UDC 547-327

DOCKING, SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY EVALUATION FOR NEW AMIDE COMPOUNDS

Ahmed A. J. Mahmood and Safaa P. Bahnam

Pharmaceutical Chemistry Department, Pharmacy College, Mosul University, Iraq
e-mail: ahmedsot@uomosul.edu.iq

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Abstract: *The beta-lactams are recognized as effective antibiotics for treating infections, yet bacterial production of β-lactamase, which hydrolyzes the beta-lactam ring, can render these drugs inactive. Combining these antibiotics with a β-lactamase inhibitor, such as clavulanic acid, mitigates this resistance. In a docking study involving Temoniera-1 (TEM-1), 1pzp, we induced the synthesis of eight amide compounds by reacting acid chloride derivatives with sulphathiazol or oxadiazol amine, forming an amide bond. The newly synthesized compounds were differentiated using physical and spectroscopic methods and verified biologically by estimating their minimum inhibitory concentration (MIC) against four strains of β-lactamase G(+)ve and G(-)ve bacteria. Their anti β-lactamase behaviors were then compared with that of clavulanic acid as a co-inhibitor with amoxicillin against the same four strains of bacteria. The results indicate that four of the new amides exhibit excellent anti-β-lactamase activity and contain one or more hydrophobic residues in their structures. Halogen atoms enhance the selectivity of tower β-lactamases. Derivatives of both oxadiazole and sulfathiazole scaffolds show promising anti-β-lactamase activity as non-β-lactam inhibitors.*

Keywords: *Sulfathiazole, Oxadiazole, TEM-1 β-lactamase, amides, docking, anti- β-lactamase.*
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Introduction

β-lactam antibiotics, the original native antibacterial compounds still distinguish an exceptional class of antibiotics, gratitude to both their exceptional antibacterial action and selectivity. Nevertheless, their resistance can quickly extend on a universal level [1]. With chief bacterial pathogens, this bacterial resistance has nowadays touched terrifying stages [2]. Illnesses initiated by drug-resistant bacteria have developed international hazards to community healthiness [3].

β-lactamase indicates the chief associated procedure of resistance [4]. Consequently, two approaches to overwhelm β- lactamase-mediated resistance: (a) improving the persisting β- lactamase-resistant antibiotics and (b) initiation of β-lactamase specifying inhibitors (BLIS) in order to co-administer together with a β-lactam antibiotics [4,5]. Consequently, here is an critical demand to purpose and improve additional antimicrobial or anti-β-lactamases to control this condition [4,6].

Serine β-lactamases were classified under the classes A, C, and D. They are accountable for the braking of the β-lactam moiety. This backing route includes the creation of a covalent acyl-intermediate among the catalytic serine and the β-lactam moiety in the enzyme's bocket [7]. Alternatively, the metallo-β-lactamases of class B utilize single or double of the zinc ions for accomplishing the β-lactam braking [7,8].

The β-lactamases Temoniera-1 (TEM-1) 1pzp belongs to the serine β-lactamases of the category A which braking the β-lactam group by developing an acyl-intermediate structure with the catalytic serine in the enzyme pocket [7,8].

BLIS targeting the β-lactamase enzymes when co-administer beside the β-lactam antibiotics. Nevertheless, because the presently consumed BLIS comprise β-lactam ring, they

were prone to time-limited appliance as the β-lactam antibiotics themselves [9]. Moreover, the β-lactamases pocket is subjected to transmutations that equivocates the attachment of BLIS. Inhibitor-resistant β-lactamases (IRTs) have been established and continuously increasing by time [10].

Talented approaches include the initiation of non-β-lactam inhibitors; they will be effective versus a broad range of β-lactamases. Various inhibitors have now been defined [11]. Boronic acid derivatives are effective for β-lactamases categories A, C and D [12]. A new acylated phenoxyaniline derivatives presenting β-lactamase TEM-171 inhibition and theoretically attached to the allosteric site of enzyme was newly defined [13,14].

Indeed, searching the various heterocyclic molecules has been widely performed, however sulfathiazole and oxadiazoles are continuing as reserved platforms in different medicinal discovery for invention of a new candidates [15]. Sulfathiazoles are original structure with numerous activities [16]. Several heterocyclic sulfonamide compounds was therapeutically applied, of which around 30 medications are currently available [15,16]. Sulfathiazole derivatives have many pharmacological implications as antiglaucoma, antimicrobial, diuretics, antiparasitic, anti-Alzheimer, antioxidant, antiviral, antiobesity, anticonvulsant, antiinflammatory, antitubercular, antidiabetic, anticancer, etc. [16-20]. While heterocyclic oxadiazole platform was described to show a wide variety of biological events as anticonvulsant, anti-inflammatory, antimalarial, and anticancer [20-24].

Considering the extensive pharmacological profiles of sulfathiazole and oxadiazoles, this research aims to purposefully (through docking) synthesize original non-β-lactam inhibitors derived from sulfathiazole and oxadiazole scaffolds. The study also involves assessing their ability to inhibit β-lactamases.

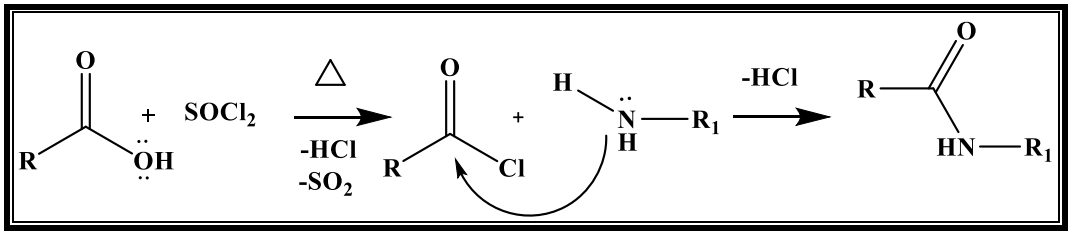
Materials and Methods

Docking analysis results:

Docking analysis was accomplished utilizing the online Mcule platform (https://mcule.com/ apps/1-click-docking/) [3]. The docking was generated with β-lactamase TEM-1 (1PZP). The chemical constructions of all compounds in this work were drown by ChemDraw program (PerkinElmer company), version 16.0.0.82(68). The choice of the best compounds will realize on the docking scores of the binding energies besides the geometric shape complementarity visualized by discovery studio visualizer (BIOVIA) v20.1.0.19295.

Synthesis:

All substances utilized in this work were bought from commercial sours and their sources are Scharlau (Spain), Merck (Germany), Alpha (India) and Fluka (Switzerland). Melting points were calculated using open capillaries. FTIR spectra were verified on a PerkinElmer infrared spectrophotometer, ^{1}H NMR and ^{13}C NMR spectra in DMSO-d6 on a Bruker, Avance DPX 400 MHz spectrometer (TMS was used as an internal reference).



Preparation of acid chloride derivatives [25]:

The carboxylic acids (2-3) mmol were dissolved in thionyl chloride (10-15 ml), the resultant combination was reflexed (30 min) within the hood (which contain the activated carbon fibers that trapped the resultant HCl and SO_2 byproducts). The excess amount of thionyl chloride was removed under reduced pressure. The remaining yields was occupied after cooling and consumed freshly in the subsequent

step of the amides synthesis.

Preparation of amides products (Ad1-Ad8) [26]:

The consequent acid chloride (2.5×10^{-3}) mole dissolved in dichloromethane (5 ml) and adds up drop by drop to a mixture of sulfathiazole or oxadiazole (2.5×10^{-3}) mole plus pyridine (2.5×10^{-3}) mole dissolved in dichloromethane (30 ml) in ice bath. The yielded mixture mixed at 25 ºC to the next day. Afterward the solvent was evaporated and the yield was washed 2-3 times with cold ethanol for removing the west products.

Biological study

Bacterial selection (*β*-lactamases detection):

Acidimetric method was used to identify β-lactamases in one G(+)ve (*Staphylococcus aureus*) and 3 G(-)ve (*E.coli and K. pneumonia*, *P. aeroginosa)* isolates of pathogenic bacteria [27]. Converting the mixture color from pink➔ yellow in 5 min reveals β-lactamase existence. (+)ve control was participated in equivalence [28]. Additionally these isolates were selected to become resistant to amoxicillin and sensitive to Augmentin for one certain strength, this is confirming that the clavulanic acid (in the Augmentin) will mask the β-lactamases enzymes in the tested bacteria and without its action there is no effects for amoxicillin as antibiotic.

Minimum inhibitory concentration (MIC) determination [29]**:**

To evaluate the MIC a broth microdilution method was utilized. A consecutive of ten replication diluted concentrations belong to the new amides and the typical antibacterial (Augmentin and Amoxicillin) was formulated, the dilution initiated from concentration of 2000 µg/ml. Bacterial colonies were inserted to the previous test tubes at of 5×10^{5} CFU/ml concentration next to the adding 1 ml of Mueller–Hinton media. Afterward at 37°C they incubated for 18 h [20,21]. The agar plus the bacterial isolates was used as a (+)ve control, while the (-)ve control including the agar only. By utilizing this technique, investigators were capable to establish the MIC, which characterizes the lowermost strength of stuff necessary to inhibit the growth of specific bacteria. The MIC determining stage was performed to check the antibacterial activity for both the standard and the synthesized compounds. The MIC for the standard compound will represent the strength at which the Augmentin will be sensitive and surely the amoxicillin will be resistance as indicated in the prior step. The MIC estimation for the new compounds (synthesized) judged their antibacterial activities (if there is any), and to identify the intensity of these compounds that will be utilized in the following step [30,31]. This concentration must be lower than their MIC and overhead the clavulanic acid MIC of the Augmentin. This is to guarantee that the antibacterial activities of the amoxicillin that performed in the subsequent phase will be initiated by the inhibition of the β-lactamase by either the clavulanic acid or the newly synthesized compound when inoculating individually with the amoxicillin [31].

Anti β-lactamase activities evaluation:

In the paper, the disk diffusion method was occupied to evaluate the anti β-lactamase activity of the new amides [32,33]. To develop the experimentation, every investigated compound was employed as a co-inhibitor along with 1000 µg of amoxicillin (equal to that of Augmentin) formulated as disks (5µl per disk). These disks including the amoxicillin and the investigated compound (in their sub MIC concentration) were subsequently positioned on Mueller-Hinton agar medium in a Petri dish (inoculated with the bacterial strains in earlier times) using sterile cotton swabs. The whole setup was then incubated for of 24 hours at 37°C. The zones of microbial progress inhibition that performed around the disk were restrained and documented as the diameters of inhibition [34,35]. For control, disks comprising 1000 µg of amoxicillin alone (5µl/disk) were also organized and involved in the trial. Dimethyl sulfoxide was employed to dissolve the new compounds, with a guarantee of its final concentration less than 2% to certify that it did not undesirably influence bacterial growth. This precaution was occupied to preserve the reliability and precision of the experiment [32,36].

Results and discussion

Docking Study results:

The designed amides along with clavulanic acid, sulbactam, tazobactam, avibactam were docked with β-lactamase TEM-1 (1pzp). The substituents used for the designing the amides are listed in Table (1), whereas their docking results were representing in Table (2).

Table 1. The substituents used for amides designation

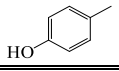
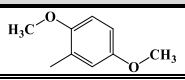
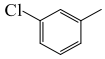
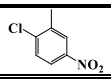
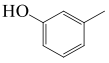
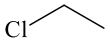
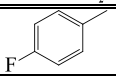
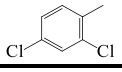
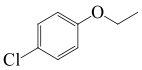
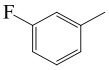
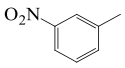
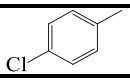
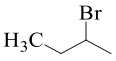
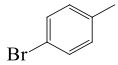
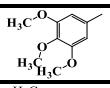
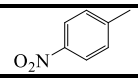
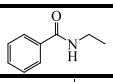
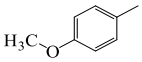
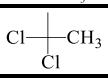
Item	R	Item	R	Item	R
R1		R11		R21	
R2		R12		R22	
R3		R13		R23	
R4		R14		R24	
R5		R15		R25	
R6		R16		R26	
R7		R17		R27	
R8		R18		R28	
R9		R19		R29	
R10		R20		R30	

Table 2. The docking results for the oxadiazole and the sulfathiazole amides compounds with TEM-1 β- lactamase (1PZP) enzymes (kcal/mol)

No.	Docking Score		No.	Docking Score		No.	Docking Score	
	oxadiazole	Sulfathiazole		oxadiazole	sulfathiazole		oxadiazole	sulfathiazole
R1	-7.2	-8.0	R11	-7.0	-6.0	R21	-6.8	-6.5
R2	-7.0	-9.1	R12	-7.4	-9.0	R22	-7.6	-6.1
R3	-8.0	-8.1	R13	-7.8	-8.4	R23	-6.4	-6.6
R4	-7.6	-9.4	R14	-8.1	-8.3	R24	-6.8	-6.5
R5	-7.5	-7.0	R15	-7.4	-8.0	R25	-9.2	-9.0
R6	-8.5	-7.1	R16	-7.4	-8.4	R26	-7.0	-7.7
R7	-8.5	-7.0	R17	-7.8	-7.0	R27	-6.4	-6.6
R8	-7.0	-7.7	R18	-7.4	-8.0	R28	-7.8	-8.3
R9	-7.4	-9.0	R19	-9.2	-9.1	R29	-7.2	-8.0
R10	-8.4	-8.4	R20	-7.3	-7.9	R30	-7.0	-7.7

The docking of the standard inhibitor sulbactam, avibactam, tazobactam and clavulanic acid were -5.5, -5.4, -4.3 and -6.1 kcal/mol respectively. The standard inhibitors docking was not performed for achieving its score as reference control only but moreover their binding poses consumed for the definition of the active pocket site amino acids for β-lactamases TEM-1 (1pzp). The amino acids residues (LEU 196, ALA 199, IEU 200, GLY 211, ALA 253 and GLY 256) were found to bind mainly all of the standard inhibitors in the

(1pzp) β-lactamase [34]. The overhead 6 residues will describe the active binding site for the connection with this enzyme.

Regarding the collaboration of the tested amides with enzymes (Table 2), the results demonstrated that 8 compounds with the substituents (R19 and R25) for the oxadiazole and the substituents (R2, R4, R9, R12, R19 and R25) for the sulfathiazole represent the best docking scores, additionally they bound 4-5 out of the 6 amino acids that defined the binding site of β-lactamases TEM-1 (1pzp), besides to 3-5 additional amino acids residues for every compound, those may play a role in the potentiation the contact with the enzyme (Figure 1).

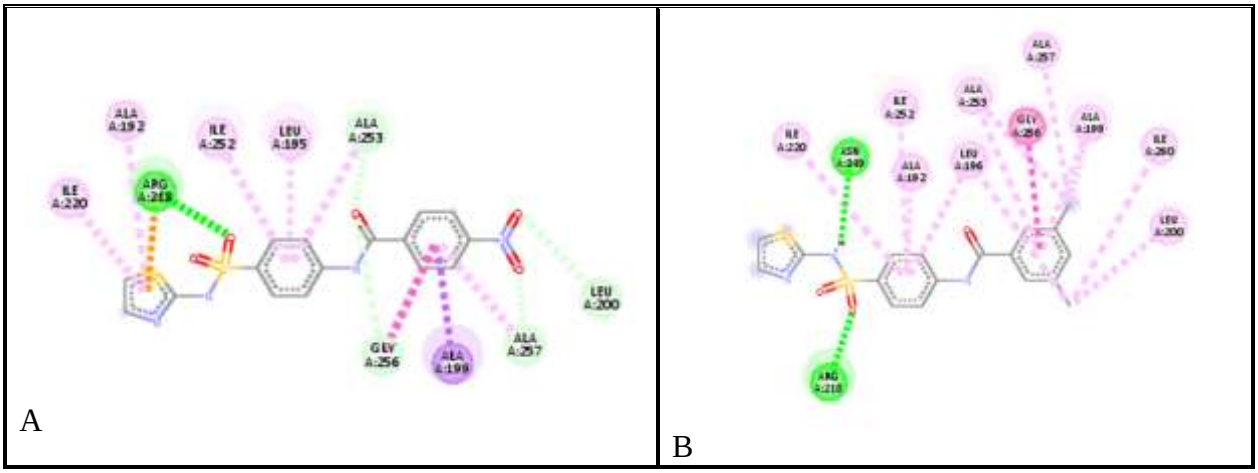
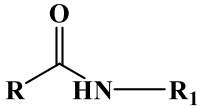


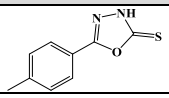
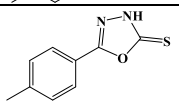
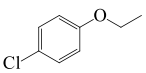
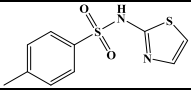
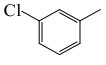
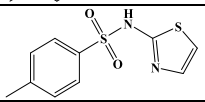
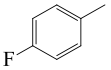
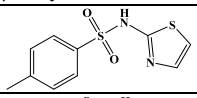
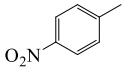
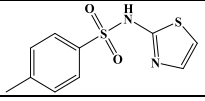
Fig. 1. 2 dimensional poses for selected R9 (A) R19 (B) with the 1pzp enzyme.

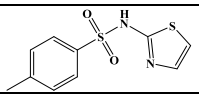
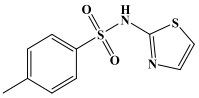
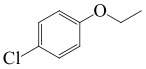
Chemistry:

Table (3) demonstrates the most important physical characteristic and the documented representative FT-IR values as ν cm^{-1} belongs to the newly synthesized amides.

Table 3. The most important physical characteristic and the documented representative FT-IR values as ν cm-1 belongs to the amides (Ad1-Ad8).



Amides	R_1	R	m.p(°C)	Yield %	Color	N-H ν cm^{-1}	C=O ν cm^{-1}	Other ν cm^{-1}
Ad1			225-227	72	Off White	w 3397	m, sh 1675	------
Ad2			247-249	85	White	w 3359	m , sh 1679	C-Cl m 400 C-O-C s, sh 1228
Ad3			218-220	65	Pinkish off white	w 3370	m, sh 1668	C-Cl m 406
Ad4			271-273	70	Pinkish off white	w 3391	m, sh 1676	C-F m 507
Ad5			254-256	87	Pink	w 3396	m, sh 1661	C-F m 513
Ad6			160-162	63	Off white	w 3313	m, sh 1694	------

Ad7			**266-268**	**82**	**White**	**w 3370**	**m, sh 1688**	**------**
Ad8			**269-271**	**74**	**White**	**w 3372**	**m, sh 1693**	**C-O-C m 1238 C-Cl m 400**

The FTIR denote the amide bonds creation by the vanishing of the 1° NH_2 at 3356 cm^{-1} and 3259 cm^{-1} of the original oxadiazole and sulfathiazole compounds respectively, and switched by the 2° amine NH at 3313-3397 cm^{-1}, in addition to the emergence of carbonyl at 1661-1694 cm^{-1} which document amide establishments. Besides to additional peaks for every compound that identify to the substitutions of the acid chlorides consumed (Table 3).

^{1}H-NMR and ^{13}C-NMR of amides (Ad1-Ad8) designate the development of the amide bonds, as the HNMR performance the lone proton emergence belongs to the NH oxadiazoles amide (compounds Ad1 and Ad2) at 7.81-7.82 ppm and the NH of the sulfathiazole amides (compounds A3-A8) at 8.34-10.49 ppm.

Whereas in the ^{13}C-NMR the attendance of the amide carbonyl C=O of the oxadiazoles (compounds Ad1 and Ad2) at 172.09 ppm, and the sulfathiazole amide carbonyl C=O (compounds Ad3-Ad8) at 161.34-168.85 ppm. This also will authorize the development of amide bond.

The spectral representation of the new amides compounds Ad1-Ad8

Ad1 [3,5-dimethyl-N-(4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)benzamide] **The ^{1}H-NMR of Ad1 (δ, ppm) (DMSO-*d*6):** δ 14.69 (s, 1H, S 12), δ 10.49 (s, 1H, N 13), δ 7.87 (s, 4H, C 2, 3, 5 & 6), δ 7.65 (d, 2H, C 17 & 21), δ 7.12 (m, 1H, C 19), δ 2.30 (s, 6H, C 22 & 23).**The ^{13}C-NMR of Ad1 (δ, ppm) (DMSO-*d*6):** δ 179.14 ppm (C9), δ 166.60 ppm (C14), δ 161.07 ppm (C7), δ 140.34 ppm (C1), δ 137.23 ppm (C18,20), δ 132.24 ppm (C16), δ 131.91 ppm (C19), δ 127.82 ppm (C3,5), δ 127.71 ppm (C17,21), δ119.77 ppm (C4), δ 119.63 ppm (C2,6), δ 20.92 ppm (C22,23).

Ad2 [2-(4-chlorophenoxy)-N-(4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol -2-yl)phenyl)acetamide].**The ^{1}H-NMR of Ad2 (δ, ppm) (DMSO-*d*6):** δ 14.69 (s, 1H, S 12), δ 10.49 (s, 1H, N 13), δ 7.87 (s, 4H, C 2, 3, 5 & 6), δ 7.37 (d, 2H, C 20 & 22), δ 7.04 (d, 2H, C 19 & 23), δ 4.77 (s, 2H, C 16).**The ^{13}C-NMR of Ad2 (δ, ppm) (DMSO-*d*6):** δ 179.14 ppm (C9), δ 168.04 ppm (C14), δ 161.07 ppm (C7), δ 157.21 ppm (C18), δ 138.40 ppm (C1), δ 129.54 ppm (C20,22), δ 127.75 ppm (C3,5), δ 127.29 ppm (C21), δ 119.77 ppm (C4), δ 119.09 ppm (C2,6), δ 116.05 ppm (C19,23), δ 67.49 ppm (C16).

Ad3 [3-chloro-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)benzamide].**The ^{1}H-NMR of Ad3 (δ, ppm) (DMSO-*d*6):** δ 12.72 (s, 1H, N 11), δ 10.45 (s, 1H, N 1), δ 8.10 (s, 1H, C 21), δ 8.04 (s, 1H, C 25), δ 7.92 (d, 2H, C 3 & 7), δ 7.78 (d, 2H, C 4 & 6), δ 7.48 (s, 1H, C 24), δ 7.45 (s, 1H, C 23), δ 7.20 (d, 1H, C 15), δ 6.80 (d, 1H, C 16). **The ^{13}C-NMR of Ad3 (δ, ppm) (DMSO-*d*6):** δ 166.24 ppm (C18), δ 162.17 ppm (C10), δ 142.23 ppm (C2), δ 138.93 ppm (C15), δ 133.34 ppm (C20), δ 132.77 ppm (C5), δ 132.63 ppm (C22), δ 130.99 ppm (C23), δ 129.96 ppm (C24), δ 128.85 ppm (C4,6), δ 127.64 ppm (C21), δ 126.57 ppm (C25), δ 120.26 ppm (C3,7), δ 113.94 ppm (C16).

Ad4 [4-fluoro-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)benzamide]. **The ^{1}H-NMR of Ad4 (δ, ppm) (DMSO-*d*6):** δ 12.70 (s, 1H, N 9), δ 9.02 (s, 1H, N 1), δ 8.00 (d, 2H, C 21 & 25), δ 7.83-7.89 (m, 4H, C 3, 4, 6 & 7), δ 7.24 (d, 1H, C 15), δ 7.13 (d, 2H, C 22 & 24), δ 6.69 (d, 1H, C 16). **The ^{13}C-NMR of Ad4 (δ, ppm) (DMSO-*d*6):** δ 166.34 ppm (C18), δ 164.82 ppm (C23), δ 162.17 ppm (C10), δ 142.43 ppm (C2), δ 138.93 ppm (C15), δ 132.77 ppm (C5), δ 130.64 ppm (C20), δ 129.84 ppm (C21,25), δ 128.85 ppm (C4,6), δ 120.26 ppm (C3,7), δ 115.97 ppm (C22,24), δ 113.94 ppm (C16).

Ad5 [2-methyl-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)benzamide]. **The ^{1}H-NMR of Ad5 (δ, ppm) (DMSO-*d*6):** δ 12.70 (s, 1H, N 11), δ 10.46 (s, 1H, N 1), δ 7.92 (d, 2H, C 3 & 7), δ 7.82 (s, 1H, C 25), δ 7.78 (d, 2H, C 4 & 6), δ 7.51 (dd, 1H, C 23), δ 7.32 (s, 1H, C 24), δ 7.27 (s, 1H, C 22), δ 7.24 (d, 1H, C 15), δ 6.81 (d, 1H, C 16). **The ^{13}C-NMR of Ad5 (δ, ppm) (DMSO-*d*6):** δ 164.26 ppm (C18), δ 162.17 ppm (C10), δ 160.22 ppm (C21), δ 142.14 ppm (C2), δ 138.93 ppm (C15), δ 132.93 ppm (C23), δ 132.77 ppm (C5), δ 129.88 ppm (C25), δ 128.85 ppm (C4,6), δ 124.54 ppm (C24), δ 120.92 ppm (C20), δ 120.14 ppm (C3,7), δ 115.93 ppm (C22), δ 113.94 ppm (C16).

Ad6 [4-nitro-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)benzamide]. **The ^{1}H-NMR of Ad6 (δ, ppm) (DMSO-*d*6):** δ 12.70 (s, 1H, N 9), δ 9.00 (s, 1H, N 1), δ 8.31 (d, 2H, C 22 & 24), δ 8.15

(d, 2H, C 21 & 25), δ 7.83- 7.89 (m, 4H, C 3, 4, 6 & 7), δ 7.13 (d, 1H, C 15), δ 6.69 (d, 1H, C 16). **The ^{13}C-NMR of Ad6 (δ, ppm) (DMSO-*d*6):** δ 166.37 ppm (C18), δ 162.17 ppm (C10), δ 149.31 ppm (C23), δ 142.23 ppm (C2), δ 138.93 ppm (C15), δ 138.62 ppm (C20), δ 132.77 ppm (C5), δ 129.66 ppm (C21,25), δ 128.85 ppm (C4,6), δ 123.66 ppm (C22,24), δ 120.26 ppm (C3,7), δ 113.94 ppm (C16).

Ad7 [3,5-dimethyl-N-(4-(N-(thiazol-2-yl) sulfamoyl) phenyl) benzamide]0. **The ^{1}H-NMR of Ad7 (δ, ppm) (DMSO-*d*6):** δ 12.70 (s, 1H, N 11), δ 10.47 (s, 1H, N 1), δ 7.92 (d, 2H, C 3 & 7), δ 7.78 (d, 2H, C 4 & 6), δ 7.56 (s, 2H, C 21 & 25), δ 7.26 (s, 1H, C 23), δ 7.24 (d, 1H, C 15), δ 6.82 (d, 1H, C 16), δ 2.36 (s, 6H, C 26 & 27). **The ^{13}C-NMR of Ad7 (δ, ppm) (DMSO-*d*6):** δ 166.65 ppm (C18), δ 162.17 ppm (C10), δ 142.43 ppm (C2), δ138.93 ppm (C15), δ 137.23 ppm (C22,24), δ 132.77 ppm (C5), δ 132.24 ppm (C20), δ 131.91 ppm (C23), δ 128.85 ppm (C4,6), δ 127.71 ppm (C21,25), δ 120.26 ppm (C3,7), δ 113.94 ppm (C16), δ 20.92 ppm (C26,27).

Ad8 [2-(4-chlorophenoxy)-N-(4-(N-(thiazol-2-yl) sulfamoyl) phenyl) acetamide]. **The ^{1}H-NMR of Ad8 (δ, ppm) (DMSO-*d*6):** δ 12.70 (s, 1H, N 11), δ 10.44 (s, 1H, N 1), δ 7.45 (d, 1H, C 15), δ 7.36 (d, 2H, C 4 & 6), δ 7.24 (d, 2H, C 3 & 7), δ 7.02 (d, 2H, C 24 & 26), δ 6.18 (d, 2H, C 23 & 27), δ 6.56 (d, 1H, C 16), δ 3.37 (s, 2H, C 20). **The ^{13}C-NMR of Ad8 (δ, ppm) (DMSO-*d*6):** δ 168.05 ppm (C18), δ 162.17 ppm (C10), δ 157.21 ppm (C22), δ 140.26 ppm (C2), δ 138.93 ppm (C15), δ 132.77 ppm (C5), δ 129.54 ppm (C24,26), δ 128,84 ppm (C4,6), δ 127.29 ppm (C25), δ 120.11 ppm (C3,7), δ 116.05 ppm (C23,27), δ 113.94 ppm (C16), δ 67.49 ppm (C20).

Biological study results:

The MIC results indicate that Augmentin exhibits significant activity against all bacterial strains, whereas Amoxicillin shows negligible antibacterial activity against all bacterial strains except for K. pneumonia. This implies that clavulanic acid (in Augmentin) acts as an anti-β-lactamase agent, protecting Amoxicillin from enzyme action. However, the new amides exhibit minimal antibacterial activity. MIC analysis was conducted for the synthesized amides to ensure that when co-administered with Amoxicillin as an anti-β-lactamase, they do not develop any antibacterial activity (their own sub MIC strengths of 400 µg and 800 µg will be used in the subsequent incubation step) (Table 4).

Table 4. MIC outcomes for the standard antibacterial and the newly amides compounds

Compds	Minimum inhibitory concentration				Compds	Minimum inhibitory concentration			
	G (-)ve			G (+)ve		G (-)ve			G (+)ve
	E. coli	*Klep. Pnem.*	*Psed. Aerog.*	*Staph. Aur.*		*E. coli*	*Klep. Pnem.*	*Psed. Aerog.*	*Staph. Aur.*
Augmentin	750	1000	500	500	Ad4	1000	1000	1000	1000
Amoxicillin	>2000	1000	>2000	>2000	Ad5	>2000	>2000	>2000	>2000
Ad1	>2000	1000	>2000	>2000	Ad6	1000	>2000	1000	>2000
Ad2	>2000	>2000	>2000	>2000	Ad7	>2000	1000	>2000	>2000
Ad3	1000	1000	1000	1000	Ad8	1000	>2000	>2000	>2000

In this phase, the β-lactamase inhibitory action of the new amides against the tested pathogenic bacterial isolates was assessed [29]. Each tested amides was utilized as co-inhibitor with 1000 µg of amoxicillin formulated as disks (5µl/disk) at 400 µg and 800 µg strengths.

Amoxicillin 1000 µg alone and Amoxiclav (1000/200mg) were incubated too, the outcomes would be respects as a control for the newly synthesized amides (Table 5).

Table 5. The zones of inhibition for the amides when with the Amoxicillin against bacterial isolates

Compds	Diameter of Inhib. zones (mm)								Compds	Diameter of Inhib. zones (mm)							
	G (+)ve		G (-)ive							G (+)ve		G (-)ive					
	Staph. Aureus		*E. coli*		*K. pneumonia*		*P. aeruginosa*			*Staph. Aureus*		*E. coli*		*K. pneumonia*		*P. aeroginosa*	
	1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2		1:1	1:2	1:1	1:2	1:1	1:2	1:1	1:2
Augmentin	18	18	21	22	20	22	21	22	Ad4	15	18	17	19	9	10	5	8
Amoxicillin	9	10	0	0	0	0	0	0	Ad5	8	10	5	8	18	19	0	5
Ad1	18	19	19	19	18	21	20	20	Ad6	10	12	10	14	16	19	8	10
Ad2	18	20	18	20	9	12	20	22	Ad7	19	21	18	20	19	19	18	20
Ad3	20	21	21	22	12	15	21	21	Ad8	9	12	21	22	9	10	8	8

1:1 = 1000 µg/ml Amoxicillin: 400 µg/ml amides
1:2 = the same 1000 µg/ml Amoxicillin: 800 µg/ml amides

Generally, the above outcomes specified the fact that totally the new amides developed good action as β-lactamase inhibitors versus all bacterial strains signified that these compounds stop the β-lactamases in these bacterial strains.

Notably, two compounds, Ad1 and Ad7 (oxadiazole and sulfathiazole with R19), demonstrated promising activities resembling those of clavulanic acid, despite having no antibacterial activities when used alone at this strength. Conversely, compounds Ad2 and Ad3 (oxadiazole with R25 and sulfathiazole with R2) exhibited negative anti-β-lactamase activities against K. pneumonia only, with beneficial activities against the remaining strains. The remaining amides demonstrated β-lactamase inhibition in only one or two strains, classifying them as weak inhibitors. All these amides incorporate one or more hydrophobic residues in their structures, along with halogen atoms or a nitro group in some compounds. This aligns with docking results and other research, indicating that increased hydrophobicity enhances selectivity for the β-lactamase enzyme. These findings suggest promising anti-β-lactamase activities for both oxadiazole and sulfathiazole scaffolds as non-β-lactam inhibitors, emphasizing their potential in combating bacterial resistance.

Conclusion

In conclusion, our findings suggest that the active pocket of β-lactamases favors hydrophobic residues, supported by the observation that four of the new amides with effective β-lactamase inhibitory activities contain one or more hydrophobic components. Additionally, the presence of halogens enhances the affinity towards β-lactamases. Derivatives from both oxadiazole and sulfathiazole scaffolds exhibit promising anti-β-lactamase activities, positioning them as potential non-β-lactam inhibitors. These results underscore the significance of hydrophobicity and halogen entities in designing effective compounds to combat β-lactamase-mediated bacterial resistance.

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YENİ AMID BİRLƏŞMƏLƏRİNİN β-LAKTAMAZ İNHİBİTOR AKTİVLİYİNİN QİYMƏTLƏNDİRİLMƏSİ, SİNTEZİ VƏ DOKİNQİ

Əhməd A. J. Mahmud, Safaa P. Bəhnam

Mosul Universiteti, Əczaçılıq Kolleci, Əczaçılıq Kimyası Departamenti, İraq
e-poçt: ahmedsot@uomosul.edu.iq

Xülasə: Beta-laktamlar infeksiyaların müalicəsi üçün effektiv antibiotiklər kimi tanınır, lakin beta-laktam halqasını hidroliz edən β-laktamazanın bakterial istehsalı bu dərmanları təsirsiz hala sala bilər. Bu antibiotikləri klavulan turşusu kimi bir β-laktamaz inhibitoru ilə kopmbinasiyası bu müqaviməti azaldır. Temoniera-1 (TEM-1), 1pzp-ni əhatə edən tədqiqatlarda biz turşu xlorid törəmələrini sulfatiazol və ya oksadiazol amin ilə reaksiyaya daxil edərək, amid rabitəsi yaradaraq səkkiz amid birləşməsinin sintezini təmin etmişik. Yeni sintez edilmiş birləşmələr fiziki və spektroskopik üsullarla differensiallaşdırılmış, β-laktamaz G(+) və G(-) bakteriyalarının dörd ştamına qarşı onların minimum inhibitor qatılığı (MİK) qiymətləndirilmiş və bioloji cəhətdən yoxlanılmışdır. Daha sonra onların anti β-laktamaz davranışları eyni dörd bakteriya ştamına qarşı

amoksisillinlə birgə inhibitor kimi klavulan turşusu ilə müqayisə edilmişdir. Nəticələr göstərir ki, yeni amidlərdən dördü əla anti-β-laktamaz aktivliyi nümayiş etdirir və strukturlarında bir və ya daha çox hidrofobik qalıq ehtiva edir. Halogen atomları artıqlığı β-laktamazların seçiciliyini artırır. Həm oksadiazol, həm də sulfatiazol karkaslarının törəmələri qeyri-β-laktam inhibitorları kimi perspektivli anti-β-laktamaz xassəsi göstərir.
Açar sözlər: Sulfatiazol, Oksadiazol, TEM-1 β-laktamaza, amidlər, dokinq, anti-β-laktamaza.

ДОКИНГ, СИНТЕЗ И ОЦЕНКА β-ЛАКТАМАЗНОЙ ИНГИБИРУЮЩЕЙ АКТИВНОСТИ НОВЫХ АМИДНЫХ СОЕДИНЕНИЙ

Ахмед А.Дж. Махмуд, Сафаа П. Бахнам

Кафедра фармацевтической химии, Фармацевтический колледж,
Мосульский университет, Ирак
e-mail: ahmedsot@uomosul.edu.iq

Резюме: Бета-лактамы признаны эффективными антибиотиками для лечения инфекций, однако бактериальная продукция β-лактамазы, которая гидролизует бета-лактамное кольцо, может сделать эти препараты неактивными. Сочетание этих антибиотиков с ингибитором β-лактамаз, таким как клавулановая кислота, снижает эту резистентность. В исследовании стыковки с участием Temoniera-1 (TEM-1), 1pzp, мы индуцировали синтез восьми амидных соединений путем взаимодействия производных хлорангидридов с сульфатиазолом или оксадиазоламином, образуя амидную связь. Вновь синтезированные соединения были дифференцированы физическими и спектроскопическими методами и биологически проверены путем оценки их минимальной ингибирующей концентрации (МПК) в отношении четырех штаммов бактерий β-лактамаз G(+)ve и G(-)ve. Затем их анти-β-лактамазное поведение сравнивали с действием клавулановой кислоты в качестве со-ингибитора с амоксициллином против тех же четырех штаммов бактерий. Результаты показывают, что четыре из новых амидов проявляют превосходную анти-β-лактамазную активность и содержат в своей структуре один или несколько гидрофобных остатков. Увеличение атомов галогенов повышают селективность β-лактамаз. Производные как оксадиазольных, так и сульфатиазольных каркасов демонстрируют многообещающую анти-β-лактамазную активность в качестве не-β-лактамных ингибиторов.
Ключевые слова: сульфатиазол, оксадиазол, β-лактамазы TEM-1, амиды, докинг, анти-β-лактамазы.