Design, synthesis, and evaluation the anti-β-lactamase activity of new sulphathiazole-derived monobactam compounds

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Abstract

Objectives: β-Lactams are the most successful antibiotics for the management of infectious diseases. Unfortunately, the bacterial production of β-lactamase that hydrolyzes the β-lactam ring can inactivate these drugs. The use of β-lactamase inhibitors like (clavulanic acid) in combination with the β-lactams may reduce this inactivation. The prevalent β-lactamase phenotype is the TEM-1 of class A released by Gram-positive and Gram-negative bacteria.

Methods: The docking study with TEM-1 β-lactamase lead to synthesize of new 5 monobactam compounds as the acid chloride derivatives reacted with the Schiff bases compound forming the monobactam ring. The final 5 synthesized compounds were characterized using physical and spectroscopic methods and tested biologically by evaluating their MIC values against 4 strains of β-lactamase Gram-positive and Gram-negative bacteria. The results were compared with those acquired from using clavulanic acid as a co-inhibiter with amoxicillin against the tested bacteria.

Results: The results revealed that 2 synthesized compounds showed an anti β-lactamase effect resemble to that of clavulanic acid.

Conclusion: As conclusion; the β-lactamase active pocket prefers hydrophobic substituents, as the synthesized products with these groups appeared to have the highest affinity.

Keywords: TEM-1 β-lactamase, sulphathiazole, monobactam, Schiff base, acid chloride, anti- β-lactamase, antibacterial.

تصميم ، تحضير وفعالية نشاط بيتا- لاكتاميز لمشتقات السلفاثايزول كمركبات احادية الاكتام

الأهداف: تعتبر بيتا- لاكتام من انجح المضادات الحيوية في علاج الامراض المعدية، ولسوء الحظ فأن الانتاج البكتيري لل- بيتا- لاكتام تعطل عمل هذه الادوية. أن استخدام مثبطات بيتا –لاكتاميز (حامض الكلافانيليك) بالاشتراك مع بيتا –للاكتام يعمل على يقلل من هذه المقاومة ، وتعتبر خميرة (تي أي أم -1) السائدة من النوع (أ) حيث تعمل على تحلل حلقة البيتا- لاكتام.

طريقة العمل: من خلال دراسة تالف المركبات المقترحة مع الانزيم بيتا- الاكتاميز نوع (تي أي أم -1) وجد بان خمس مركبات لها فعالية متوقعة. حضرت المركبات من خلال تكوين قواعد شيف للسلفاثايزول مع الالديهايدات ، ثم اجراء التحولق

للحلقة الرباعية. تم تشخيص المركبات المحضرة من خلال الخواص الفيزيائية والطيفية ودراسة الخواص المضادة للبكتريا لاربع سلاسل من البكتريا الموجبة والسالبة الكرام ومقارنتها بمواد قياسية .
النتائج: تشير النتائج إلى أن مركبين من المركبات المحضرة لها تاثير كمثبطات لانزيم البيتا- لاكتاميز.
الخلاصة: نستنتج أن المركبات الفعالة كمثبطات لانزيم البيتا- لاكتاميز يجب ان تكون كارهة للماء، حيث تظهر المركبات المحضرة ذات الفعالية كمثبطات لانزيم البيتا- لاكتاميز هذه الخاصية .
الكلمات المفتاحية : بيتا لاكتاميز (تي أي أم) النوع (أ) ، سلفاثايزول ، احادي الحلقة ، قواعد شف، مضادات بيتا-لاكتاميز.

Introduction:

Antibiotics, particularly the β-lactam classes are a cornerstone of modern medicine for fighting the infectious diseases [1]. Their mechanism of action involves inhibition of the synthesis of peptidoglycan (the chief constituent of the bacterial cell wall), thus producing permanent damage and death of the bacteria (bactericidal)[1,2]. The β-lactam antibiotics group includes four families of antibiotics: the penicillins, cephalosporins, carbapenems and monobactams. All of them are containing a four-membered β-lactam ring (azetidin-2-one), which is essential for their antibacterial activity[1,3].

Of the several manifestations of β-lactams bacterial resistance currently known, the production of beta-lactamases is the most important [2,3] The Ambler system makes use of amino acid sequence to categorize β-lactamases into four broad classes: A, B, C, and D[3]. The A, C, and D classes are serine β-lactamases (SBLs) whereas the class B enzymes are metallo β-lactamases (MBLs)[3,4].

The β-Lactamase inhibitors are affect the capability of the bacteria to inactivate β-lactam antibiotic, and their administration in combination with β-lactam antibiotics by co-administration are now the most effective procedures to fight a particular resistance mechanism[4,5]. However, their restricted spectrum of effectiveness, which is restricted to class A enzymes necessitates the search for further broadly effective β-lactamase inhibitors[5]. Therefore, there is an urgent need to discover and develop new antibiotics or anti β-lactamases to handle this situation.

Monocyclic β-lactams are more stable to hydrolysis by β-lactamases compared to other β-lactams [4].The monobactams like Aztreonam belong to a subclass of monocyclic beta-lactam moieties are the only U.S. Food and Drug Administration-approved monobactam in clinical use [5,6]. This study aims to design, dock (computer calculation), synthesize, and study the

biological activities of new β-lactamase inhibitors.

Material and methods:

All employed chemicals were purchased from commercial sources and their suppliers are Fluka (Switzerland), Alpha (India), Scharlau (Spain), and Merck (Germany). Melting points of the synthesized compound were determined by the Electrochemical CIA 9300 melting point apparatus (UK) by using an open capillary method and they were uncorrected. FTIR spectra were recorded on a PerkinElmer infrared spectrophotometer. 1H NMR and 13C NMR spectra in DMSO-d6 on a Bruker Avance DPX 400 MHz spectrometer using TMS as an internal reference. All the products were synthesized by the method given in the literature and identified by ^{1}H NMR ^{13}C NMR and IR spectra and microanalyses of these compounds were in satisfactory agreement with the structures. The purity of the compounds and the completion of the reactions were monitored by TLC using pre-coated silica gel plate.

Docking Study:

The Computational Docking Study was carried out using the online platform Mcule by AutoDockVina logarithm (https://mcule.com/ apps/1-click-docking/). The structure of the bacterial Penicillin Binding Proteins PBP (1qmf) and β-lactamases TEM-1 (1pzo) were retrieved from PDB[7].

Docking Study on Penicillin Binding Protein (PBP):

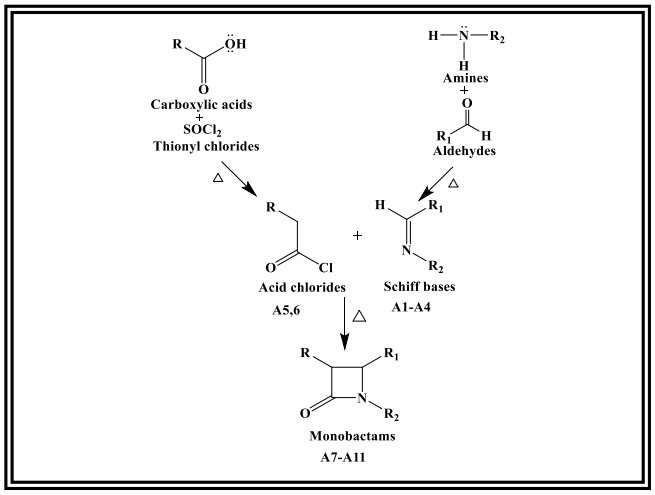
The docking study of the investigated products on PBP (1qmf) was conducted to test the antimicrobial activities of investigated products [8], and to be compared with reference antibiotics of various activities to validate this approach.

Docking Study on β-Lactamases TEM-1:

The docking study of the investigated products on β-lactamases TEM-1 (1pzo) was conducted to investigates the affinity toward the TEM-1 β-lactamases to be compared with reference anti β-lactamases of various activities.

The docking scores of the binding energies due to the interaction between the active ligands (inhibitors) and the enzymes pockets were chosen with the highest score based on geometric shape complementarity [9].

Chemical study:



General procedure for the preparation of Schiff base derivatives (A1-A4) [10, 11]:

A (0.004) mole of benzaldehyde derivatives was dissolved by heating in 20 ml absolute ethanol before mixing with (0.004) mole of sulphathiazole which was also dissolved by heating in 20 ml absolute ethanol, then the mixture was refluxed with stirring for 10-15h (the proceeding of the reaction was monitored by TLC using 1:1:1 methanol: diethylether : DCM as eluent), after cooling the precipitate was filtered, dried and then washed once with cold ethanol and 3 times with distilled water and dried.

General procedure for the preparation of acid chloride derivatives (A 5 and A6)[12]:

A (3-5 mole) of individual carboxylic acids was dissolved in 10-15 ml of thionyl chloride, the mixture was reflxed for 30 min in the hood, then the excess of thionyl chloride was distilled under reduced pressure, after cooling the yielded products was taken and used freshly in the next step.

General procedure for the preparation of monobactam derivatives (A7-A11)[13]:

The corresponding acid chloride (0.0016) mole in 10 ml dichloroethane was added drop by drop to a mixture of a corresponding Schiff base (0.0016) mole with (0.0016) mole pyridine in 30 ml dichloroethane at 0˚C. The resulting mixture was reflexed for 20-25 h (as

monitored by TLC using 1:2:2 ethanol: diethyl ether: DCE as eluent). After cooling the solution was washed twice with water (30 mL), saturated aqueous NaHCO3 (30 mL) and finally with saturated solution of NaCl (30 mL). The organic layer was removed and dried using anhydrous sodium sulphate. After solvent evaporation, the dry product was washed with cold ethanol.

Antimicrobial Study

Detection of β-lactamases in bacterial isolate:

The detection of β-lactamases in bacterial isolates was done for Gram-positive (*Staphylococcus aurous*) and 3 Gram-negative (*Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeroginosa*) pathogenic bacteria isolates using acidimetric method [14]. Were 2 mL of 0.5% (w/v) aqueous phenol red solution was diluted with 16.6 mL distilled water and 1.2 g of benzylpenicillin is added. The pH is adjusted to 8.5 with 1 M NaOH. Then 100 µl of resulting violet solution was distributed into tubes and inoculated with several colonies of bacteria from culture to get a dense suspension. A yellow color within 5 min indicates β-lactamase activity. Positive controls were run in parallel[15].

Determination of minimum inhibitory concentration (MIC)[16]:

A broth microdilution method was used to evaluate the MIC according to the CLSI (CLSI document M7-MIC, Clinical Laboratory Standards Institute). A serial of 10 doubling dilution of the synthesized compounds and standard antibacterial (Amoxiclav (Amoxicillin 2000mg + clavulanic acid 400mg), Amoxicillin, Cefotaxime, Ceftriaxone, and Ciprofloxacin) was prepared in test tubes with final concentration starting from 2000 µg/ml. A 1 ml of Mueller–Hinton agar was added. Bacterial isolates were diluted and added to the test tubes to give a final concentration 5×10^5 CFU/ml. The test tubes incubated at 37°C for 18 h[17]. The (+)ve control containing Mueller–Hinton agar and bacterial isolates only, whereas the (-)ve control containing only Mueller–Hinton agar.

Determination of anti β-lactamase activities:

The anti β-lactamase activity of the synthesized compounds against human pathogenic bacterial isolates was evaluated by measuring the zones of inhibition in the disk diffusion method[16]. Each tested compound was used as co-inhibiter with 1000 or 2000 µg of amoxicillin prepared as

disks (5µl/disk) at a concentration equal or below their MIC, and then placed on Petri dish with Mueller–Hinton agar medium (previously inoculated with the tested bacterial strains by sterile cotton swabs). After incubation at 37°C for 24 h, zones of microbial growth produced around the tested substances were measured and recorded as the diameters of inhibition [18]. Disks containing 1000 or 2000 µg of amoxicillin (5µl/disk) was prepared and used as control.

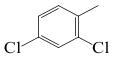
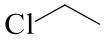
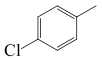
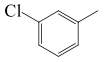
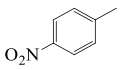
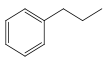
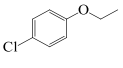
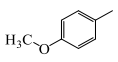
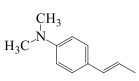
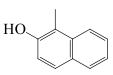
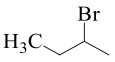
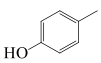
In all the above microbial study DMSO was used as a solvent for the synthesized compounds with a final concentration of less than 2 % in order to ensure that it has no effect on bacterial growth.

Results and discussion:

Molecular Docking Study for standard inhibitors:

The docking for both clavulanic acid and sulbactam were carried out on both bacterial PBP (1qmf) and β-lactamases TEM-1 (1pzo). The results are (-6.1) and (-5.1) for clavulanic acid and for sulbactam are (-6.6) and (-6.2) respectively. Docking for monobactam derivatives was carried out on both bacterial PBP (1qmf) and β-lactamases TEM-1 (1pzo). The chemical structures of the designated substitutions are listed in Table (1), while the results of docking are listed in Table (2).

Table (1): The chemical structures of the designated substitutions.

R	Structure	R	structure	Z	structure
R1		**R9**		**Z1**	
R2		**R10**		**Z2**	
R3		**R11**		**Z3**	
R4		**R12**		**Z4**	
R5		**R13**		**Z5**	
R6		**R14**		**Z6**	

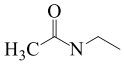
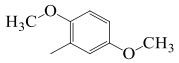
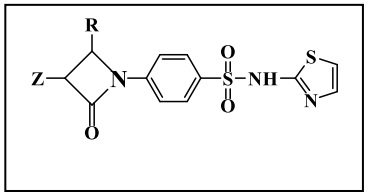
R7				**Z7**	
R8				**Z8**	

Table (2): Docking Study results for monobactam sulphathiazole derivatives.



Comp.	Docking Scour		Comp.	Docking Scour		Comp.	Docking Scour	
	PBP	B-Lactm.		PBP	B-Lactm.		PBP	B-Lactm.
R1 , Z1	- 8.8	- 7.9	R5 , Z7	- 8.3	- 6.8	R10 , Z5	- 8.6	- 8.4
R1 , Z2	- 8.3	- 8.2	R5 , Z8	- 8.9	- 8.0	R10 , Z6	- 8.7	- 8.4
R1 , Z3	- 8.6	- 9.4	R6 , Z1	- 9.2	- 8.8	R10 , Z7	- 9.1	- 8.6
R1 , Z4	- 8.1	- 8.1	R6 , Z2	- 9.3	- 8.5	R10 , Z8	- 9.0	- 8.7
R1 , Z5	- 8.3	- 8.3	R6 , Z3	- 9.3	- 8.6	R11 , Z1	- 7.9	- 8.5
R1 , Z6	- 7.8	- 8.0	R6 , Z4	- 9.2	- 8.3	R11 , Z2	- 8.3	- 8.0
R1 , Z7	- 9.6	- 7.9	R6 , Z5	- 9.2	- 8.2	R11 , Z3	- 8.9	- 9.7
R1 , Z8	- 9.4	- 8.7	R6 , Z6	- 8.5	- 8.0	R11 , Z4	- 8.5	- 8.8
R2 , Z1	- 8.5	- 8.3	R6 , Z7	- 9.3	- 8.0	R11 , Z5	-8.3	- 8.0
R2 , Z2	- 8.8	- 8.3	R6 , Z8	- 9.4	- 7.6	R11 , Z6	- 8.7	- 8.9
R2 , Z3	- 8.6	- 9.8	R7 , Z1	- 8.1	- 7.7	R11 , Z7	- 8.8	- 8.6
R2 , Z4	- 8.3	- 8.2	R7 , Z2	- 8.3	- 7.9	R11 , Z8	- 8.3	- 9.5
R2 , Z5	- 8.3	- 8.4	R7 , Z3	- 8.9	- 8.6	R12 , Z1	- 8.4	- 8.3
R2 , Z6	- 8.1	- 7.9	R7 , Z4	- 9.2	- 7.8	R12 , Z2	- 8.1	- 7.9
R2 , Z7	- 9.2	- 8.4	R7 , Z5	- 8.8	- 7.6	R12 , Z3	- 8.3	- 8.3
R2 , Z8	- 8.9	- 8.1	R7 , Z6	- 8.3	- 7.8	R12 , Z4	- 8.9	- 8.0
R3 , Z1	- 8.5	- 8.4	R7 , Z7	- 8.6	- 8.3	R12 , Z5	- 9.2	- 7.6
R3 , Z2	- 8.5	- 8.4	R7 , Z8	- 8.1	- 8.3	R12 , Z6	- 9.3	- 8.0
R3 , Z3	- 9.5	- 8.4	R8 , Z1	- 8.3	- 7.7	R12 , Z7	- 9.3	- 8.1
R3 , Z4	- 9.1	- 8.3	R8 , Z2	- 7.8	- 7.6	R12 , Z8	- 9.2	- 9.7
R3 , Z5	- 8.0	- 8.0	R8 , Z3	- 9.6	- 8.4	R13 , Z1	- 9.2	- 8.6
R3 , Z6	- 8.0	- 8.0	R8 , Z4	- 9.4	- 7.9	R13 , Z2	- 8.5	- 8.0
R3 , Z7	- 8.8	- 7.9	R8 , Z5	- 8.5	- 7.7	R13 , Z3	- 9.3	- 8.7
R3 , Z8	- 8.6	- 8.8	R8 , Z6	- 8.8	- 7.3	R13 , Z4	- 9.4	- 8.9
R4 , Z1	- 8.6	- 8.2	R8 , Z7	- 8.6	- 8.0	R13 , Z5	- 8.8	- 8.6
R4 , Z2	- 8.7	- 8.3	R8 , Z8	- 8.3	- 7.7	R13 , Z6	- 8.3	- 8.6
R4 , Z3	- 9.1	- 8.0	R9 , Z1	- 8.3	- 8.5	R13 , Z7	- 8.6	- 8.4
R4 , Z4	- 9.0	- 8.2	R9 , Z2	- 8.1	- 7.9	R13 , Z8	- 8.1	- 8.2
R4 , Z5	- 7.9	- 8.3	R9 , Z3	- 9.2	- 8.4	R14 , Z1	- 9.1	- 8.1
R4 , Z6	- 8.3	- 7.6	R9 , Z4	- 8.9	- 7.9	R14 , Z2	- 9.0	- 8.0
R4 , Z7	- 8.9	- 7.4	R9 , Z5	- 8.5	- 7.6	R14 , Z3	- 7.9	- 8.1
R4 , Z8	- 8.5	- 7.7	R9 , Z6	- 8.5	- 7.7	R14 , Z4	- 8.3	- 8.0
R5 , Z1	-8.3	- 7.1	R9 , Z7	- 9.5	- 8.2	R14 , Z5	- 8.9	- 7.5
R5 , Z2	- 8.7	- 7.5	R9 , Z8	- 9.1	- 8.7	R14 , Z6	- 8.5	- 7.7
R5 , Z3	- 8.8	- 7.6	R10 , Z1	- 8.0	- 8.3	R14 , Z7	- 9.5	- 7.9
R5 , Z4	- 8.3	- 7.5	R10 , Z2	- 8.0	- 8.6	R14 , Z8	- 9.1	- 8.0
R5 , Z5	- 8.4	- 7.5	R10 , Z3	- 8.8	- 8.3			
R5 , Z6	- 8.1	- 7.4	R10 , Z4	- 8.6	- 8.5			

The docking results showed that as the hydrophobicity increases in the tested compounds the score will decrease. The compounds bound variably to the region located between H10, H11 and H12 helices of TEM-1, indicating that the pocket is mostly hydrophobic in nature [19], and the residue participated in the binding with our compounds were almost the same as those combined with the standard inhibitors (clavulanic acid and sulbactam).

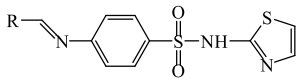
Chemical results:

Preparation of Schiff base derivatives (A1-A4):

This reaction was occurred through nucleophilic addition mechanism. The unshared pair of electron of the amino group attacks the electron seeking carbon of the carbonyl group of the aldehyde to form the aminal intermediate, which losses water molecule to form the imine (Schiff base). The physical properties and the most characteristic peaks of the FTIR spectrum for compounds (A1-A4) are shown in the table (3).

In general these compounds were disposed the absence of the N-H (NH2 group) bond stretching at 3272 cm^{-1} of the sulphathiazol and appearance of absorption bond at 1580-1590 cm^{-1} related to the C=N bond stretching which indicating the formation of Schiff base. There was an addition of characteristics peaks for each substitution of the aldehydes used.

Table (3): physical properties and the most characteristic peaks (ν cm^{-1}) of the FT-IR spectrum for the Schiff base derivatives (A1-A4).



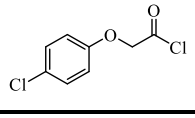
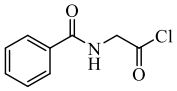
Compd	R	m.p(°C)	Yield%	Color	Time to end of the reaction	R_f (Methanol 1 : Ether 1 : DCM 1)	N-H	C-H	C=N imine	O=S=O	C=S or C-SH	C-Cl
A1	R1	261-263	93	White	10 h	0.64	w 3355	w 3106	s 1583	s 1332, 1131	s 1077	------
A2	R2	162-164	81	Off White	13 h	0.63	w 3349	w 3118	s 1583	s 1336, 1131	s 1077	m 1091
A3	R11	170-172	90	Yellow	12 h	0.47	w 3355	w 3106	s 1587	m 1330, 1130	s 1080	------
A4	R12	260-262	82	Dark yellow	10 h	0.42	w 3348	m 3105	s 1584	m 1334, 1131	s 1077	------

Preparation of acid chloride derivatives (A5 and A6):

This reaction was occurred via nucleophilic addition-elimination mechanism which involves, firstly

displacement of chloride from the sulfur by the hydroxyl group to form the acyl chloro-sulfite intermediate. In the second step the chloride ion (Cl^-) attacks the carbonyl carbon via addition step to form a tetrahedral intermediate, which losses SO2 and HCl molecules in the elimination step to form the acid chloride (Table 4).

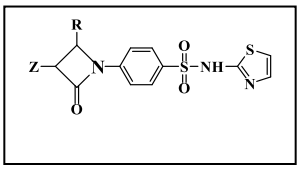
Table 4: The chemical structures and the colors of the synthesized acid chlorides (A5 and and A6).

Compd. No.	Chemical structure	Color	Compd. No.	Chemical structure	Color
A5		Gray	**A6**		Dark red

Preparation of monobactam compounds (A7-A11):

This reaction was occurred as the nitrogen of the imine (Schiff base) acts as a nucleophile attacking the acid chloride carbonyl and eliminating the chloride ion as a result. This anion attacks the electron seeking carbonyl carbon via intramolecular nucleophilic addition reaction to form the four membered ring azetidine moiety (monobactam)[20]. The physical properties and the most characteristic peaks of the FT-IR spectrum for these compounds are shown in the Table (5).

Table (5): Physical properties and the most characteristic peaks (ν cm^{-1}) of the FT-IR spectrum for the monobactam derivatives (A7-A11)



Compd	Z , R	m.p(°C)	Yield%	Color	Time to end of the reaction	R_f (Ethanol 1 : Ether 2 : DCE 2)	N-H	C-H Stretch.	C=O Lactam	C=N Imine	C-O-C	O=S=O	C=S or C-SH	C-Cl
A7	**Z3 , R1**	**210-212**	**67**	**Drack-yellow**	**25 h**	**0.74**	**w 3306**	**w 3104**	**m 1690**	**s 1585**	**m 1231, 1130**	**s 1330, 1136**	**s 1077**	**m 1085**
A8	**Z3 , R2**	**110-112**	**75**	**Yellow**	**22 h**	**0.63**	**w 3372**	**w 3103**	**m 1690**	**s 1583**	**m 1233, 1128**	**s 1335, 1138**	**s 1078**	**m 1092**

A9	**Z3 , R11**	**197-200**	**67**	**Yellow**	**24 h**	**0.47**	**w 3370**	**w 3100**	**m 1694**	**s 1582**	**m 1235, 1133**	**s 1334, 1131**	**s 1076**	**m 1097**
A10	**Z8 , R11**	**151-154**	**63**	**Dark-yellow**	**24 h**	**0.44**	**w 3339, 3200**	**w 3024**	**m 1686**	**m 1581**	**------**	**s 1330, 1132**	**s 1071**	**------**
A11	**Z8 , R12**	**207-210**	**72**	**Dark-yellow**	**23 h**	**0.55**	**w 3339, 3230**	**w 3059**	**m 1691**	**m 1588**	**------**	**s 1336, 1131**	**s 1078**	**------**

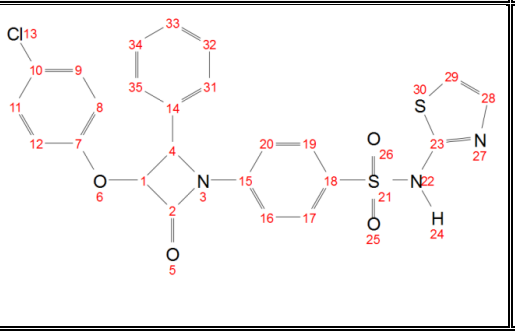
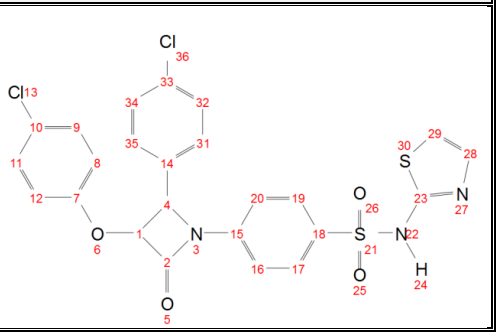
The result from the Table (5) indicated that the formation of monobactam ring as its carbonyl group C=O appears at 1686-1694 cm^{-1} which added to the original Schiff bases C=N at 1580-1590 cm^{-1}. Also Z8 acid chloride compound.

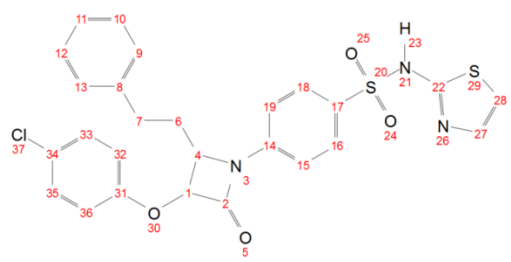
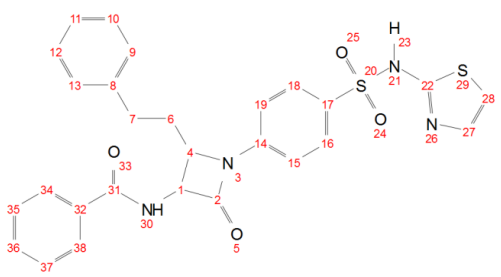
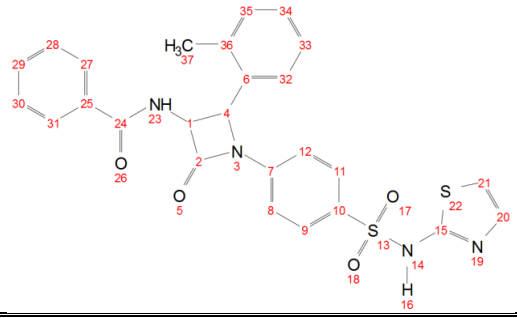
^{1}H-NMR indicate the formation of the four membranes monobactam rings, as the proton NMR shows the appearance of the single proton of the C4 and C1 in the compounds A7-11 at 3.90-5.12 ppm and 4.19-5.84 ppm respectively, confirming the reaction of the acid chloride with the Schiff base derivatives forming the ring the appearance of the C-O-C at 1231-1235 cm^{-1} and the C-Cl at 1085-1097 cm^{-1} for Z3 acid chloride compound, in addition to the appearance of N-H at 3200-3230 cm^{-1} for

(Table 6).

While in the ^{13}C-NMR and for compounds A7-11 the appearance of the carbonyl C2 at 162.98-166.61 ppm, and the appearance of C1 and C4 at 59.06-74.16 ppm and 57.79-60.77 ppm respectively also confirm the formation of the monobactam ring (Table 6).

Table 6: The chemical structures synthesized monobactam compounds.

Compd	ST	Compd	ST
A7		**A8**	

A9		**A10**	
A11			

Compound A7: N-(2-oxo-1-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-4-(o-tolyl) azetidin-3-yl)benzamide. **The ^{1}H-NMR of A7 (δ, ppm) (DMSO-d6) showed the chemical shift for the following protons:** 12.62 (s, 1H, N 22), 7.92 (d, 2H, C 17 & 19), 7.62 (dd, 2H, C 16 & 20), 7.18 (dd, 4H, C 8, 9, 11 & 12), 6.95-6.98 (m, 5H, C 31, 32, 34 & 35), 6.81 (d, 1H, C 28), 6.75 (d, 1H, C 29), 5.84 (d, 1H, C 1), 4.16 (d, 1H, C 4).. **The ^{13}C-NMR of A7 (δ, ppm) (DMSO-d6) reported the chemical shift for the following carbons:** 167.69 (C23), 162.98 (C2), 156.03 (C7), 139.40 (C15), 134.32 (C14), 130.85 (C18), 129.87 (C9,11), 129.21 (C28), 129.11 (C17,19), 128.39 (C32,34), 127.88 (C33), 127.48 (C10), δ127.17 (C31,35), 121.33 (C16,20), 118,62 (C8,12), 109.30 (C29), 74.15 (C1), 60.77 (C4).

Compound A8: 4-(3-(4-chlorophenoxy)-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-N-(thiazol-2-yl) benzenesulfonamide **The ^{1}H-NMR of A8 (δ, ppm) (DMSO-d6) showed the chemical shift for the following protons:** 12.71 (s, 1H, N 22), 7.69 (d, 2H, C 17 & 19), 7.53 (d, 2H, C 16 & 20), 7.46 (d, 1H, C 28), 7.42 (d, 1H, C 29), 7.33 (d, 2H, C 31 & 35), 7.25 (d, 2H, C 32 & 34), 7.16 (d, 2H, C 9 & 11), 6.96 (d, 2H, C 8 & 12), 4.80 (d, 1H, C 1), 4.16 (d, 1H, C 4).**The ^{13}C-NMR of A 8 (δ, ppm) (DMSO-d6) reported the chemical shift for the following carbons:** 167.69 (C23), 162.98 (C2), 156.03 (C7), 139.38 (C15), 134.70 (C33), 132.65 (C14), 130.85 (C18), 129.87 (C9,11), 129.21 (C28), 129.11 (C17,19), 128.52 (C32,34), 128.51 (C31,35), 127.48 (C10), 121.33 (C16,20), 118.62 (C8,12), 109.30 (C29), 74.16 (C1), 60.44 (C4).

Compound A9: 4-(3-(4-chlorophenoxy)-2-oxo-4-phenethylazetidin-1-yl)-N-(thiazol-2-yl) benzenesulfonamide **The ^{1}H-NMR of A9 (δ, ppm) (DMSO-d6) showed the chemical shift for the following protons:** 12.64 (s, 1H, N 21), 8.11 (dd, 2H, C 16 & 18), 8.00 (dd, 2H, C 15 & 19), 7.16 (m, 5H, C 9-13 aromatic), 7.02 (dd, 4H, C 32, 33, 35 & 36), 6.85 (d, 1H, C 27), 6.81 (d, 1H, C 28), 4.80 (d, 1H, C1), 3.90 (d, 1H, C 4), 2.67 (t, 2H, C 7), 1.82 (t, 2H, C 6). **The ^{13}C-NMR of A9 (δ, ppm) (DMSO-d6) reported the chemical shift for the following carbons:** 167.69 (C22), 165.79 (C2), 156.55 (C31), 143.14 (C8), 139.33 (C14), 130.90 (C17), 129.87 (C33,35), 129.21 (C27), 129.13 (C16,18), 128.74 (C9,13), 128.68 (C10,12), 127.48 (C34), 126.57 (C11), 120.29 (C15,19), 118.63 (C32,36), 109.30 (C28), 81.53 (C1), 59.33 (C4), 33.34 (C7), 27.56 (C6).

Compound A10: N-(2-oxo-4-phenethyl-1-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)azetidin-3-yl)benzamide. **The ^{1}H-NMR of A10 (δ, ppm) (DMSO-d6) showed the chemical shift for the following protons:** 10.75 (s, 1H, N 21), 8.41 (s, 1H, N 30), 7.98 (d, 2H, C 16 & 18), 7.63 (d, 2H, C 15 & 19), 7.42-7.52 (m, 10H, C 9, 10, 11, 12, 13, 34, 35, 36, 37 & 38 aromatic), 6.76 (d, 1H, C 27), 6.56 (d, 1H, C 28), 4.19 (d, 1H, C1), 3.91 (d, 1H, C 4), 3.15 (t, 2H, C 7), 2.94 (t, 2H, C 6).**The ^{13}C-NMR of A 10 (δ, ppm) (DMSO-d6) reported the chemical shift for the following carbons:** 167.69 (C22), 166.60 (C31), 166.54 (C2), 143.06 (C8), 139.63 (C14), 134.63 (C32), 131.95 (C36), 130.90 (C17), 129.21 (C27), 129.13 (C16,18), 128.89 (C35,37), 128.74 (C9,13), 128.68 (C10,12), 127.30 (C34,38), 126.57 (C11), 120.26 (C15,19), 109.30 (C28), 59.06 (C1), 57.79 (C4), 33.24 (C7), 27.26 (C6).

Compound A11: N-(2-oxo-1-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-4-(o-tolyl) azetidin-3-yl)benzamide. **The ^{1}H-NMR of A10 (δ, ppm) (DMSO-d6) showed the chemical shift for the following protons:** 10.85 (s, 1H, N 14), 8.56 (s, 1H, C 23), 7.85 (d, 2H, C 27 & 31), 7.84 (d, 2H, C 9 & 11), 7.53 (s, 1H, C 29), 7.44 (m, 4H, C 8, 12, 28 & 30), 7.18 (m, 4H, C 32, 33, 34 & 35), 7.13 (d, 1H, C 20), 6.69 (d, 1H, C 21), 5.50 (d, 1H, C 1), 5.12 (d, 1H, C 4), 2.47 (s, 3H, C 37). **The ^{13}C-NMR of A 10 (δ, ppm) (DMSO-d6) reported the chemical shift for the following carbons:** 167.69 (C15), 166.61 (C2), 166.36 (C24), 139.81 (C7), 137.61 (C36), 136.74 (C6), δ134.55 (C25), 131.95 (C29), 130.85 (C10), 129.35 (C35), 129.21 (C20), 129.10 (C9,11), 128.89 (C28,30), 127.31 (C34), 127.31 (C27,31), 126.99 (C32), 126.54 (C33), 121.39 (C8,12), 109.30 (C21), 59.65 (C4), 59.46 (C1), 19.40 (C37).

Antimicrobial study: Determination of minimum inhibitory concentration (MIC):

After The detection of β-lactamases in bacterial isolates, a broth microdilution method was used to evaluate the (MIC), as a serial of 10 doubling dilution of the synthesized compounds and standard antibacterial (Amoxiclav) (Amoxicillin 2000mg + clavulanic acid 400mg), Amoxicillin, Cefotaxime, Ceftriaxone, and Ciprofloxacin) was tested with final concentration starting from 2000 mg/ml (and from 5000 mg/ml for Amoxicillin). The results were summarized in table (7).

Table (7): Minimum inhibition concentration (MIC) for the synthesized compounds and the antibacterial.

Compounds No.	MIC (µg/ml)				Compounds No.	MIC (µg/ml)			
	Gram(+)ve	Gram (-) ve				Gram(+)ve	Gram (-) ve		
	Staph. aueus	*E. coli*	*K. pneumonia*	*P. aeroginosa*		*Staph. aureus*	*E. coli*	*K. pneumonia*	*P. aeroginosa*
Amoxiclav	1000	2000	2000	2000	A7	>2000	>2000	>2000	>2000
Amoxicillin	2000	4000	4000	4000	A8	2000	2000	2000	>2000
Cefotaxime	7.8	125	3.9	3.9	A9	>2000	>2000	>2000	>2000
Ceftriaxone	31.25	125	3.9	7.8	A10	>2000	>2000	>2000	>2000
Ciprofloxacin	< 7.812	< 7.812	< 7.812	< 7.812	A11	>2000	>2000	>2000	>2000

Compounds A8 had an antibacterial activity and has Cl group which may be responsible for its anti-bacterial activity [21].

Determination of anti β-lactamase activities:

Firstly amoxicillin and amoxiclav (2000/400mg) were incubated with the bacterial isolates by using three concentrations 3000, 2000, and 1000 µg/ml, the results would be conceders as a control for the results of the incubation of the synthesized compounds table (8).

Table (8): Inhibition zones for Amoxiclave and Amoxicillin against Gram(+)ve and Gram(-)ve bacteria.

Compounds No.	Inhibition zone diameter (mm)											
	Gram(+)ve			Gram (-) ve								
	Staph. Aueus			*E. coli*			*K. pneumonia*			*P. aeroginosa*		
	3000µg/ml	2000µg/ml	1000µg/ml	3000µg/ml	2000µg/ml	1000µg/ml2	3000µg/ml	2000µg/ml	1000µg/ml	3000µg/ml	2000µg/ml	1000µg/ml
Amoxiclave	34	30	28	23	20	12	22	15	6	22	16	5
Amoxicillin	33	30	27	0	0	0	0	0	0	0	0	0

Amoxicillin shows antibacterial activity against *Staph. Aureus* only with no activity against Gram (-)ve strains. Amoxiclav on the other hand shows the same amoxicillin activity against *Staph. Aureus*, but also show

activity against Gram (-)ve bacterial strains.

Depending on the above results, amoxicillin was used in two concentrations in the next step, 1000µg/ml for Gram (+)ve bacteria and 2000µg/ml for Gram (-)ve bacteria Table (9).

Table (9): Inhibition zones for the synthesized compounds as co-inhibitors with Amoxicillin against Gram-positive and Gram-negative pathogenic bacteria.

Com. No.	Inhibition zone diameter (mm)								Com. No.	Inhibition zone diameter (mm)							
	Gram(+)ve		Gram (-)ive							Gram(+)ve		Gram (-)ive					
	Staph. aueus		*E. coli*		*K. pneumonia*		*P. aeroginosa*			*Staph. aueus*		*E. coli*		*K. pneumonia*		*P. aeroginosa*	
	1:1	1:2	1:1/2	1:1	1:1/2	1:1	1:1/2	1:1		1:1	1:2	1:1/2	1:1	1:1/2	1:1	1:1/2	1:1
A7	28	30	25	28	0	0	0	0	A10	28	30	0	0	0	0	0	0
A8	28	30	0	0	0	0	0	0	A11	28	30	28	30	0	0	0	0
A9	28	30	0	0	0	0	0	0									

1:1 = 1000 µg/ml Amoxicillin : 1000 µg/ml synthesized compound for Gram(+)ve bacteria
1:2 = 1000 µg/ml Amoxicillin : 2000 µg/ml synthesized compound for Gram(+)ve bacteria
1:1/2 = 2000 µg/ml Amoxicillin : 1000 µg/ml synthesized compound for Gram(-)ve bacteria
1:1 = 2000 µg/ml Amoxicillin : 2000 µg/ml synthesized compound for Gram(-)ve bacteria

In general, the results indicated that all the synthesized compounds had no activity as anti β-lactamase against *Staph. aureus, K. pneumonia,* and *P. aeroginosa*.

Concerning the anti β-lactamase activities against *E. coli* bacteria, 2 compounds which are A7, and A11 showed strong anti β-lactamase activities resembling that of clavulanic acid, although both of them have no antibacterial activities. These compounds having one or more hydrophobic residue in its structure, this is coming true with the docking results which indicate that the selectivity for the β-lactamase enzyme increases as the compounds became more hydrophobic, as the active binding site pocket of the β-lactamase are mostly hydrophobic in nature [22].

Conclusion:

It was concluded that the β-lactamase TEM-1 active pocket prefers hydrophobic substituents, as the 2 synthesized active anti β-lactamase compounds having hydrophobic residue. So that the selectivity for the β-lactamase enzyme increases as the compounds became more hydrophobic, and the Cl group will increase the antibacterial activity of the synthesized compounds.

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