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MOLECULAR DOCKING, SYNTHESIS AND BIOLOGICAL EVALUATION FOR NEW 2-ARYL THIAZOLIDINE-4-CARBOXYLIC ACID DERIVATIVES AS B-LACTAMASE INHIBITORS

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ABSTRACT

β -lactam antibiotics have become the standard treatment for bacterial infections. Production of β-lactamases as the major cause of bacterial resistance inactivates the β-lactams. There is an urgent need to design and develop anti β -lactamases (clavulanic acid-like) products. TEM-1 is a prevalent plasmid-encoded β-lactamase bacterium that efficiently catalyzes the hydrolysis of penicillin and early cephalosporin. Depending on the docking study with TEM-1 β-lactamase, we synthesize 8 compounds of 2-Aryl thiazolidine- 4-carboxylic acid derivatives (from condensing aromatic aldehydes with L- cysteine). The synthesized compounds were characterized using physical and spectroscopic methods. The biological activity of the 8 new compounds was tested *in vitro* and their MIC was evaluated against 4 strains of β-lactamase G(+)ve and G(-)ve pathogenic bacteria, then the anti β-lactamases activities were tested and compared with that of clavulanic acid as a co-inhibitory with amoxicillin. The results of the biological activity revealed that 3 of them showed anti β-lactamase effect comparable to that of clavulanic acid. Our results revolve that the β-lactamase active pocket has hydrophobic space which prefers hydrophobic substituents, as products with these group show the highest affinity.

KEYWORDS: TEM-1 β-lactamase , 2-Aryl thiazolidine- 4-carboxylic acid, anti- β-lactamase, L-cysteine

INTRODUCTION

Antibacterial resistance has now reached alarming levels, with major bacterial pathogens.[1,2] β -lactam antibiotics, the first natural antibacterial compounds still represent an outstanding class of antibiotics, thanks to both their excellent antibacterial activity and selectivity. However, their resistance can rapidly spread on a global scale.[3,4]

β -lactamase production represents the most relevant mechanism of resistance. For that reason, two strategies to overcome β- lactamase-mediated resistance: (a) the optimization of β- lactamase-stable antibiotics and (b) the development of selective β-lactamase inhibitors (BLIS) to be co-administered with a β-lactam antibiotics.[5] This research aims to design, dock (computer calculation), synthesize, and study the biological activities of new β-lactamase inhibitors.

MATERIALS 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 were determined in open capillaries. FTIR spectra were recorded on a PerkinElmer infrared spectrophotometer, ^{1}H NMR and ^{13}C NMR spectra in DMSO-d6 on a Bruker, Avance DPX 400 MHz spectrometer using TMS as an internal reference. All the starting materials 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 with default parameters (https://mcule.com/ apps/1-click-docking/). The structure β-lactamases TEM-1 (1pzo) and PBP (1qmf) were retrieved from PDB.[6]

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[7], 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.[8]

Chemical study

Preparation of 2-Aryl thiazolidine-4-carboxylic acid derivatives (A 13-20)[9]

A mixture of L-cysteine (27mmol) and appropriate aldehyde (27mmol) in absolute ethanol (50 mL) and distilled water (5 mL) was stirred at room temperature for 2-12 h, and the solid precipitated out was collected, washed 3 times with distilled water, and then dried.

Antimicrobial study

Detection of β-lactamases in bacterial isolate

The detection of β-lactamases in bacterial isolates was done for Gram-positive (*Staphylococcus aureus*) and 3 Gram-negative pathogenic bacteria (*Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeroginosa*) isolates using acidimetric method.[10] A yellow color within 5 min indicates β-lactamase activity. Positive controls were run in parallel.[11]

Determination of minimum inhibitory concentration (MIC)[12]

A broth micro dilution 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, then 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.[12,13] A (+)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 was evaluated by measuring the zones of inhibition in the disk diffusion method.[12] 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.[14] Disks containing 1000 or 2000 µg of amoxicillin (5µl/disk) was prepared and used as control. DMSO was used as a solvent for the synthesized compounds with a final concentration of less than 2 % to ensure that it does not affect bacterial growth.

RESULTS AND DISCUSSION

Docking study

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.

Molecular Docking results of 2-Aryl thiazolidine- 4-carboxylic acid derivatives

The docking for 2-Aryl thiazolidine- 4-carboxylic acid derivatives was carried out on on both bacterial PBP (1qmf) and β-lactamases TEM-1 (1pzo). The chemical structures of the designated product are listed in Table (1), while the results of docking are listed in Table (2). Compounds with R 2, 4, 5, 6, 9, 10, 12, and 13 substitutions show the highest score.

Table (1): Substitution used to design the 2-Aryl thiazolidine-4-carboxylic acid derivatives.

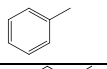
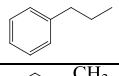
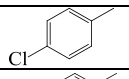
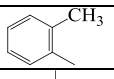
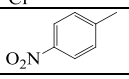
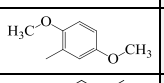
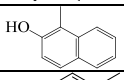
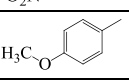
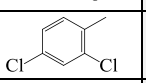
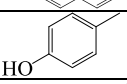
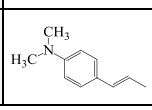
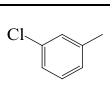
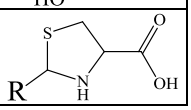
R	Structure	R	structure	R	Structure
R1		R6		R11	
R2		R7		R12	
R3		R8		R13	
R4		R9		R14	
R5		R10			

Table (2): Docking Study results for the 2-Aryl thiazolidine-4-carboxylic acid derivatives with the β-Lactamases (TEM-1) and PBP.

R	Docking Scour		R	Docking Scour		R	Docking Scour	
	PBP	β-lactm.		PBP	β-lactm.		PBP	β-lactm.
R1	- 6.5	- 5.7	R6	- 7.7	- 8.2	R11	- 7.0	- 6.3
R2	- 8.7	- 8.7	R7	- 6.1	- 5.5	R12	- 6.8	- 8.1
R3	- 7.5	- 6.0	R8	- 6.7	- 6.0	R13	- 7.7	- 8.2
R4	- 8.7	- 8.9	R9	- 6.8	- 8.3	R14	- 6.2	- 5.6
R5	- 7.5	- 8.2	R10	- 8.6	- 8.0			

The docking results showed that as the hydrophobic substitutions increase in the tested compound the score will decrease as a result. The compounds bound variably to the region located between H10, H11, and H12 helices of TEM-1, indicating that the pocket is mostly hydrophobic[15], 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 study

Preparation of 2-Aryl thiazolidine-4-carboxylic acid derivatives (A1-8)

This reaction was occurred in two step mechanism. In the first step the unshared pair of electron of the amino group of L-cysteine attacks the carbonyl's carbon atom of the aldehyde to form the aminol intermediate which losses water molecule to form imine compounds (Schiff base). In the second step the sulfur in this case acts as nucleophile and attacks the imine carbon in intramolecular cyclization step to form the thiazolidine compound. The physical properties and the most characteristic peaks of the FTIR spectrum for compounds (A1-8) are shown in the table (3).

Table (3): physical properties and the most characteristic peaks (ν cm^{-1}) of the FT-IR spectrum for the 2-Aryl thiazolidine- 4-carboxylic derivatives (A 1-8).

Comp No.	Compound structure	m.p(°C)	Yield%	Color	Time to end of the reaction	R_f (Ether 1:DCM 1)	N-H	C-H	C=O acid	Other
A1	R=2	171-173	90	White	3 h	0.76	m 3284	m 2993	s 1524	C-Cl s 1091
A2	R=3	183-185	92	Yellow	4.5 h	0.54	m 3273	w 3015	s 1620	N-O s 1343
A3	R=4	163-164	85	Off white	5 h	0.67	m 3286	w 2993	s 1615	C-O m 1241
A4	R=5	180-182	60	Brown	12 h	0.43	m 3271	m 3004	s 1609	C-N m 1304
A5	R=6	174-176	97	Off white	5 h	0.47	m 3242	w 2996	s 1626	------
A6	R=10	177-179	96	White	5 h	0.71	m 3281	m 2993	s 1614	C-Cl s 1087
A7	R=12	164-166	77	Off white	12 h	0.42	m 3254	m 2990	s 1612	------
A8	R=13	144-147	90	Yellow	3 h	0.45	m 3267	m 3018,	s 1622	O-H m 3329

The IR spectra of compounds (A1-8) characterized by the absence of the absorption bands of the S-H bond stretching of the cysteine at 2537 cm-1indicating the reaction of the S atom with the aldehyde and then forming the five-membered ring is common for all the series, and appearance of absorption bands for each individual compound depending on the substitutions of the aldehyde used.

The ^{1}H-NMR spectra indicates the formation of the five membered thiazolidine rings via the appearance of the singlet chemical shifts in the ranges 4.10-5.83 ppm and 7.68-8.52 ppm for the proton of the C1 carbon and N3 nitrogen respectively, confirming the reaction of cysteine with the aldehyde forming the thiazolidine ring.

While in the ^{13}C-NMRspectra disposed the appearance of the chemical shifts at 65.85-69.00 ppm and 64.16-65.78 ppm for the carbon atoms C1 and C4 respectively, this is also confirmed the formation of the thiazolidine moiety.

The other chemical shifts appeared in the both ^{1}H-NMR

and ^{13}C-NMR spectra for each compound depending on the substitutions of the aldehyde used and appeared in their ordinary positions.

Compound A1: 2-(4-chlorophenyl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A1 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 7.78 (s, 1H, N3), 7.28-7.32 (m, 4H, aromatic), 5.35 (d, 1H, C1), 3.94 (dt, 1H, C4), 3.23 (d, 2H, C5). **The ^{13}C-NMR of A1 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.36 (C7), 140.99 (C2), 134.11 (C12), 129.22 (C11,13), 128.89 (C10,14), 68.71 (C1), 65.76 (C4), 35.39 (C5).

Compound A2: 2-(4-nitrophenyl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A2 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 8.17 (s, 1H, N3), 7.67-8.05 (m, 4H, aromatic), 5.33 (t, 1H, C1), 3.90 (s, 1H, C4), 3.31 (dd, 2H, C5). **The ^{13}C-NMR of A2 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.36 (C7), 147.55 (C12), 146.20 (C2), 129.04 (C10,14), 124.23 (C11,13), 68.95 (C1), 65.78 (C4), 35.38 (C5).

Compound A3: 2-(4-methoxyphenyl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A3 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 7.80 (s, 1H, N3), 6.28-7.30 (m, 4H, aromatic), 5.33 (d, 1H, C1), 3.94 (dt, 1H, C4), 3.78 (s, 3H, C16), 3.23 (d, 2H, C5). **The ^{13}C-NMR of A3 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.36 (C7), 159.39 (C12), 136.82 (C2), 128.61 (C10,14), 114.35 (C11,13), 69.00 (C1), 65.75 (C4), 55.32 (C16), 35.39 (C5).

Compound A4: (E)-2-(4-(dimethylamino) styryl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A4 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 7.85 (s, 1H, N3), 6.71-7.33 (m, 4H, aromatic), 6.58 (m, 1H, C10), 6.09 (dd, 1H, C2), 4.79 (dd, 1H, C1), 3.90 (d, 1H, C4), 3.30 (dd, 2H, C5) 3.01 (s, 6H, C18 & 19). **The ^{13}C-NMR of A4 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.52 (C7), 151.29 (C14), 130.46 (C10), 128.12 (C12,16), 127.84 (C11), 123.95 (C2), 111.61 (C13,15), 65.85 (C1), 64.16 (C4), 40.10 (C18,19), 34.32 (C5).

Compound A5: 2-(naphthalen-1-yl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A5 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 7.74 (s, 1H, N3), 7.59-8.21 (m, 7H, aromatic), 4.10 (s, 1H, C1), 4.28 (t, 1H, C4), 3.33 (dd, 2H, C5). **The ^{13}C-NMR of A5 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.36 (C7), 135.69 (C2), 134.07 (C14), 134.02 (C13), 128.93 (C15), 127.80 (C12), 126.82 (C10), 126.76 (C16), 126.53 (C11), 126.38 (C17), 125.46 (C18), 68.13 (C1), 65.64 (C4), 35.33 (C5).

Compound A6: 2-(3-chlorophenyl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A6 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 8.52 (s, 1H, N3), 7.42-7.49 (m, 4H, aromatic), 5.15 (s, 1H, C1), 4.14 (m, 1H, C4), 3.33 (dd, 2H, C5). **The ^{13}C-NMR of A6 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.36 (C7), 142.82 (C2), 134.21 (C13), 129.88 (C11), 128.67 (C12), 128.11 (C14), 126.99 (C10), 68.79 (C1), 65.69 (C4), 35.38 (C5).

Compound A7: 2-(o-tolyl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A7 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 8.30 (s, 1H, N3), 7.17-7.29 (m, 4H, aromatic), 5.50 (d, 1H, C1), 3.99 (dt, 1H, C4), 3.22 (d, 2H, C5) 2.46 (s, 3H, C15). **The ^{13}C-NMR of A7 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.36 (C7), 140.58 (C2), 138.04 (C14), 130.09 (C13), 127.63 (C10), 127.39 (C12), 126.91 (C11), 67.58 (C1), 65.78 (C4), 35.32 (C5), 19.37 (C15).

Compound A8: 2-(2-hydroxynaphthalen-1-yl)thiazolidine-4-carboxylic acid. **The ^{1}H-NMR of A8 (δ, ppm) (DMSO-*d*6) showed the chemical shift for the following protons:** 9.31 (s, 1H, O3), 7.68 (s, 1H, N3), 6.99-7.50 (m, 6H, aromatic), 5.83 (d, 1H, C1), 4.01 (d, 1H, C4), 3.25 (dd, 2H, C5). **The ^{13}C-NMR of A8 (δ, ppm) (DMSO-*d*6) reported the chemical shift for the following carbons:** 173.36 (C7), 156.37 (C10), 134.51 (C14), 129.47 (C13), 129.21 (C12), 129.16 (C15), 127.23 (C17), 124.95 (C16), 124.39 (C18), 117.62 (C2), 115.02 (C11), 68.53 (C1), 65.57 (C4), 35.28 (C5).

BIOLOGICAL STUDY RESULTS

Minimum inhibitory concentration (MIC) was determined after the detection of β-lactamases in the pathogenic bacteria, a broth microdilution method was used to evaluate the MIC, A serial of 10 doubling dilution of the synthesized compounds and standard antibacterial Amoxiclave (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 (4).

Table (4): MIC results for the synthesized compounds and standard antibacterial agents.

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	A3	2000	1000	1000	1000
Amoxicillin	2000	4000	4000	4000	A4	>2000	>2000	>2000	>2000
Cefotaxime	7.8	125	3.9	3.9	A5	>2000	>2000	>2000	>2000
Ceftriaxone	31.25	125	3.9	7.8	A6	1000	1000	1000	1000
Ciprofloxacin	< 7.812	< 7.812	< 7.812	< 7.812	A7	>2000	>2000	>2000	>2000
A1	2000	1000	1000	2000	A8	>2000	>2000	>2000	>2000
A2	>2000	>2000	>2000	>2000					

The MIC results showed that few of them had an antibacterial activity with a low sensitivity level (high MIC) as compared with the slandered antibacterial agents. A6 and with less extend A1 and A3 showed the highest antibacterial activity (low MIC) for all bacterial strains, this is may be caused by the presence of Cl group, as it helps in increasing antibacterial activity or it is important for this activity.[16]

The anti β-lactamase activity was evaluated by measuring the zones of inhibition in the disk diffusion method.[12] 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.

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 (5).

Table 5: Inhibition zones for Amoxiclave and Amoxicillin against Gram-positive and Gram-negative pathogenic bacteria.

Compounds No.	Inhibition zone diameter (mm)											
	Gram(+)ve			Gram (-)ve								
	Staph. aureus			*E. coli*			*K. pneumonia*			*P. aeroginosa*		
	3000 µg/ml	2000 µg/ml	1000 µg/ml	3000 µg/ml	2000 µg/ml	1000 µg/ml	3000 µg/ml	2000 µg/ml	1000 µg/ml	3000 µg/ml	2000 µg/ml	1000 µg/ml
Amoxiclav	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 concentration in the next step, 1000µg/ml for Gram (+)ve bacteria and 2000µg/ml for Gram (-)ve bacteria table (6).

Table (6): 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 (-)ve							Gram(+)ve		Gram (-)ve					
	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*
A1	28	30	0	0	0	0	0	0	A5	28	30	28	32	0	0	0	0
A2	28	30	0	0	0	0	0	0	A6	28	30	0	0	0	0	0	0
A3	28	30	0	0	0	0	0	0	A7	28	30	26	33	0	0	0	0
A4	28	30	0	0	0	0	0	0	A8	28	30	28	32	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*, and the only anti β-lactamase activities have appeared against *E. coli* species.

Concerning the anti β-lactamase activities against *E. coli* bacteria, the 3 synthesized compounds A5, A7 and A8 showed strong anti β-lactamase activities resembling or more than that of clavulanic acid, although all of them have no antibacterial activities. All 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.[15]

CONCLUSION

We can conclude that the β-lactamase TEM-1 active pocket prefers hydrophobic substituents, as the 3 synthesized active anti β-lactamase compounds all 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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