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ORIGINAL ARTICLE

Synthesis, Characterization, and Computational, Biological Studies of Four-membered Cyclic Amides 2- azetinones

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KEYWORDS

β-Lactams; Azetidin-2-ones; Tetrahydro furan-3-carboxylic acid; Gaussian program

ABSTRACT: Two compounds,3-benzoyl-2-(4-bromophenyl)-3-phenyl-6 – oxa-2-azaspiro[3.4]octan-1-one and 1',1'''-bis(3-bromo-4-(dimethylamino)phenyl)-4,4''',5,5'''-tetrahydro-2H,2'''H-tetraspiro[furan-3,2'-azetidine-4',1''-acenaphthylene-2'',2'''-azetidine-3''',3'''-furan]-3',4'''-dione, were synthesized by tetrahydro furan-3-carboxylic acid and appropriate imines. Two studies were conducted on the prepared compounds, one of them is the study of their anti-biological efficacy using two types of bacteria *Staphylococcus aureus* and *Escherichia coli*, The other study that was conducted on the compounds is a computational study to calculate some of the thermodynamic parameters of synthesized derivatives by using a Gaussian program.

INTRODUCTION

Staudinger produced a beta-lactam ring by cyclic loading [2+2] by using chitins and imines as shown in Figure 1. Thus the first beta-lactam compound was prepared in 1907, and these are among the heterocyclic compounds known as 2-Azetidinones [1, 2].

β-lactam compounds have many uses, including antibacterial, as well as increasing genetic mutations and transfer genes by increasing the number of resistant strains to bacteria. Examples of these compounds are (penicillin and cephalosporins) (Figure 2).[4]

The second group of naturally occurring beta-lactams and cephalosporins were discovered and chemical modifications to them provided effective semi-synthetic derivatives against different types of bacteria known to be resistant to penicillin. Many properties of ampicillin and amoxicillin are similar Figure 3.

However, the two antibiotics are different, in that, the latter is more completely absorbed than the former when given orally. In the case of amoxicillin, the drug levels in the blood are twice as high as the corresponding dose of ampicillin. Several other biological activities such as antifungal [5] and anticancer, synthesis have been based on the construction of the β-lactam ring Figure 4. [6-8]

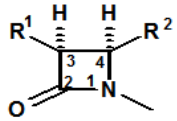

Figure 1. β-lactam compound.

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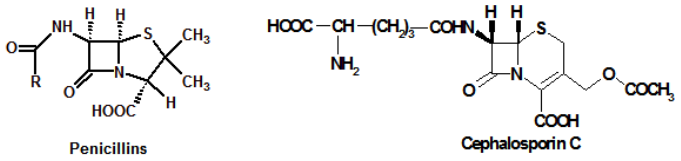

Figure 2. Examples of β-lactam compounds.

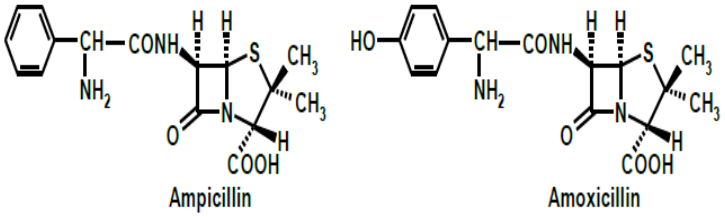

Figure 3. Chemical structure of Ampicillin and Amoxicillin.

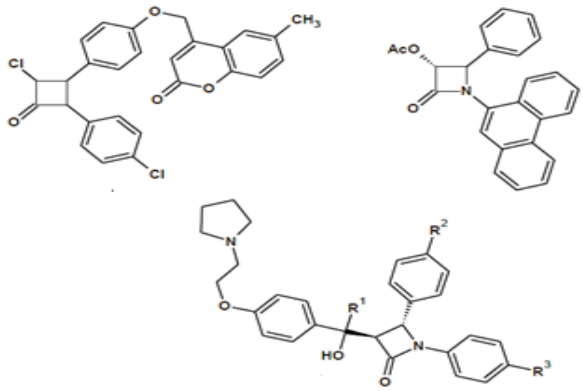

Figure 4. Chemical structure of some β -lactam compounds is anti-fungal and anti-cancer.

MATERIALS AND METHODS

***Synthesis of imines [9]*(I, II)**

1-(2E)-2-[(4-bromophenyl) imino]- 1, 2-diphenylethanone (I)

Prepared by reaction Benzil (1g, 4.76 mmol) with 4-bromoaniline (0.818 g, 4.76mmol) and add (10 -15) drops of glacial acetic the product was purified by recrystallization with methanol [m.p = 112 -114˚C, R_f= 0.8, IR (KBr disk): (C=N) 1620 cm^{-1}.

2-(1Z,2E)-N,N'-bis(4-(dimethylamino) phenyl)-2a,5 dihydroacenaphthylene-1,2-diimine(II)

Prepared by reaction Acenaphthenquinone (1g, 5.49 mmol) with 2-bromo- N,N-dimethylbenzene-1,4-diamine (2.36g , 10.98 mmol) and add (10 -15) drops of glacial acetic The product was purified by recrystallization with methanol [m.p = 119 -121˚C, R_f= 0.9, IR (KBr disk): (C=N)1654 cm^{-1}.

General Procedure of of β-lactam [10, 11]

3-benzoyl-2-(4-bromophenyl)-3 – phenyl – 6- oxa –2 – azaspiro[3.4]octan-1-one

The compound was prepared by reactant (1 g , 2.747 mmole) (E)-2-((4-bromophenyl)imino)-1,2-diphenylethan-1-one with (0.2627 mL ,0.31 g , 2.747 mmole) Tetrahydro furan-3-carboxylic acid and (1.14mL, 0.83241g, 8.241mmole) triethylamine in (25mL) of dichloromethane (DCM) , were added under nitrogen(N_2) atmosphere at 0˚C drop wise, Then add (0.384mL, 0.630g, 4.1205mmole) a solution of $POCl_3$ in 10 mL of dry dichloromethane with stirring at 0˚C.

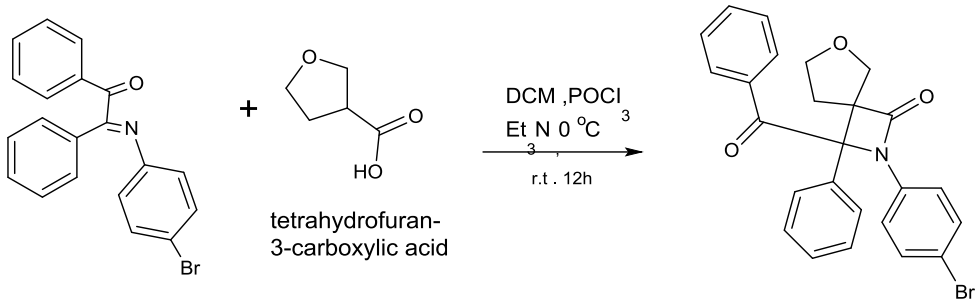

Figure 5. Preparation of compound 1

1',1'''-bis(3-bromo-4-(dimethylamino)phenyl)-4,4'''',5,5''''-tetrahydro-2H,2''''H-tetraspiro[furan-3,2'-azetidine-4',1''-acenaphthylene-2'',2'''-azetidine-3''',3''''-furan]-3',4'''-dione

The compound was prepared by reactant (1g,3.81mmole) 2-bromo-4-(((1E,2Z)-2-((3-bromo-4-(1-methylhydrazinyl) phenyl)imino)acenaphthylen-1(2H)-ylidene)amino)-N,N-dimethylaniline with (0.885mL,0.73g, 7.62mmole) Tetrahydro furan-3-carboxylic acid and (3.17mL, 2.308g, 22.86mmole) triethylamine in (25mL) of dry dichloromethane (DCM) , were added under nitrogen (N_2) atmosphere at 0˚C drop wise, Then add (1.066 mL, 1.748 g, 11.43mmole) a solution of $POCl_3$ in 10mL of dry dichloromethane with stirring at 0˚C.

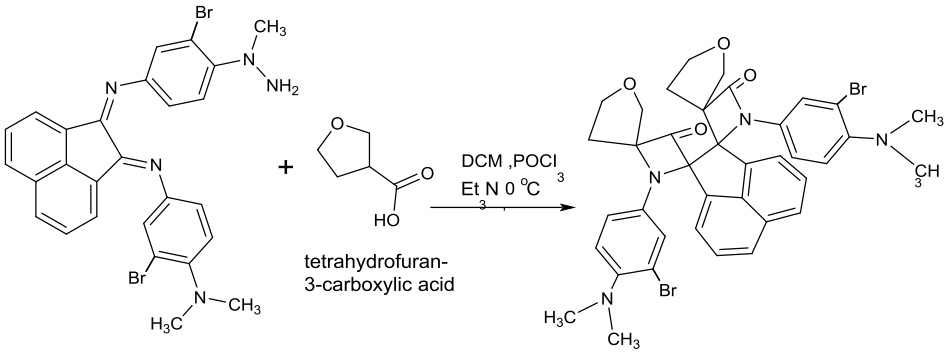

Figure 6. Preparation of compound 2.

Antibacterial activity

Two types of bacteria were used to determine the biological activity of the prepared β-lactam compounds. The bacteria used are (*Staphylococcus aureus* and *Escherichia coli*) and the results were compared. Dimethyl sulfoxide as a control and the concentrations of the compounds (0.1mL) were added to the cups. In this study, the results appeared after incubation for 48 hours at 37˚C, Antibacterial testing was carried out using the agar diffusion technique. Antimicrobial activity was evaluated based on the observed zone of inhibition value by measuring the diameter of the zone of inhibition against test organisms [12, 23].

Determination of Cytotoxicity using human erythrocytes

Different concentrations of the prepared compounds (0.2, 0.4, and 0.6) $mgml^{-1}$ were prepared and diluted with phosphate buffered saline. An Eppendorf tube was used, where a volume of 0.8ml of all concentrations was placed, and then human red blood cells were added to make the final volume of 1ml. The tubes were examined to detect the decomposition of red blood cells after a 30- minute and at37°C a temperature. The results were compared with negative control (saline) and positive control (tap water) [13].

Quantum chemical calculations

All calculations in this work were performed by the implementation of DFT (Density Function Theory) using Gaussian 09 W which has been linked to Gauss view 5.0for the purpose of calculating the most important parameters such as EHOMO, ELUMO, energy gap (ΔE), and the other parameters.

RESULTS AND DISCUSSION

Synthesis of imines

Compounds (I) were prepared from the reaction of Benzil with 4- bromoaniline while PreparedCompound (II)byreactionAcenaphthenquinone with2-Bromo- N, N-dimethylbenzene-1,4-diamine and presence of glacial acetic acid in absolute ethanol as shown in Figure 7.

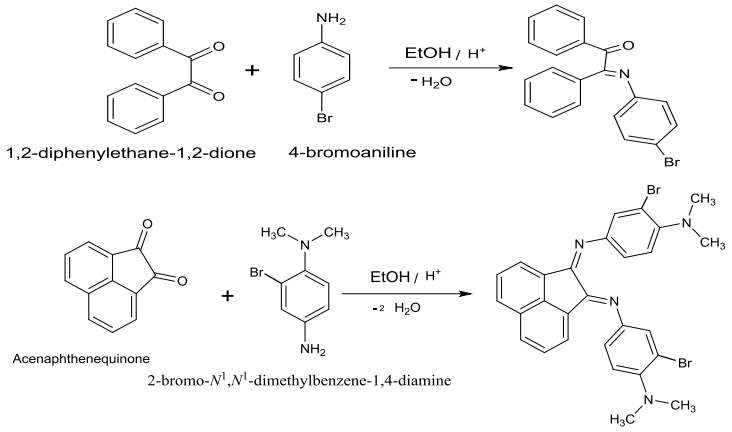

Figure7. Synthesis of imine.

Involves the reaction of a nucleophilic attack of the amine group on the carbon of the carbonyl group of the ketone to form a compound N-(substituted hemiaminals) which loses a water molecule to give the stable compound. Mechanism of imine formation is shown in Figure 8.

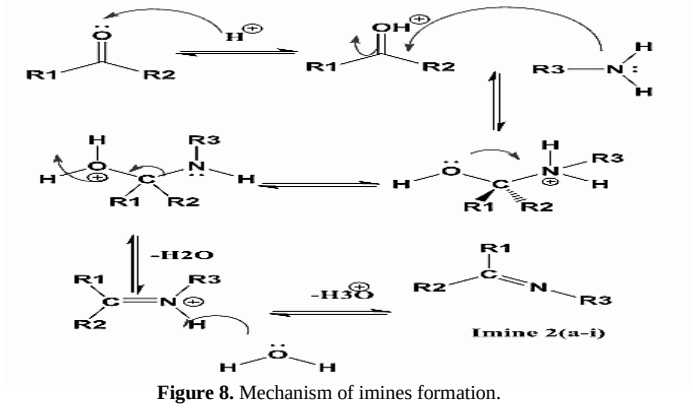

Figure 8. Mechanism of imines formation.

measureds the melting point Compounds (I,II) as show in Table 1 diagnosed by specifying (FT-IR) shown in Table 2.It features ranges corresponding to the expansion vibrations bands aliphatic (C- H), (aromatic C-H), (C=C)) and (azomethineband C=N). These bands occur (2852.72, 2800.64, 2835.36), (3026. 31,3062.96,3001.24)), (1573.91, 1512. 19, 1600.92), (1618.28, 1604.77 ,1616.35) respectively.

Tabel 1. Shows the physical date imines.

Comp.	m.p.°C	Color	RF	Reaction time	Yield%
I	220-222	Yellow	0.9	25h	80
II	248-250	Brown	0.7	25h	77

Table 2. Shows IR spectra of Imines.

Comp.	Aromatic (C-H) stretching (cm^{-1})	Aliphatic(C-H) stretching (cm^{-1})	Azomethine(C=N) stretching (cm^{-1})	Aromatic (C=C) stretching (cm^{-1})
I	3055 3078	2951	1620	1581
II	3007 3062	2989	1658	1593

Synthesis β-lactam

Benefiting from previous studies ketene-imine cyclization in the presence of triethylamine furnishing C3–C4 bond formation of β-lactam as steps for the syntheses β-lactam Compounds. The synthesis involves the treatment of imines with Tetrahydro furan-3-carboxylic acid or Tetrahydro furan-2-carboxylic acid triethylamine with phosphorus oxychloride POCl3 by using solvent dichloromethane under a dry system nitrogen (N_2) atmospheres and mechanism of formation β-lactams shown in Figures 9 & 10 [14].

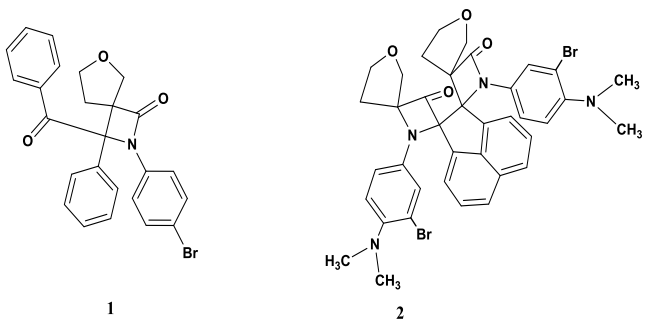

Figure 9. Structure synthesized β-lactams compounds.

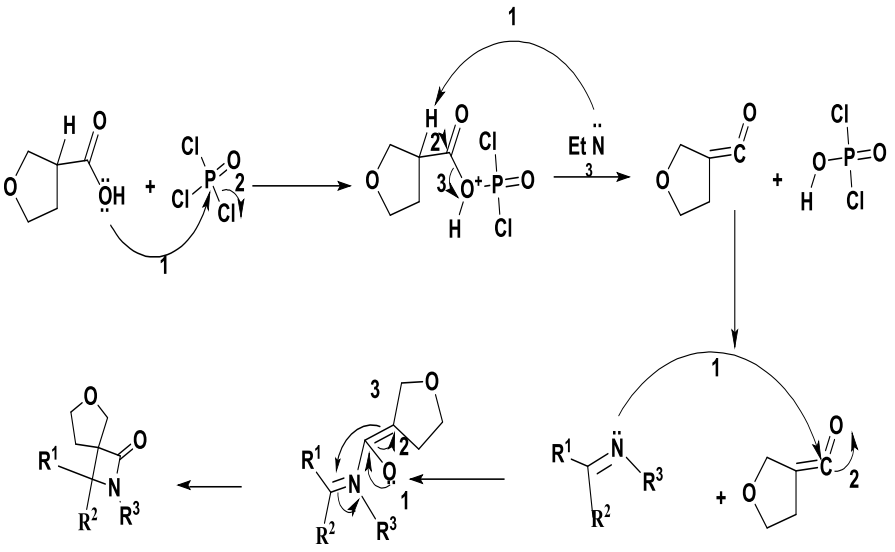

Figure 10. Mechanism of β-lactams formation.

FT-IR data and bonding

Infrared spectra of β-lactam showed the presence of the aromatic (C-H) group, the aliphatic (C-H) group, the carbonyl group, the alkene, and the substituent ring occurring within the ranges at 3002-3070, 2993-2927, 1727-1751, 1458-1618, and 748-858cm^{-1}, respectively [15-17].FT-IR spectrum data of β-lactam are shown in Table 3.

Also measured physical properties of the prepared compounds (1, 2) are shown in Table 4.

^{1}H NMR spectral

^{1}H-NMR results of β-lactam compounds are shown in Table 5.

Table 3. FTIR spectral data of β-lactam.

(Comp.)	Aromatic (C-H) stretching (cm^{-1})	Aromatic (C=C) stretching (cm^{-1})	Aliphatic (C-H) stretching (cm^{-1})	Amide (C=O) stretching (cm^{-1}) (β-lactam)
1	**3008**	**1588**	**----**	1674
2	**3010**	**1602**	**2938**	1718

Table 4. Physical properties of β-lactam.

Comp	m.p.°C	Colour	Yield%
1	244-246	Yellow	60
2	152-154	Yellow	65

Table5. ^{1}H-NMR spectrum data of β-lactam.

Comp.	tetrahydrofuran ring proton (ppm)	Aromatic proton(ppm)	Aliphatic proton(ppm)
1	1.06- 3.41	7.58-7.95	---
2	1.07– 3.39	6.80-8.58	2.14–N-$(CH_3)_2$

Antibacterial activity

The results showed that the prepared compounds of different concentrations and both compounds had different biological activity against positive Bacteria (*Staphylococcus aureus*) and negative Bacteria. Table6 shows the obtained results.

Table 6. Biological activities for β-lactams

NO.	Comp.	Gram- positive bacteria Compd. No. Klebsiella pneumonia *Staph.aureus* Gram- positive bacteria *staph.aureus*	Gram-negative bacteria *E.coli*
1	a	22	24
2	b	19	21

Determination of Cytotoxicity using Human erythrocytes

The prepared beta-lactam compounds did not show any toxicity toward red blood cells at concentrations (0.2, 0.4, and 0.6) mg ml^{-1}. This method is considered one of the quick ways to know the decomposition of red blood cells and depends on several factors, including temperature, incubation period, and concentration of the substance

Quantum chemical calculations

The global parameters for the chemical reactivity were calculated from LUMO and HOMO energies and the results are summarized in Table7.Figure 5 shows the frontier molecule orbital density distributions of the investigated compounds.

The HOMO-LUMO energygap indicates the chemical reactivity of the molecule whether the molecule is “hard” or “soft”. On the contrary, a large (LUMO – HOMO) energygap corresponds to high kinetic stability and low chemical reactivity. Compound 2 is characterized by a small energy gap (ΔE gap=2.44 eV) while sample 1 has the highest energy gap (ΔE gap=2.59 eV). So, compound 2 known as a "soft" molecule has a high ability to polarize because its excitation energy is small, unlike the hard molecule, and thus affects the activity of the molecule from a Biological aspect [18].

Ionization potential is an important parameter of the chemical reactivity of molecules, high ionization potential values indicate chemical inertness and high stability, and small ionization potential values indicate high reactivity of the molecules, As illustrated in Table 7, we can note that compound1 has the highest ionization potential values, therefore, these molecules are high stability and less reactivity. Also, compound 2 has the lowest ionization potential, so, these molecules are showing less stability and more reactivity.

The definition of electron affinity (EA) is the energy released when an electron is added to a neutral molecule [19]. It has been found that the lower electron affinity values enhanced the electron-donating property of the molecule, small values the electron-donating property of the molecule, so, in our results, and we can expect that compound 2 is the more donating molecule while compound 1 is the more accepting molecule [20].

The chemical softness (ς) and hardness (η) are very significant parameters to explain the stability and reactivity of the molecule. As Soft molecule is a more reactive molecule because it can easily offer electrons [21, 22]. Hence, compound 2(η =1.22 eV, ς =0.38 eV) is the lowest hardness, more softness, less stability, and the more reactive molecule. Meanwhile, compound 1 (η= 1.29 eV, ς =0.40 eV) is the more hard, less soft, the more stability, and less reactive molecule as shown in Table7, the geometries of the molecules were fully optimized and the optimized structures are shown in Figures 11-16.

Table 7. Selected quantum chemical parameters for compounds.

Compound	HOMO	LUMO	Eg	IP	EA	χ	η	ω	ς	μ
1	-5.69	-8.28	-2.59	5.69	8.28	6.98	-1.29	-31.6	-0.40	-6.98
2	-5.66	-8.10	-2.44	5.66	8.10	6.88	-1.22	-28.9	-0.38	-6.88

Hint: energy gap (Eg), ionization potential (IP), electron affinity (EA), electronegativity (χ), hardness (η), softness (S), and chemical potential (μ).

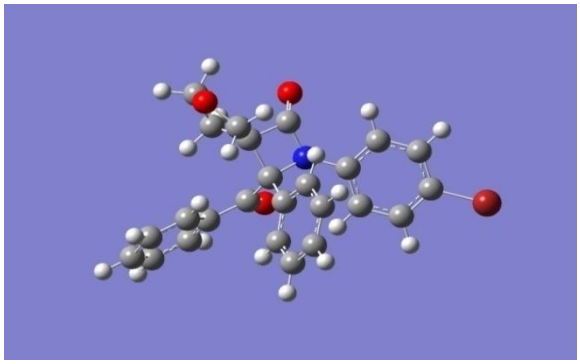

Figure 11. Optimized structure of compound 1.

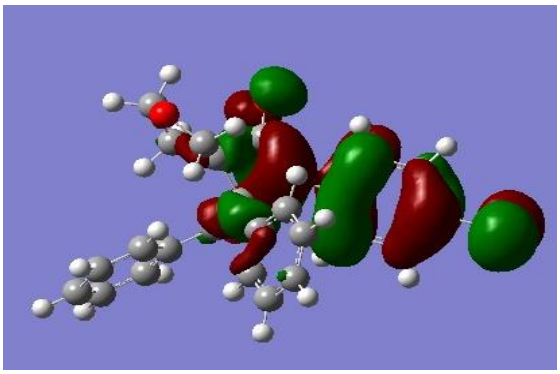

Figure 12. HOMO molecular orbital of compound 1.

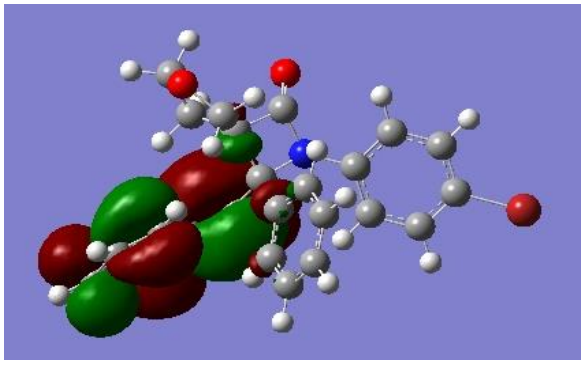

Figure 13. LUMO molecular orbital of compound 1.

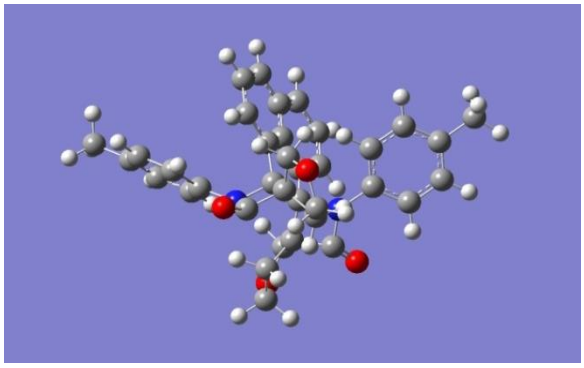

Figure 14. Optimized structure of compound 2.

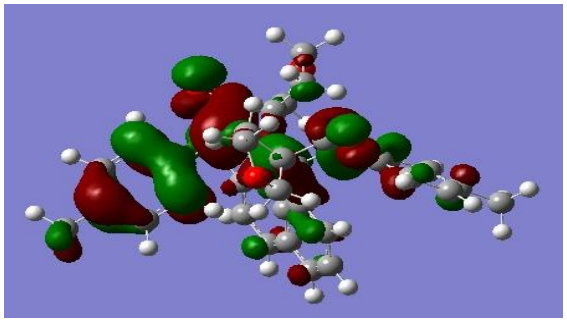

Figure 15. HOMO molecular orbital of compound 2.

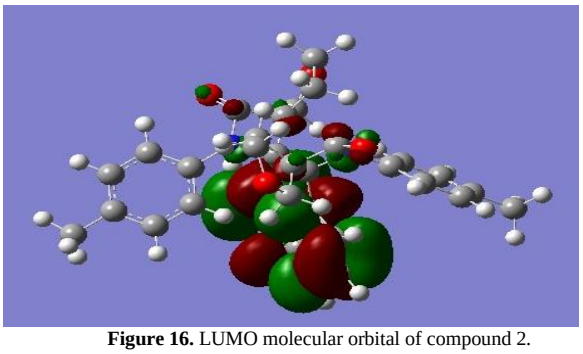

Figure 16. LUMO molecular orbital of compound 2.

CONCLUSIONS

In this study we mentioned the preparation of B-Lactam derivatives. The work included the preparation of imine compounds from a mixture of the amine with ketone as the first step and then the cyclization process for these

compounds. These derivatives were confirmed from spectral data analysis; FT-IR and H1 NMR.

To assess the properties of biological, two types of pathogenic bacteria were used in this study and the results obtained showed that the complexes possess antibacterial activity. As well as Computational study was performed to calculate some of the thermodynamic parameters of synthesized derivatives by using a Gaussian program

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Troisi L., Granito C., Pindinelli E., 2010. Novel and Recent Synthesis and Applications of b-Lactams In Banik, B. K., Ed. Heterocyclic Scaffolds *I*. 22, 101-209.

2. Morin R.B., Gorman M., 1982.Chemistry and Biology of β-Lactam Antibiotics. Eds.; Academic Press: New York.1-3.

3. Staudinger H., 1908.Contribution to our Knowledge of Ketenes. First Paper. Diphenylketene. Liebigs Ann Chem. 356, 51-123.

4. Mechergui A., Touati A., Baaboura R., Achour W., Hassen A.B., 2011. Phenotypic and molecular characterization of β-lactams resistance in commensal *Neisseria* strains isolated from neutropenic patients in Tunisia.AnnalMicr.61, 695–697.

5. Keri R.S., Hosamani K.M., Reddy H.S., Shingalapur R.V., 2010. Synthesis, *in-vitro* Antimicrobial and Cytotoxic Studies of Novel Azetidinone Derivatives. Arch Pharm. 343, 237-247.

6- Banik I., Becker F.F.; Banik B.K., 2003.Stereoselective Synthesis of β-Lactams with Polyaromatic Imines: Entry to New and Novel Anticancer Agents. J Med Chem. 46, 12-15.

7. Meegan M.J., Carr M., Knox A.J.S., Zisterer D.M., Lloyd D.G., 2008. β-Lactam type molecular scaffolds for antiproliferative activity: Synthesis and cytotoxic effectsin breast cancer cells. J Enzyme Inhib Med Chem. 23, 668-685.

8. John M. Beale, John H. Block., 2011.Organic Medicinal and Pharmaceutical Chemistry, 12th Edition. Wilson and Gisvold's, 260.

9. Reddy R., Saravanan P., Corey J., 2004.A Simple Stereo controlled Synthesis of Salinosporamide A. J Am Chem Soc. 126, 6230- 6231.

10. Turos E., Coates C., Shim J., Wang Y. Leslie M., Long T., Kumar S., Alex O., Marci C., Sonja D., Daniel V., Eduardo A., Javier G., 2005. *N*-Methylthio β-lactam antibacterials: Effects of the C_3/C_4 ring substituents on anti-MRSA activity Bioorg. Med. Chem., 13, 6289-6308.

11. Jarrahpour A., Shekarriz M., Taslimi A., 2004. Synthesis and Antimicrobial Activity of Some New Sugar-Based Monocyclic β-Lactams. Molecules. 9, 29.

12. Ali R., 2011. Synthesis of New Schiff's Bases Derived from 4-Phthalimidyl Acetophenone. Journal of Al-Nahrain. 14(3), 1-8.

13. Xian-gno H., Ursella M., 1994. Antifungal compounds from saloumnigrescence.J Ethnopharm.43, 173-177

14. Enders D., Gries G., 2005.Asymmetric Synthesis of Substituted Azetidine Type α- and β-Amino Acids, Synthesis. 3508-3516.

15. Maowad E.B., 1989. Synthesis of some new heterocyclic compounds with expected potential biological activity, J Islamic AcadSci. 24, 237-240.

16. Sajda S. Afatt., Muntaha Y., Ibrahim A., 2016. Syntheses, Characterization of a New Ligand(3-Hydrazino-N-isopropylidene-5-methyl-4H-1,2,4-trizole-4-amine) and its complex with (Fe(III), Co(III) and Ni(II)), Journal of Thi-Qar Science. 5(4), 1991- 8690.

17. Sajda S. Afatt, Haider A., 2012. Synthesis and characterization of a new Schiff base {N-(2-{[(4bromophenyl)imino]methyl}phenyl)acetamide}and its compolexes with some transition metal. Journal of Education for Pure Science. 2(4), 110-117

18.Saranya M., Ayyappan S., Nithya R., Sangeetha K., Gokila A., 2018. Molecular structure, NBO and HOMO-LUMO analysis of quercetin on single layer grapheme by

density functional theory. Molecular Structure. 13(1), 97–105.

19. Bendjeddou A., Abbaz T., Drissi S., Gouasmia A., Villemin D., 2016.Quantum chemical studies on molecular structure and reactivity descriptors of a series of trimethyltetrathiafulvalene functionalized by conjugated substituent. J Adv Chem Scie. 318 - 322.

20. Nowotny J., Bak T., Nowotny K., Sheppard R., 2008. Defect Chemistry and Electrical Properties of Titanium Dioxide. 1. Defect Diagrams, J Phys Chem C. 112(2), 590-601.

21. Jensen F., 2017.Introduction to computational chemistry, John Wiley & sons. ISBN: 978-1-118-82599-0.3rd Edition. pp. 664.

22.Gotwals R., Sendlinger C., 2007.The North Carolina School of Science and Mathematics, Durham, NC.