Chloro-6-methyl pyrimidin-2-yl)-1- (4- ((4-methyl benzylidene) amino) phenyl) ethan-1-imine

The FT-IR data of comp. 3 showed band at 1365 cm $^{-1}$ for (CH_3), 3008 Cm^{-1} for (Ar-H), 1590 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=N) Shiff base, 2931 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 802 cm^{-1} for (C-Cl) .

Compound (4) : 4- (((4- (E)-1- ((4-Chloro-6-methyl pyrimidin-2-yl) imino) ethyl) phenyl) imino) methyl)-N, N- dimethyl aniline

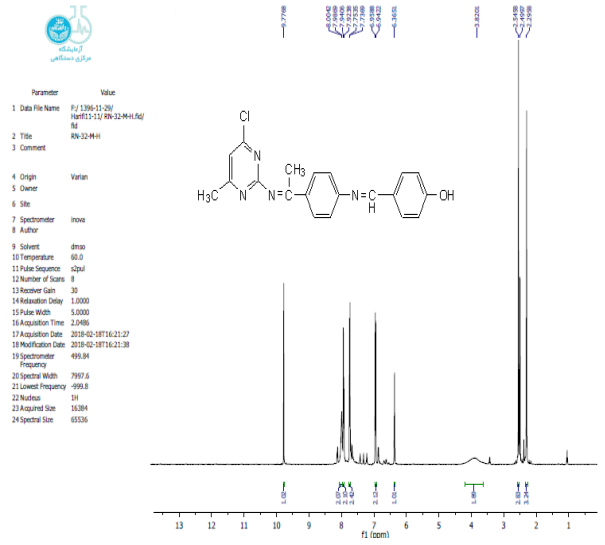
The FT-IR data of comp. 4 showed band at 1373 cm $^{-1}$ for (CH_3), 3008 Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=N) Shiff base, 2916 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 802 cm^{-1} for (C-Cl) .

Compound (5) : (1E)-1- (4- ((4-bromo benzylidene) amino)phenyl)-N- (4-Chloro-6- methyl pyrimidin-2-yl) ethan-1-imine

The FT-IR data of comp. 5 showed band at 663 cm $^{-1}$ for (C-Br), 3008 Cm^{-1} for (Ar-H), 1590 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=N) Shiff base, 2916 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 802 cm^{-1} for (C-Cl)

Compound (6) : 4- (((4- ((E) -1- ((4-Chloro-6- methyl pyrimidin-2-yl) imino) ethyl) phenyl) imino) methyl) phenol

The FT-IR data of comp. 6 showed band at 3301 cm $^{-1}$ for (OH), 3008 Cm^{-1} for (Ar-H), 1588 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=N) Shiff base, 2931 cm^{-1} for (C-H) for



^{1}H NMR spectrum of compound (6)

(CH_3), 1527 cm^{-1} due to aromatic (C=C), 801 cm^{-1} for (C-Cl) . The ^{1}H NMR (DMSO) spectrum data of compound (6) show δ : 9.7 (S, 1H, OH), 2.2 (S, 3H, N=C-CH3), 3.8 (S, 3H, CH3 pyrimidine), 6.3-7.9 (M, 9H, Ar-H), 8.004 (S, 1H, CH). The ^{13}C-NMR (DMSO) spectrum data of compound (6) show δ : 18 (C_{20}), 8 (C_{19}), 155 (C_2), 124 (C_5), 120 (C_{12}), 161 (C_1), 150 (C_9), 153 (C_{16}), 129-142 (C- arom)

Compound (7) : (1E)-N- (4-Chloro-6-methyl pyrimidin-2-yl)-1- (4- ((4-nitrobenzylidene)amino) phenyl).

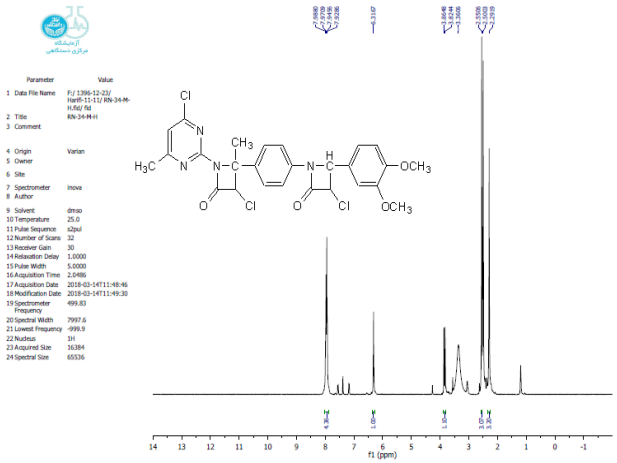
The FT-IR data of comp. 7 Showed band at 1512 cm^{-1} for (NO_2), 3008 Cm^{-1} for (Ar-H), 1590 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=N) Shiff base, 2931 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 801 cm^{-1} for (C-Cl) . The ^{1}H NMR (DMSO) spectrum data of compound (6) show δ : 0.9 (S, 3H, N=C-CH3), 2.3 (S, 3H, CH3 pyrimidine), 6.3-8.3 (M, 9H, Ar-H), 8.4 (S, 1H, CH). The ^{13} C-NMR (DMSO) spectrum data of compound (7) show δ : 26 (C_{20}), 18 (C_{19}), 155 (C_2), 120 (C_5), 124 (C_{12}), 161 (C_1), 153 (C_9), 150 (C_{16}), 129-142 (C-arom)

Compound (8) :3-Chloro-4- (4- (3-Chloro-2- (3, 4-dimethoxy phenyl)-4-oxoazetidin-1-yl)phenyl)-1- (4-Chloro-6-methyl pyrimidin-2-yl)-4- methylazetidin-2-one.

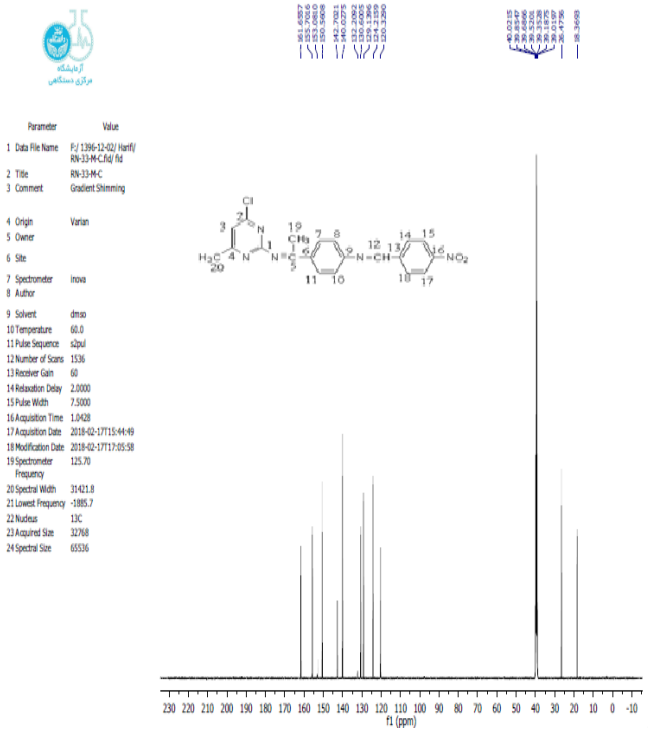
The FT-IR data of comp. 8 Showed band at 1272 cm^{-1} for (OCH_3), 3009 Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=O), 2939 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 725cm^{-1} for (C-Cl), 1272 cm^{-1} for (C-O) . The ^{1}H- NMR (DMSO) spectrum data of compound (8) show δ : 3.0 (S, 6H, OCH3), 1.1 (S, 3H, CH3 β-lactam), 2.2 (S, 3H, CH3 pyrimidine), 3.8 (d, 1H, CH), 3.8 (d, 1H, CH -Cl), 6.3 (S, 1H, CH-Cl), 7.92-7.98 (m, 8H, Ar-H). The^{13} C-NMR (DMSO) spectrum data of compound (8) show δ : 26 (C_{24}, C_{23}), 18 (C_{25}), 8, 4 (, C_{26}), 45 (C_{14}), 55 (C_5), 97 (C_{15}, C_6), 191 (C_{16}, C_7), 154 (C_{20}, C_{21}), 156 (C_2), 161 (C_1), 109-149 (C_{Arom}).

Compound (9) :3-Chloro-4- (4- (3-Chloro-2-oxo-4-(p-tolyl)azetidin-1-yl)phenyl)-1- (4-Chloro-6-methyl pyrimidin-2-yl)-4-methylazetidin

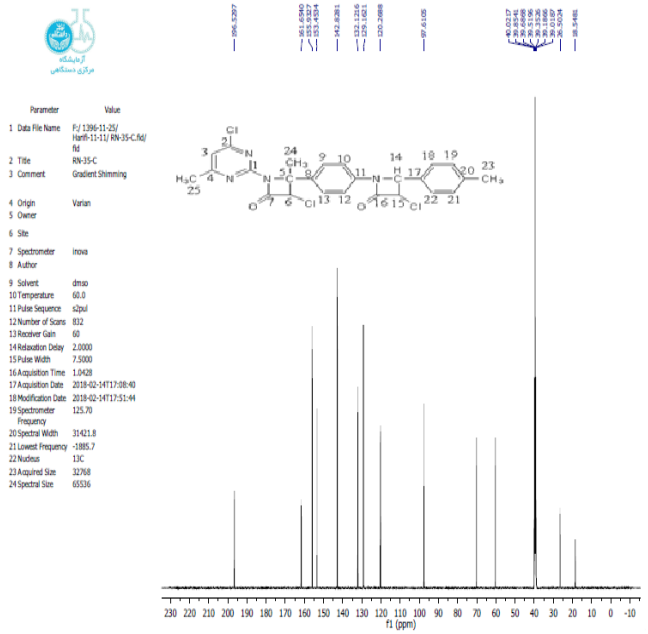
The FT-IR data of comp. 9 Showed band at 1365 cm^{-1} for (CH_3), 3008 Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=O), 2939 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 801cm^{-1} for (C-Cl) . The ^{1}H- NMR (DMSO) spectrum data of compound (9) show δ: 1.01 (S, 43H, CH3), 1.2 (S, 3H, CH3 β-lactam), 2.2 (S, 3H, CH3 pyrimidine), 3 (d, 1H, CH), 3.4 (d, 1H, CH -Cl), 3.5 (S, 1H, CH-Cl), 6.3, 8 (m, 9H, Ar-H) . The^{13} C-NMR (DMSO) spectrum data of compound (9) show δ : 26 (C_{24}), 18 (C_{23}), 39 (C_{25}), 60.3 (C_{14}),



^{1}H NMR spectrum of compound (8)



C- NMR spectrum of compound (7) ^{13}

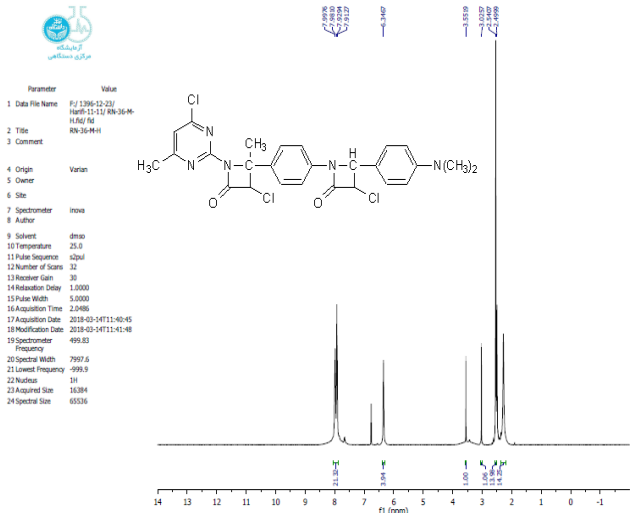


^{13}C- NMR spectrum of compound (9)

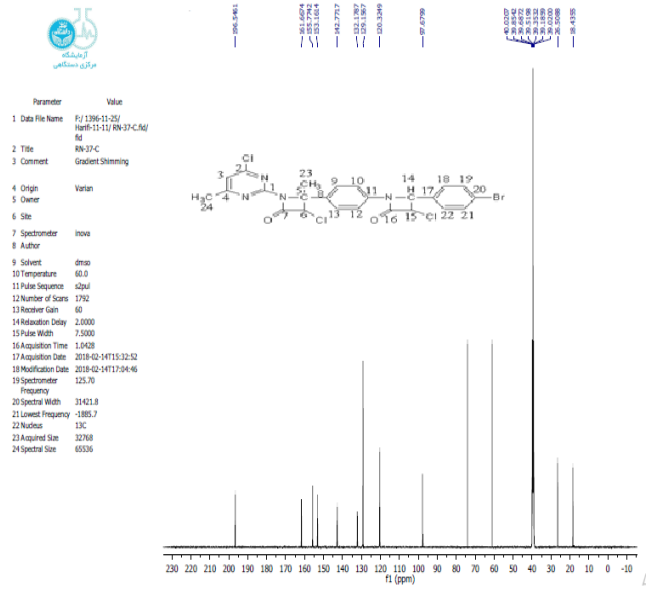
70.1 (C_5), 97 (C_{15}, C_6), 196 (C_{16}, C_7), 155 (C_2), 161 (C_1), 120-153 (C_{Arom}) .

Compound (10) :3-Chloro-4- (4- (3-Chloro-2- (4-(dimethyl amino)phenyl)-4-oxoazetidin-1-yl)phenyl)-1-(4-Chloro-6-methylpyrimidin-2-yl)-4-methylazetidin-2-one

The FTIR data of comp. 10 showed band at 1365 cm $^{-1}$ for (CH_3), 3009 Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=O), 2939 cm^{-1} for (C-H) for (CH_3), 1504 cm^{-1} due to aromatic (C=C), 750cm^{-1} for (C-Cl) . The ^{1}H- NMR (DMSO) spectrum data of compound (10) show δ : 2.2 (S, 6H, N (CH_3)$_2$), 1.1 (S, 3H, CH3 β-lactam), 1.2 (S, 3H, CH3 pyrimidine), 3.0 (d, 1H, CH), 3.5 (d, 1H, CH__-Cl), 6.3 (S, 1H, CH-Cl), 7.91, 7.99 (m, 9H, Ar-H) . The ^{13}C-NMR (DMSO) spectrum data of compound (10) show δ : 39 (C_{24}, C_{23}), 18 (C_{25}), 60 (C_{14}), 71 (C_5), 97 (C_{15}, C_6), 196 (C_{16}, C_7), 156 (C_2), 161 (C_1), 153 (C_{11}), 120-142 (C_{Arom}).



^{1}H NMR spectrum of compound (10)



^{13}C- NMR spectrum of compound (11)

Compound (11) :4- (4- (2- (4-bromo phenyl)-3-Chloro-4-oxoazetidin-1-yl)phenyl)-3-Chloro-1- (4-Chloro-6-methyl pyrimidin-2-yl)-4- methyl azetidin-2-one.

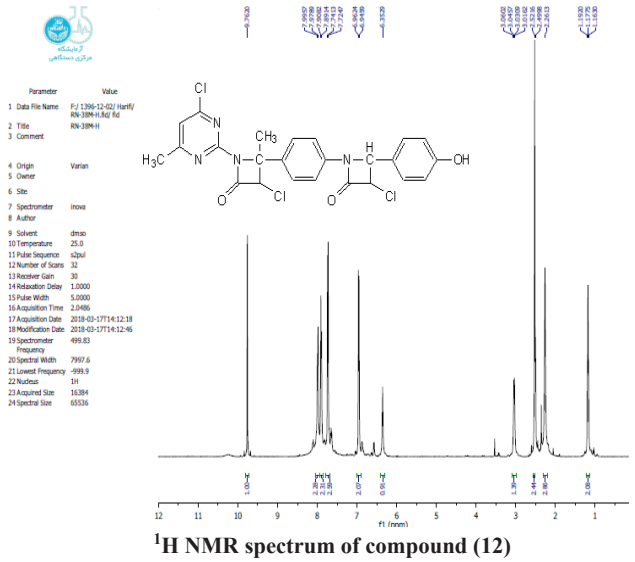
The FTIR data of comp. 11 showed band at 663 cm $^{-1}$ for (C-Br), 3008 Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=O), 2939 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 755cm^{-1} for (C-Cl) . The ^{1}H- NMR (DMSO) spectrum data of compound (11) show δ : 1.1 (S, 3H, CH3 β-lactam), 2.28 (S, 3H, CH3 pyrimidine), 3.4 (d, 1H, CH), 3.5 (d, 1H, CH__-Cl), 6.3 (S, 1H, CH-Cl), 7.91, 8 (m, 9H, Ar-H) . The^{13} C-NMR (DMSO) spectrum data of compound (11) show δ : 26 (C_{24}), 18 (C_{23}), 39 (C_{14}), 61 (C_5), 76 (C_{15}, C_6), 196 (C_{16}, C_7), 155 (C_2), 161 (C_1), 153 (C_{11}), 120-142 (C_{Arom}).

Compound (12) :3-Chloro-4- (4- (3-Chloro-2- (4-hydroxy phenyl)-4-oxoazetidin-1-yl)phenyl)-1- (4-Chloro-6-methyl pyrimidin-2-yl)-4-methylazetidin-2-one.

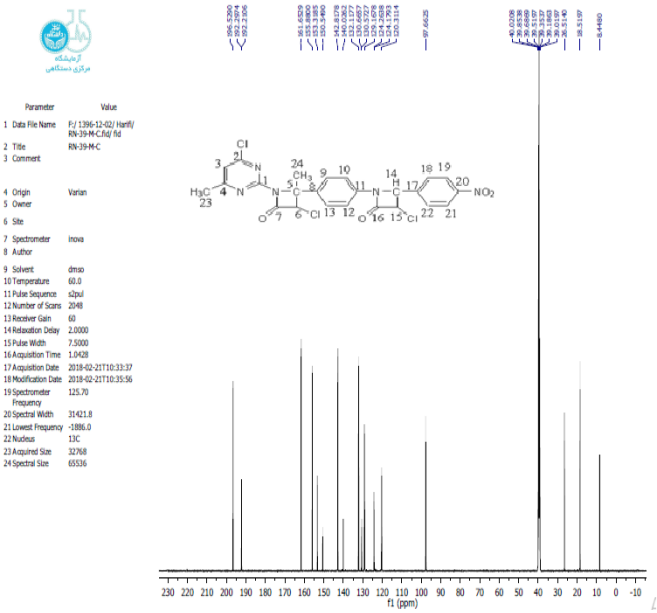
The FTIR data of comp. 12 showed band at 3317cm $^{-1}$ for (OH), 3008 Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside pyrimidin ring, 1658 cm^{-1} for (C=O), 2917 cm^{-1} for (C-H) for (CH_3), 1527 cm^{-1} due to aromatic (C=C), 755cm^{-1} for (C-Cl) . The ^{1}H- NMR (DMSO) spectrum data of compound (12) show δ :9.7 (S, 1H, OH), 1.16 (S, 3H, CH3 β-lactam), 2.2 (S, 3H, CH3 pyrimidine), 3.01 (d, 1H, CH), 3.02 (d, 1H, CH__-Cl), 3.06 (S, 1H, CH-Cl), 6.3-7.9 (m, 9H, Ar-H) . The ^{13}C-NMR (DMSO) spectrum data of compound (12) show δ : 18 (C_{24}), 8.5 (C_{23}), 26 (C_{14}), 45 (C_5), 97 (C_{15}, C_6), 190- 196 (C_{16}, C_7), 161 (C_2), 163 (C_1), 154 (C_{11}), 115-143 (C_{Arom}).

Compound (13) :3-Chloro-4- (4 (3-Chloro-2- (4-nitro phenyl)-4-oxoazetidin-1-yl) phenyl)-1- (4-Chloro-6-methyl pyrimidin-2-yl)-4- methylazetidin-2-one.

The FTIR data of comp. 13 showed band at 1512cm^{-1} for (NO_2), 3009Cm^{-1} for (Ar-H), 1589 cm^{-1} (C=N) inside Pyrimidin ring, 1658 cm^{-1} for (C=O), 2939 cm^{-1} for (C-H) for (CH_3), 1504 cm^{-1} due to aromatic (C=C), 750 cm^{-1} for (C-Cl) . The ^{1}H- NMR (DMSO) spectrum data of compound (13) show δ : 1.1 (S, 3H, CH3 β-lactam), 1.3 (S, 3H, CH3 pyrimidine), 2.2 (d, 1H, CH),



^{1}H NMR spectrum of compound (12)



^{13}C- NMR spectrum of compound (13)

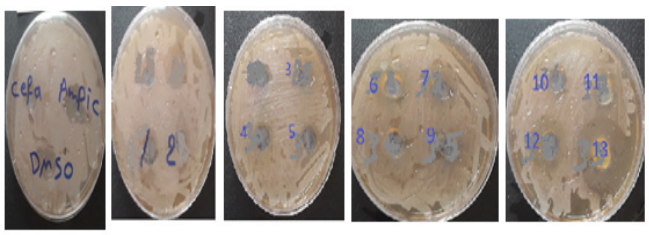


Figure 14: Biological activity of compound prepared against staphylococcusureus bacteria

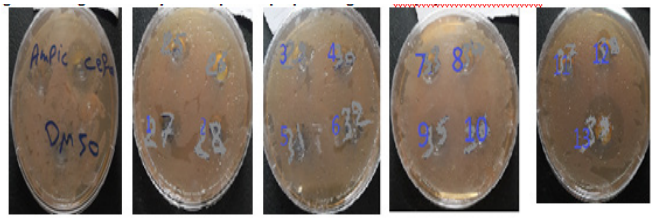


Figure 15: Biological activity of compound prepared against Escherichia coli bacteria

3.34 (d, 1H, CH -Cl), 6.3 (S, 1H, CH-Cl), 7.9, 8.4 (m, 9H, Ar-H). The ^{13}C-NMR (DMSO) spectrum data of compound 13 show δ : 8.4 (C_{24}), 18.5 (C_{23}), 26 (C_{14}), 39 (C_5), 97 (C_{15}, C_6), 192- 196 (C_{16}, C_7), 155 (C_2), 161 (C_1), 150 (C_{11}), 120-142 (C_{Arom}).

BIOLOGICAL ACTIVITY

From the above studies it can be concluded that the synthesized compounds exhibit significant antibacterial activity against bacteria staphylococcus aurous and Escherichia coli, the compounds that appeared good activity are 13 against (staphylococcus aurous) on other hand, compound 13 show good activity against (Escherichia coli), the results of the antibacterial activity are shown in the Figure 14 and 15.

Effect of Compound (13) on the Effectiveness Of Cholinesterase Ch.E in Human Serum

The percentage of inhibition of the enzyme was calculated using the concentrations used for compound (13) based on the percentage of inhibition by comparing the efficacy of the enzyme by using the inhibitory substance without the inhibitory substance under the same condition as shown in the equation below.

$$\text{Inhibition \%} = 100 - \frac{\text{Effectiveness using inhibitory substance} \times 100}{\text{Effectiveness without using inhibitory substance}}$$

The Results of this study are shown in Table (1) below

Biologic Study on Cancer Cells

After studying the biologic activity of the compounds prepared on two types of bacteria and identifying the most effective compound of the Bylogical compound 13 the pharmacological efficacy of the enzyme as well as its pharmacological efficacy

Table 2: Effect of compound (13) as a stimulant

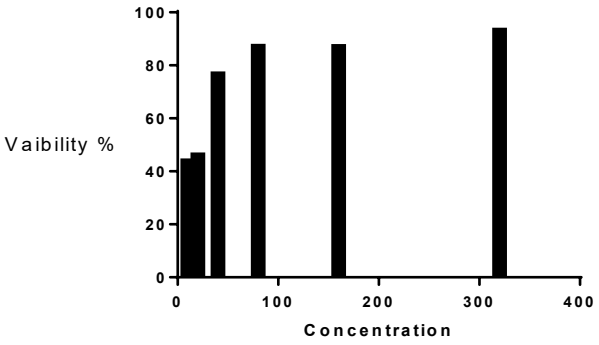
Concentration (M)	*Effectiveness µmol/ml/3min*	*% Inhibition*
None	8.23	0.0
$4.5x10^{-2}$	0.2942	96.42
$4.5x10^{-3}$	15.739	91.23
$4.5x10^{-4}$	1.471	82.12
$4.5x10^{-5}$	2.942	64.25

Table 1: Show Biological activity for compounds (1)

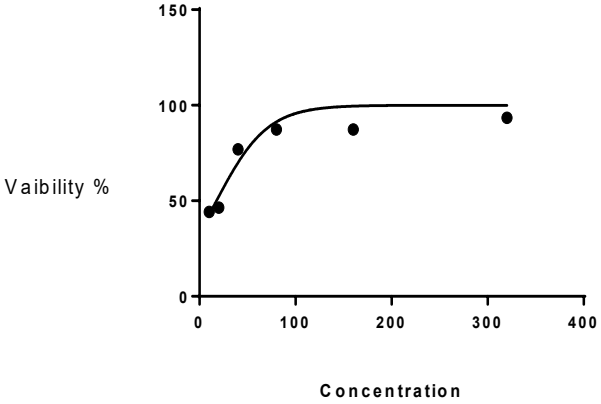
Compounds No.	*Bacterial species E. Coli*	*Staph . aureus*	*Compounds No.*	*Bacterial species E. Coli*	*Staph. aureus*
Ampicillin	+++	+++	8	-	_
Cephalixin	+++	-	9	-	+++
DMSO	-	+++	10	-	_
1	+++	+++	11	-	+++
2	+++	+++	12	+++	+++
3	-	_	13	+++	+++
4	-	_			
5	-	_			
6	-	+++			
7	-	+++			

-=No inhibition = inactive, ++= (11-20) mm= moderately active, +++ = (more than 20) mm good active

on the infected and normal hepatocellular carcinoma this study showed that this compound has a high pharmacological efficacy .the effect of this compound on cancer cells was studied in six different concentrations. The results showed that when the concentration of 320 Mg/ml cells infected the response rate



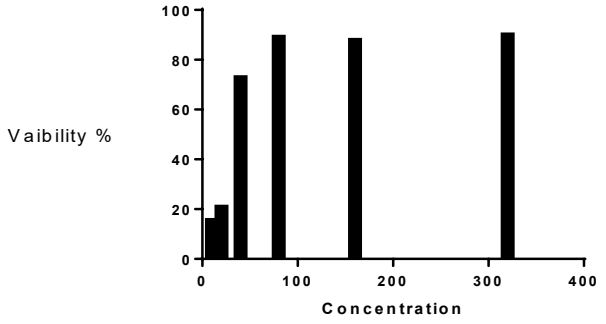
Shows the percentages of cells that remain alive versus different concentrations on the Infected liver cancer cell line as well as showing the moral difference between the concentration



Shows the greatest concentration of treatment that eliminates half the cells (IC50) Infected Cell.IC50=4.630

Table 3: Physical properties of compound (2)

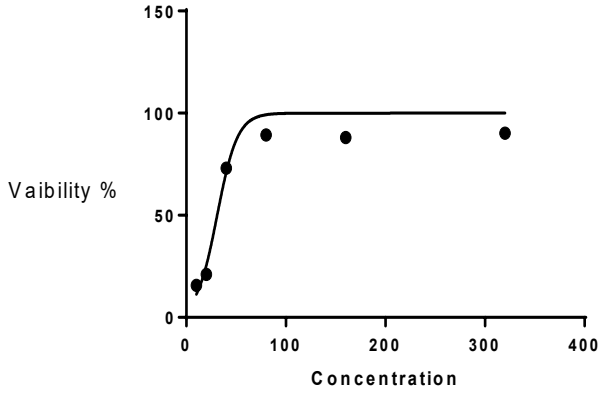
No	*Name of Comp .*	*M.f*	*M.W*	*M.P (^0C)*	*R_f*	*Color*	*%*
1	(E)-4- (1- ((4-Chloro-6-methyl pyrimidin-2-yl) imino)ethyl) aniline	$C_{13}H_{13}ClN_4$	260.73	167-165	0, 33	Yellow	95
2	(1E)-N- (4-Chloro-6-Methyl pyrimidin-2-yl)-1- (4- ((3, 4-di methoxy benzylidene) amino) phenyl) ethan-1- imine	$C_{22}H_{21}ClN_4O_2$	408.89	201 – 204	0.22	Yellow	80
3	(1E)-N- (4- Chloro-6-methyl pyrimidin-2-yl) -1- (4- ((4-methyl benzylidene) amino) phenyl) ethan-1-imine	$C_{21}H_{19}ClN_4$	362.86	163- 164	0.33	Yellow	93
4	4- (((4- (E)-1- ((4-Chloro-6-methyl pyrimidin-2-yl) imino)ethyl)phenyl) imino)methyl)-N, N- di methyl aniline	$C_{22}H_{22}ClN_5$	391.90	86- 88	0.21	Brown	93
5	(1E)-1- (4- ((4-bromo benzylidene) amino) phenyl) –N- (4-Chloro-6- methyl pyrimidin -2- yl) ethan-1- imine	$C_{20}H_{16}BrCl\ N_4$	427.73	220 – 222	0.23	Yellow	85
6	4- (((4- ((E) -1- ((4-Chloro-6-methyl pyrimidin-2-yl) imino) ethyl) phenyl) imino) methyl) phenol	$C_{20}H_{17}ClN_4O$	364.83	131- 133	0.34	Brown	87
7	(1E) –N- (4-Chloro-6-Methyl pyrimidin-2-yl) -1- (4- ((4-nitro benzylidene) amino) phenyl) ethan -1- imine	$C_{20}H_{16}ClN_5O_2$	393. 83	127- 130	0.24	Yellow	92
8	3-Chloro-4- (4- (3-Chloro-2- (3, 4-di methoxy phenyl)-4- oxoazetidin-1-yl) phenyl)-1- (4-Chloro-6-methyl pyrimidin-2-yl)-4- methyl azetidin-2-one	$C_{26}H_{23}Cl_3N_4O_4$	561.84	116 -119	0.26	Yellow	65
9	3-Chloro-4- (4- (3- Chloro-2-OxO-4- (p-tollyl) azetidin-1-yl) phenyl)-1- (4-Chloro -6-methyl pyrimidin -2-yl)-4-methyl azetidin-2-one	$C_{25}H_{21}Cl_3N_4O_2$	515.82	136-134	0.33	Yellow	66
10	3-Chloro-4- (4- (3-Chloro-2- (4- (di methyl amino) phenyl)-4-oxoazetidin-1-yl) phenyl) -1- (4-Chloro-6-methyl pyrimidin-2-yl)-4- methylazetidin-2-one	$C_{26}H_{24}Cl_3N_5O_2$	544.86	171- 173	0.22	Yellow	58
11	4- (4- (2- (4-bromo phenyl) -3-Chloro-4-oxoazetidin-1-yl) phenyl)-3-Chloro-1- (4-Chloro-6-methyl pyrimidin-2-yl) -4- methylazetidin-2-one	$C_{24}H_{18}BrCl_3N_4O_2$	580.69	140- 142	0.34	Yellow	55
12	3-Chloro-4- (4- (3-Chloro-2- (4- hydroxy phenyl)-4- Oxoazetidin-1-yl) phenyl)-1- (4- Chloro -6-methyl pyrimidin -2-yl)-4- methyl azetidin-2-one	$C_{24}H_{19}Cl_3N_4O_3$	517.79	107- 104	0.31	Yellow	63
13	3-Chloro-4- (4- (3 Chloro-2- (4-nitro phenyl)-4 oxoazetidin-1-yl) phenyl)-1- (4-Chloro-6-Methyl pyrimidin-2-yl)-4- methyl azetidin-2 – one	$C_{24}H_{18}Cl_3N_5O_4$	546.79	121 -123	0.32	Yellow	93



Shows the percentages of cells that remain alive versus different concentrations on the natural liver cancer cell line as well as showing the moral difference between the concentration.

(93%) and the same concentration of natural cells response rate (90%) as shown in figure (16) also shows the differences in the differences between the Concentrations

The mean difference between these concentrations which is unique among the concentrations of (320) for both lines gave the inhibition rate of injured line (93%) and the inhibition rate of the normal line (90%).



Shows the greatest concentration of treatment that eliminates half the cells (IC_{50}) natural Cells IC_{50}=9.533

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