Sys Rev Pharm 2020; 11(2): 487 – 494
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Synthesis, Characterization and Antibacterial Evaluation of Oxoazetidin – Benzene Sulfonamide Derivatives as a Hybrid Antimicrobial Agents

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Article History: *Submitted: 30.12.2019* *Revised: 18.01.2020* *Accepted: 13.02.2020*

ABSTRACT
Purpose: To preparation of oxoazetidine-benzene sulfonamide conjugates that have the beta lactam and sulfonamide moieties as a possible hybrid antibacterial agent
Method: Eight derivatives of oxoazetidine-benzene sulfonamide have been synthesized by coupling the benzene or toluene with chlorosulfonic acid, and then treated with hydrazine to get hydrazide derivatives. Hydrazide derivatives, then, treated with benzaldehyde derivative to form Schif base intermediate, which will be cyclized with chloroacetyl chloride to obtain the target compounds. The chemical structures had been identified by ^{1}H-NMR, FT-IR and Elemental Analysis and evaluation of their antibacterial activity against Gram-positive, Gram-negative and penicillin-resistant bacteria obtained from clinical specimens using disc diffusion method.
Results: Of these eight compounds, compound 4b4 showed good activity against all types of tested bacteria, compound 4a4 also had a good action against all species tested. Compounds 4a1, 4b1, 4a2, 4b2 showed a greater activity against Gram-negative bacteria as compared to amoxicillin.
Conclusion: The prepared compounds have a potential antibacterial activity, mainly against Gram negative species.
Keywords: Heterocyclic compounds, antibacterial, beta lactams, Oxoazetidin, benzene sulfonamide
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DOI: 10.5530/srp.2020.2.74

INTRODUCTION
An antibiotic is a sort of antimicrobial agent that is active against bacteria and is the most significant form of antibacterial agent for bacterial infection control. In the therapy and prevention of such diseases, antibiotic medicines are commonly used. Antibiotics can either kill or deter bacteria's production. There is also a few number of antibiotics that have antiprotozoal activity. Antibiotics are not active against viruses such as common cold or influenza; so-called antiviral drugs or antivirals rather than antibiotics are drugs that inhibit viruses. [1] [2]
Beta lactams are a group of antibiotics that share a beta lactam ring in their core chemical structure. Antibiotics that have a beta lactam (2-azetidinone) heterocyclic ring include: Penicillins, Cephalosporins, Carbenems and Monobactams. Most of these beta lactam-containing antibiotics have a broad spectrum of activity against bacteria and very effective for treatment of a wide range of infections in humans. [3]
Monobactams are cell wall inhibitor betalactam antibiotics that are active against gram negative bacteria. Monobactams are β-lactam antibiotics that are monocyclic and produced by bacteria. Unlike most other β-lactams, the β-lactam loop is not linked to another ring. Monobactams only work against Gram-negative aerobic bacteria (e.g., Neisseria, Pseudomonas). Siderophore-conjugated monobactams are promising to treat multidrug-resistant pathogens. Aztreonam is a monobactam antibiotic that is commercially available. Tigemonam, nocardicin A, and tabtoxin are other types of monobactams. Skin rash and occasional unusual liver dysfunction may be considered as adverse effects of monobactams. [4]
Among the first antibacterial agents were the sulfonamides, widely known as sulfa drugs, which paved the way for newer antibiotics in the future. Antibiotics of sulfonamide skeleton inhibit essential steps in the synthesis of bacterial folic acid. Their actions against facultative gram-negative bacteria and staphylococci as considered bactericidal. They have unanticipated action against streptococci and no activity against enterococci or anaerobic species.[5]
The aim of this research lies under the umbrella of synthesizing a new antibiotic that is effective against a broad range of Gram-positive and Gram-negative bacteria as well as penicillin-resistant *Pseudomonas aeruginosa*. The target compounds to be synthesized are molecules composed from a sulfonamide skeleton and a beta lactam heterocyclic ring, both of which are known to have antibacterial activity.

Experimental
Table (1) List of Abbreviations and Chemical Names

Abbreviation	Chemical Name
1a	Benzene sulfonyl chloride
1b	p-Toluene sulfonyl chloride
2a	Benzene sulfonohydrazide
2b	p-Toluene sulfonohydrazide

3a1	N'-benzylidenebenzenesulfonohydrazide
3a2	N'-(4-chlorobenzylidene)benzenesulfonohydrazide
3a3	N'-(4-nitrobenzylidene)benzenesulfonohydrazide
3a4	N'-(4-methoxybenzylidene)benzenesulfonohydrazide
3b1	N'-benzylidene-4-methylbenzenesulfonohydrazide
3b2	N'-(4-chlorobenzylidene)-4-methylbenzenesulfonohydrazide
3b3	4-methyl-N'-(4-nitrobenzylidene)benzenesulfonohydrazide
3b4	N'-(4-methoxybenzylidene)-4-methylbenzenesulfonohydrazide
4a1	N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)benzenesulfonamide
4a2	N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)benzenesulfonamide
4a3	N-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)benzenesulfonamide
4a4	N-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)benzenesulfonamide
4b1	N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-4-methylbenzenesulfonamide
4b2	N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-4-methylbenzenesulfonamide
4b3	N-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-4-methylbenzenesulfonamide
4b4	N-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)-4-methylbenzenesulfonamide

Synthesis of Compound 1 Derivatives (Sulfonylchloride)

30 mmol of chlorosulfonic acid (3480 mg) is placed in a flask which is placed in a cool water bath at 20-25^{0}C with continuous stirring. 10 mmol of benzene (780 mg) [or toluene 920 mg] is placed in dropping funnel and the benzene is allowed to drop slowly on chlorosulfonic acid over 120 minutes. After completing benzene addition, the mixture was left stirring for 60 minutes. The mixture was poured over 200 ml of crushed ice in a separatory funnel and 150 ml of dichloromethane was added. The mixture was shaken very well and the organic layer was separated quickly and washed with sodium bicarbonate. The organic solution was left in a beaker overnight to allow the evaporation of dichloromethane. The next day, a colorless oily liquid (or white crystalline powder from toluene reaction) was left in the beaker. The white powder was recrystallized by ethanol. The yield was 77% (1355 mg) of compound 1a and 75% (1425 mg) of compound 1b.[6]

Synthesis of Compound 2 Derivatives (sulfonohydrazide)

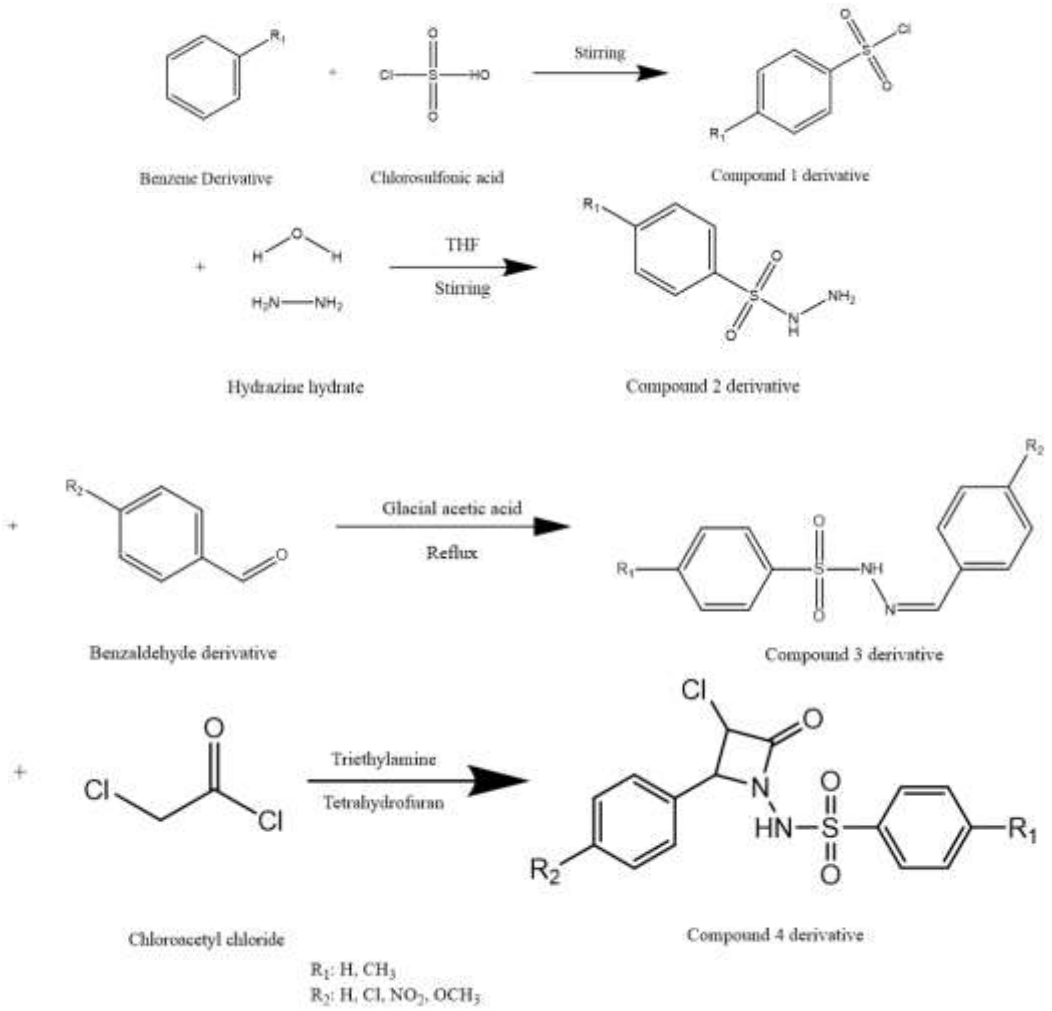
10 mmol of a compound derivative (1760 mg from 1a, 1900 mg from 1b) was dissolved in 25 ml of tetrahydrofuran and placed in a round bottom flask in a cold water bath at 10^{0}C and stirred for 15 minutes. 20 mmol of hydrazine hydrate 80% (1000 mg) in distilled water (25 ml) was placed in a dropping funnel and added to the previous mixture drop by drop over a period of 30 minutes. After completing the addition, the mixture was stirred for additional 30 minutes the transferred to a separatory funnel. The aqueous layer was discarded and the organic tetrahydrofuran layer was filtered to remove any foreign particles. The solution was placed in a round bottom flask with stirring and 50 ml of distilled water was added slowly over 10 minutes. Compound 2 derivative began to form as a white needles in the solution. The product is filtered under reduced pressure and washed with water several times and left to dry in the air. The dried while crystalline powder was recrystallized by dichloromethane. The yield was 80% for compound 2a (1375 mg) and 83% for compound 2b (1544 mg).[7]

Synthesis of Compound 3 Derivatives (Schiff Base)

10 mmol of a compound 2 derivative (1720 mg from 2a, 1860 mg from 2b) was dissolved in 30 ml of glacial acetic acid and placed in a round bottom flask with a magnetic stirring rod. 12 mmol of a benzaldehyde derivative (1272 mg benzaldehyde, 1680 mg 4-chlorobenzaldehyde, 1812 mg 4-nitrobenzaldehyde and 1632 mg 4-methoxybenzaldehyde) was added to the solution and the mixture was refluxed for 6 hours and then left overnight at room temperature. The next day, a crystalline powder was formed in the solution and collected by filtration under reduced pressure. The product was washed with sodium bicarbonate and with water then left to dry. The product was recrystallized by ethanol. The physical properties of compound 3 derivative are listed in table (2).[8]

Synthesis of Compound 4 Derivatives (Beta lactam)

10 mmol of a compound 3 derivative (3a1 2600 mg, 3a2 2940 mg, 3a3 3050 mg, 3a4 2900 mg, 3b1 2740 mg, 3b2 3080 mg, 3b3 3190 mg, 3b4 3040 mg) was dissolved in 25 ml of tetrahydrofuran and placed in a round bottom flask with a magnetic stirring bar. 20 mmol of trimethylamine (2020 mg) was added to the solution and the mixture was stirred vigorously in room temperature. 12 mmol of chloroacetyl chloride (1344 mg) was placed in a dropping funnel and left to drop to the stirring mixture slowly over 30 minutes. After completion of addition, the mixture was refluxed for 90 minutes then poured on crushed ice in a beaker. A white precipitate was formed and isolated by filtration under reduced pressure. The powder was washed with water and allowed to dry in the air the recrystallized by ethanol. The physical properties of compound 4 derivatives are listed in table (2).[9]



Scheme 1: General Reaction of synthesis

Table (2) Physical Properties of Prepared Compounds

Compound	Chemical Formula	Description	Molecular weight g/mol	Yield	Melting Point
1a	$C_6H_5ClO_2S$	Oily colorless liquid	176.61	77%	Liquid
1b	$C_7H_7ClO_2S$	White crystalline powder	190.64	75%	70-71^{0}C
2a	$C_6H_8N_2O_2S$	White crystalline powder	172.21	80%	101-103^{0}C
2b	$C_7H_{10}N_2O_2S$	White crystalline powder	186.23	83%	109-110^{0}C
3a1	$C_{13}H_{12}N_2O_2S$	White crystalline powder	260.31	81%	144-145^{0}C
3a2	$C_{13}H_{11}ClN_2O_2S$	Pale yellow crystalline powder	294.75	80%	155-157^{0}C
3a3	$C_{13}H_{11}N_3O_4S$	Light orange crystalline powder	305.31	76%	191-192^{0}C
3a4	$C_{14}H_{14}N_2O_3S$	Off-white crystalline powder	290.34	85%	159-160^{0}C
3b1	$C_{14}H_{14}N_2O_2S$	Yellowish-white crystalline powder	274.34	79%	156-157^{0}C
3b2	$C_{14}H_{13}ClN_2O_2S$	Yellow crystalline powder	308.78	72%	166-168^{0}C
3b3	$C_{14}H_{13}N_3O_4S$	Dark yellow crystalline powder	319.34	80%	197-198^{0}C
3b4	$C_{15}H_{16}N_2O_3S$	Light yellow crystalline powder	304.36	78%	169-171^{0}C
4a1	$C_{15}H_{13}ClN_2O_3S$	White crystalline powder	336.79	40%	209-201^{0}C
4a2	$C_{15}H_{12}Cl_2N_2O_3S$	Off-white crystalline powder	371.23	35%	215-216^{0}C
4a3	$C_{15}H_{12}ClN_3O_5S$	Pale white crystalline powder	381.79	35%	246-248^{0}C
4a4	$C_{16}H_{15}ClN_2O_4S$	White crystalline powder	366.82	45%	219-220^{0}C
4b1	$C_{16}H_{15}ClN_2O_3S$	White crystalline powder	350.82	40%	220-221^{0}C
4b2	$C_{16}H_{14}Cl_2N_2O_3S$	Pale white crystalline powder	385.26	38%	224-226^{0}C
4b3	$C_{16}H_{14}ClN_3O_5S$	White crystalline powder	395.81	35%	257-259^{0}C
4b4	$C_{17}H_{17}ClN_2O_4S$	White crystalline powder	380.84	45%	233-235^{0}C

Antimicrobial Disc Diffusion Test Procedure

Antibacterial activity evaluation of the synthesized compound 4 derivatives was performed to screen the effectiveness of these new compounds as antibacterials and to compare their activity with the activity of standard antibiotics. The in-vitro evaluation process was carried out by the disc diffusion method.

The antibacterial activity was screened against 4 species of bacteria obtained from clinical samples. The tested bacteria species include the Gram-positive *Staphylococcus aureus*, the Gram-negative Enterobacterecea species *Escherichia coli* and *Klepsiella pneumonia* along with the penicillin resistant *Pseudomonas aeruginosa*.

Amoxicillin and cefotaxime were used as standard antibiotics.

Solutions of 3 different concentrations from each derivative of compound 4 were prepared along with solutions of the same concentrations from the standard antibiotics were also prepared. These concentrations were 125 ug/ml, 250 ug/ml and 500 ug/ml.

6 mm in diameter-measuring discs from filter paper were prepared and sterilized then added to each of the prepared solution of the compound and standards and left soaked for 2 hours.

Petri dishes containing Muller-Hinton agar were prepared and streaked with the bacteria need for the test using a sterile swab. Then, the previously prepared discs were placed on the Petri dishes at equal distances followed by transferring the dishes to the incubator where they were left incubated for 24 hours at 37^0C.

After the incubation period, the dishes were examined and the clear inhibition zones on the surface of the agar were recorded so as to evaluate the antibacterial activity of the synthesized compound 4 derivatives and to compare it with those of the standard antibiotics.

RESULTS AND DISCUSSION

FT-IR analysis result of compound 1 derivatives showed a clear and sharp absorbance band at 3090 cm^{-1} for the stretching of aromatic C-H bond, clear absorbance bands at 1455 cm^{-1} and 1480 cm^{-1} for the aromatic C=C bond stretching, a clear band at 2915 cm^{-1} for the aliphatic C-H bond of the toluene and a sharp absorbance band for the S=O bond stretching at 1390 cm^{-1}.

Regarding compound 2 derivatives, there are 3 sharp absorbance bands at 3387 cm^{-1}, 3313 cm^{-1} and 3248 cm^{-1} which are a characteristic feature of N-H bonds stretching. These bands are an indication on the conversion of compound 1 to compound 2 after reaction with hydrazine.

All of the compound 3 derivatives show the characteristic absorbance of C=N bond stretching at 1605-1611 cm-1 which is an indication on the formation of the Schiff bases. Absorbance of N-H bond stretching appeared as a single band at nearly 3300 cm^{-1} only while it showed up as 3 bands in the spectra of compound 2 derivatives, which is another indication of the Schiff base formation.

Absorbance bands of the aromatic rings bonds stretching appear clearly in the FT-IR spectra as shown in table (3-2) along with the absorbance bands of the aliphatic C-H bond stretching and S=O bond stretching which are appearing clearly on the analysis spectra.

Compounds 3a3 and 3b3 contain a nitro group substituent and the absorbance spectra showed bands for N-O bond stretching at 1540 cm^{-1} and 1533 cm-1 respectively.

C-O stretching band (aryl alkyl ether) appeared in the absorbance spectra of compound 3a4 at 1255 cm^{-1} and at 1265 cm^{-1} for compound 3b4 as these 2 compounds have a methoxy group substituent.

The most notable criteria in the absorbance spectra of compound 4 derivatives is the disappearance of N=C bond stretching absorbance bands as compared to compound 3 derivatives spectra and the appearance of a clear and sharp band at the range of nearly 1670 cm^{-1} which is attributed to the stretching of C=O band of the cyclic amide (lactam). These changes are a clear indicator on the transformation of the Schiff bases to beta lactam derivatives after reacting with chloroacetyl chloride.[10-12]

^{1}H-NMR analysis result for compound 4 derivatives showed common characteristic features that further confirmed their structure including a signal around 8.00 ppm which is attributed to the proton attached to the Nitrogen atom, this signal is a singlet.

There are 2 clear doublet signals around 5.00 ppm that are attributed to the protons attached to the aliphatic carbons in the lactam ring. These two signals showed up as doublets because each proton had coupled with the other on the adjacent carbon atom.

Protons of the aromatic rings have a group of signals in the shape of multiplet appearing at the range from 7.00 ppm to 7.80 ppm.

Protons on the carbon atom of the methyl group in the toluene ring reflected a sharp signal in the shape of a singlet around 2.50 ppm as these protons are magnetically equivalent. [13]

Elemental Microanalysis for the final compounds was performed to confirm their structures. The results displayed in Table (3) show acceptable observed percentages compared to the calculated percentages. The deviations of the observed values from the calculated values are found to be consistent with an accurate structures of the synthesized compounds.

Table (3) Elemental Analysis of Final Compounds

Compound	Chemical Formula	Molecular Weight		C	H	N	S
4a1	$C_{15}H_{13}ClN_2O_3S$	336.79	Observed	53.31	3.92	8.35	9.60
			Calculated	53.49	3.89	8.32	9.52

4a2	$C_{15}H_{12}Cl_2N_2O_3S$	371.23	Observed	49.10	2.83	7.11	9.00
			Calculated	48.53	3.26	7.55	8.64
4a3	$C_{15}H_{12}ClN_3O_5S$	381.79	Observed	46.70	3.90	10.75	8.30
			Calculated	47.19	3.17	11.01	8.40
4a4	$C_{16}H_{15}ClN_2O_4S$	366.82	Observed	53.10	4.22	7.50	8.12
			Calculated	52.39	4.12	7.64	8.74
4b1	$C_{16}H_{15}ClN_2O_3S$	350.82	Observed	54.12	4.89	7.10	9.25
			Calculated	54.78	4.31	7.99	9.14
4b2	$C_{16}H_{14}Cl_2N_2O_3S$	385.26	Observed	50.17	4.09	7.74	8.45
			Calculated	49.88	3.66	7.27	8.32
4b3	$C_{16}H_{14}ClN_3O_5S$	395.81	Observed	48.40	3.60	11.30	8.15
			Calculated	48.55	3.57	10.62	8.10
4b4	$C_{17}H_{17}ClN_2O_4S$	380.84	Observed	53.55	4.23	8.26	8.66
			Calculated	53.61	4.50	7.36	8.42

Evaluation of antibacterial activity of the synthesized final compounds was performed using disc diffusion method. Cefotaxime and amoxicillin antibiotic discs were used as standards. The activity was evaluated against four types of bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Klepsiella pneumonia* and the penicillin-resistant *Pseudomonas aeruginosa*. These bacteria were obtained from clinical samples.

The evaluated compounds showed a variety of inhibition zones on the culture media with the different types of bacteria. The evaluation results are listed in table (4).

The compounds 4a4 and 4b4, as compared to the other derivatives, showed the largest zones of inhibition and the greatest antibacterial activity across all the concentrations range.

Compounds 4a3 and 4b3 had almost no activity against all types of bacteria tested in all concentrations except for a slight activity on *E. coli* and *K. pneumonia* in the highest concentration of 500 ug/ml only.

The Gram-Positive *S. aureus* bacteria seemed to be not affected by the majority of compound 4 derivatives except for compounds 4a4 and 4b4 in 250 ug/ml and 500 ug/ml concentrations and only slightly affected by the highest concentrations of compounds 4a2 and 4b2.

Compounds 4a1 and 4b1 showed a good activity against gram-negative *E. coli* and *K. pneumonia* bacteria in all concentrations. They are also active against the penicillin-resistant *P. aeruginosa* in high concentrations. They had no effect against gram-positive *S. aureus*.

Antibacterial activity against *E. coli*, *K. pneumonia* and *P. aeruginosa* was increased with compounds 4a2 and 4b2 as compared to the compounds 4a1 and 4b1. Only the highest concentrations of compounds 4a2 and 4b2 showed a slight effect on the gram-positive *S.aureus*.

The least effective derivatives of compound 4 are 4a3 which is only slightly effective against gram-negative bacteria in the highest concentration of 500 ug/ml and 4b3 that had effect on gram-negative and *P. aeruginosa* with the 500 ug/ml concentration only.

From the evaluation data listed in table (4), the effective compounds show a concentration-dependent inhibition fashion, which means as the concentration increases, the larger the inhibition zone would be.

Table (4) Antimicrobial Activity of Various Concentrations of Final Compounds

Compound	Concentration ug/ml	Inhibition Zone Diameter mm			
		S. aureus	*E. coli*	*K. pneumonia*	*P. aeruginosa*
	125	0	0	0	0
Cefotaxime	250	8	5	6	4
	500	13	12	10	11
	125	0	0	0	0
Amoxicillin	250	0	0	0	0
	500	5	0	0	0
	125	0	4	0	0
4a1	250	0	7	0	4
	500	0	11	6	7
	125	0	0	0	0
4a2	250	0	5	5	4
	500	5	11	10	9
	125	0	0	0	0
4a3	250	0	0	0	0
	500	0	5	4	0
	125	0	4	4	0
4a4	250	0	8	9	7
	500	6	13	12	12

	125	0	4	0	0
4b1	250	0	7	5	4
	500	0	13	11	8
	125	0	0	0	0
4b2	250	0	6	4	6
	500	5	12	11	10
	125	0	0	0	0
4b3	250	0	0	0	0
	500	0	5	5	4
	125	0	0	0	0
4b4	250	4	6	5	6
	500	9	11	11	14

All the derivative of compound 4 share the same core chemical structure and differ in the substituted groups on the aromatic rings. Thus, the difference in the antibacterial activity seemed to be reliant on the type of substituents.

Lipid solubility is an important factor affecting the activity of antibiotics. The partition coefficient is used to express the lipid solubility of the compounds. Partition coefficient (P) is defined as the tendency of a nuetral substance to distribute between organic and aqueous phases when placed in organic-aqueous biphasic solution.[14] Log P is an important physical property of an antibacterial compound because it influences the permeability of the compound through the bacterial cell wall and the bioavailability so as to exert its action. The higher the value of Log P, the higher lipophilicity and antibacterial activity of a compound.[15, 16]

Compounds 4a2 and 4b2, with para chloro substituents, have greater Log P values than the unsubstituted compound 4a1 and 4b1. This is reflected on their higher antibacterial effect of compound 4a2 and 4b2 as compared to 4a1 and 4b1. Clog P values for the synthesized compounds are shown in table (5).

Table (5) Clog P for Compound 4 Derivatives

Compound	CLogP
4a1	2.37
4a2	3.08
4a3	2.11
4a4	2.30
4b1	2.87
4b2	3.58
4b3	2.61
4b4	2.80

Table (6) Spectral Data of Prepared Compounds

Compound	Chemical Formula	FT-IR	^{1}H-NMR	Melting Point
1a	$C_6H_5ClO_2S$	3090, 1455, 1480, 1390	-	Liquid
1b	$C_7H_7ClO_2S$	3084, 2915, 1495, 1380	-	70-71^{0}C
2a	$C_6H_8N_2O_2S$	3387, 3313, 3248, 3070, 1431, 1450, 1477, 1311	-	101-103^{0}C
2b	$C_7H_{10}N_2O_2S$	3391 3322 3262 3081 2915 1435 1457 1475 1315	-	109-110^{0}C
3a1	$C_{13}H_{12}N_2O_2S$	3325 3083 1416 1421 1439 1311 1605	-	144-145^{0}C
3a2	$C_{13}H_{11}ClN_2O_2S$	3336 3089 1415 1421 1433 1310 1605	-	155-157^{0}C
3a3	$C_{13}H_{11}N_3O_4S$	3343 3070 - 1411 1417 1428 1313 1607 - 1540	-	191-192^{0}C
3a4	$C_{14}H_{14}N_2O_3S$	3330 3081 3015 1415 1422 1435 1315 1610 1255	-	159-160^{0}C

3b1	$C_{14}H_{14}N_2O_2S$	3332 1423 1430	3075 1314	2930 1605	1416	-	156-157^{0}C
3b2	$C_{14}H_{13}ClN_2O_2S$	3335 1420 1431	3080 1311	2920 1605	1413	-	166-168^{0}C
3b3	$C_{14}H_{13}N_3O_4S$	3328 1422 1433	3077 1315 1533	2915 1608	1415	-	197-198^{0}C
3b4	$C_{15}H_{16}N_2O_3S$	3334 2913 1425 1436	3085 1418 1310	3014 1611	 1265	-	169-171^{0}C
4a1	$C_{15}H_{13}ClN_2O_3S$	3300 1430 1437	3075 1315	2910 1677	1410	7.25 – 7.74 (m, 10H), 8.01 (s,1H), 5.49 (d, 1H), 5.10 (d, 1H)	209-201^{0}C
4a2	$C_{15}H_{12}Cl_2N_2O_3S$	3295 1426 1436	3065 1317	2913 1675	1411	7.47 – 7.82 (m, 9H), 8.09 (s,1H), 5.48 (d, 1H), 5.09 (d, 1H)	215-216^{0}C
4a3	$C_{15}H_{12}ClN_3O_5S$	3345 1425 1434	3100 1320 1535	2920 1675	1416	7.57 – 8.15 (m, 9H), 8.02 (s,1H), 5.41 (d, 1H), 5.04 (d, 1H)	246-248^{0}C
4a4	$C_{16}H_{15}ClN_2O_4S$	3335 3035 2925 1425 1430	3060 1417 1320	3016 1670	 1236	6.85 – 7.89 (m, 9H), 8.01 (s,1H), 5.47 (d, 1H), 5.10 (d, 1H), 3.79 (s, 3H)	219-220^{0}C
4b1	$C_{16}H_{15}ClN_2O_3S$	3325 2915 1428 1439	3080 1419 1325	3023 1673	 	7.20 – 7.64 (m, 9H), 8.03 (s,1H), 5.45 (d, 1H), 5.06 (d, 1H), 2.66 (s, 3H)	220-221^{0}C
4b2	$C_{16}H_{14}Cl_2N_2O_3S$	3344 2925 1427 1438	3070 1415 1312	3032 1677	 	7.32 – 7.64 (m, 8H), 8.08 (s,1H), 5.43 (d, 1H), 5.10 (d, 1H), 2.65 (s, 3H)	224-226^{0}C
4b3	$C_{16}H_{14}ClN_3O_5S$	3290 2911 1425 1435	3071 1411 1318 1546	3037 1675	 	7.33 – 8.21 (m, 8H), 8.02 (s,1H), 5.45 (d, 1H), 5.01 (d, 1H), 2.66 (s, 3H)	257-259^{0}C
4b4	$C_{17}H_{17}ClN_2O_4S$	3310 3019 2913 1427 1436	3095 1414 1315	3334 1672	 1274	6.81 – 7.69 (m, 8H), 8.02 (s,1H), 5.51 (d, 1H), 5.11 (d, 1H), 2.65 (s, 3H), 3.80 (s, 3H)	233-235^{0}C

Electronic configuration is very influential on the activity of an antibacterial agent. Electron-donating groups, like methoxy group, enhance the antibacterial effect of the compound, whilst the electron-withdrawing groups, like nitro and chloro, tend to decrease the antibacterial effect.[17, 18]

Para-methoxy substituted compound 4a4 and 4b4 show larger zones of inhibition on the culture media than those of the unsubstituted compounds 4a1 and 4b1 due to the enhanced action with the presence of para-methoxy electron-donating groups.

Nitro groups on the para positions of compound 4a3 and 4b3 greatly reduced their antibacterial effect due to the electron-withdrawing nature of the nitro substituents.

Steric hindrance or the bulkiness of the substituted groups on a beta lactam antibiotic is an important factor to increase the resistance to penicillinase enzyme of the bacteria. This indicates that the bulky substituents on a

compound boost its effectiveness against penicillin-resistant bacteria.[19]

Compounds 4a4 and 4b4 with para-methoxy bulky group show greater antibacterial effect than the unsubstituted 4a1 and 4b1 especially on the penicillin-resistant *P. aeruginosa*.

Methoxy groups on para positions of compound 4a4 and 4b4 made them very effective of all the other derivatives as these two particular derivatives have good lipophilicity and Log P values along with benefits of the electron-donating nature and the bulkiness of the methoxy groups that enhance both the antibacterial activity and penicillinase resistance respectively.

The presence of methyl group in 4b derivatives of compound 4 has only a slight increase in the antibacterial activity as compared to their 4a counterparts as seen from the data in table (4).

Betal actam compounds with unfused rings tend to have no antibacterial activity against gram-positive bacteria but have a good activity against gram-negative bacteria and penicillin-resistant bacteria as these compounds are stable against bacterial penicillinase enzyme.[20]

Derivatives of compound 4 share the same unfused beta lactam ring and for that they demonstrate no or very weak effect on gram-positive *S. aureus* and very good activity on gram-negative and penicillin-resistant bacteria.

Sulphanilamide skeleton has an antibacterial activity.[21] The presence of this skeleton as a part of compound 4 derivatives has a contribution to their antibacterial actions as they displayed a stronger action as compared to the standard antibiotics.

CONCLUSION

The prepared eight compounds had a good activity against the tested bacterial species with some of them showed a significant action that is greater than that of standard antibiotics in the test as indicated by the inhibition zones on the test media.

ACKNOWLEDGEMENT

Appreciation to the Department of Pharmaceutical Chemistry / College of Pharmacy / University of Basra for the support and guidance.

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