Synthesis, Characterization of New Azetidinone Derivatives and Evaluation of Their Antimicrobial Activity

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Abstract

In the present study new azetidinone derivatives containing sulfa drug moiety have been prepared by cyclocondensation of the Schiff bases derived from sulfa drugs with chloroacetyl chloride in the presence of triethylamine. The Schiff bases are prepared by the condensation reaction of the sulfa drug (sulfadiazine and sulfanilamide) with the 5-nitro-2-furancaroboxyaldehyde. The structure of the azetidinons were confirmed by elemental analysis (C. H. N.) and FT-IR, ^{1}H-NMR spectroscopy. The compounds were screened for their antimicrobial activity against Staphylococcus aureus, Escherichia coli, Aspergilus niger and Aspergilus flavus. The compounds exhibited good antimicrobial activity in comparison with standard drugs.

Keywords: azetidinone, Schiff base, β-lactam, sulfa drugs and antimicrobial activity.

تحضير وتشخيص مشتقات جديدة لمركبات الازتايدينون ودراسة فعاليتها البايولوجية

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الملخص

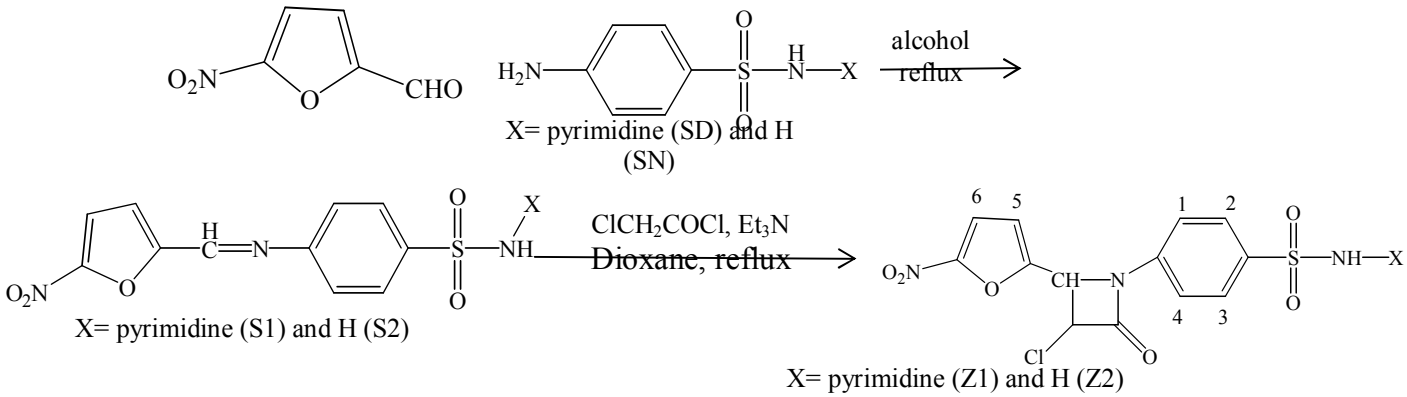
تم في هذه الدراسة تحضير اثنين من المشتقات الجديدة لمركبات ال β-لاكتام من تفاعل قواعد شيف والمحضرة ايضا في هذه الدراسة مع الكلورو اسيتايل كلورايد. حضرت قواعد شف من تفاعل 5-نايترو فورفورال مع ادوية السلفا. تم تشخيص المركبات المحضرة باستخدام التحليل العنصري الدقيق بالاضافة لمطيافيتي الاشعة تحت الحمراء والرنين النووي المغناطيسي. تم ايضا دراسة الفعالية البويولوجية للمشتقات المحضرة ضد نوعين من البكتريا ونوعين من الفطريات. اظهرت النتائج بان المركبات المحضرة ذات فعالية جيدة مضادة للبكتريا والفطريات مقارنة بالمركبات الدوائية القياسية المستخدمة في البحث.

1. Introduction

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the increasing antimicrobial resistance emergence and its dissemination among bacterial strains reduced the efficiency of treatment success of large amount of drugs. To overcome, this alarming problem, the discovery of novel active compounds is a matter urgency. Many compounds containing the β-lactam ring possess various interesting biological activities[1]. The synthesis of 2-azetidinone continues to be a very active research area because of the importance of this structural unit in penicillin and related antibiotics[2]. Azetidinones which are part of antibiotics structure are known to exhibit interesting biological activities[3, 4]. A large number of 3-chloro monocyclic β-lactam possesses powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant and anti-tubercular activities. They also function as enzyme inhibitors and are effective on the central nervous system (CNS)[5-7]. The present work is oriented toward synthesis of some azetidinones by cycloaddition of α-chloroacetyl chloride with the Schiff bases derived from sulfa drugs which result in formation of 2-azetidinone ring (β-lactam). The reaction is carried out with the base triethylamine gives β-lactam.

2. Materials and methods

Melting points were determined in an open capillary tubes on Gallenkamp apparatus. Elemental analyses (C.H.N.) were recorded in united Kingdom/ wails university. The IR spectra were recorded in KBr discs on FT-IR 8400S SHIMADZU(Japan) in Petrochemical industry company Iraq/Basrah. ^{1}H-NMR spectra was recorded on Brucker model in United Kingdom/ Wails University using $CDCl_3$ as a solvent and TMS as internal standard. The synthesis of the title azetidinons is shown in Scheme 1:



Scheme 1: Synthesis of azetidinones Z1 and Z2

2.1 Preparation of Schiff's base of sulfadiazine, S1

A solution of sulfadiazine (0.01 mole) in 30 ml ethanol and an ethanol solution of 5-nitro-2-furancarboxyaldehyde (0.01 mole) in 30 ml with few drops of sulfuric acid was refluxed for 6.5 hr. On cooling the reaction mixture overnight, the yellow product was filtered and recrystallized from ethanol, washed with diethyl ether and then dried[8]. Name and physical properties are listed in Table 1.

2.2 Preparation of Schiff's base of sulfanilamide, S2

Equimolar quantities of sulfanilamide (0.01 mole) and 5-nitro-2-furancarboxyaldehyde (0.01 mole) were dissolved in 30 ml of methanol containing few drops of glacial acetic acid as catalyst. The reaction mixture was refluxed for4.5 hrs. The yellow precipitate was filtered and recrystallized from ethanol[9]. Name and physical properties are listed in Table 1.

2.3 Preparation of azetidinones, Z1 and Z2.

A mixture of an appropriate Schiff base (0.01 mole), S1 or S2, and triethyl amine (0.02 mole) was dissolved in dry 1,4-dioxane (25 ml). To this mixture, a solution of α-chloroacetyl chloride (0.02 mole) was added in portion wise with vigorous stirring at room temperature for 20 min. The reaction mixture was refluxed for 3 hrs and then content was kept at room temperature for two days and then poured into crushed-ice water. The solid precipitate was filtered and washed with water and recrystallized from ethanol[10]. Names and physical properties are listed in Table 1.

Table 1: The names and physical properties of the synthesized compounds

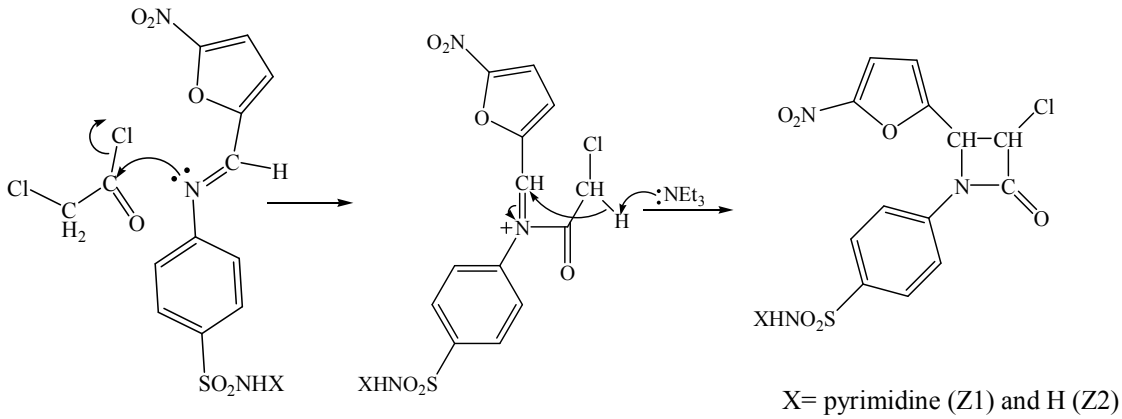
Compd.	Molecular formula	The name	m.p. (°C)	Yield %
S1	$C_{15}H_{11}N_5O_5S$	4-[(5-Nitro-furan-2-ylmethylene)-amino]-N-pyrimidin-2-yl-benzenesulfonamide	260-262	78
S2	$C_{11}H_9N_3O_5S$	4-[(5-Nitro-furan-2-ylmethylene)-amino]-benzenesulfonamide	197-199	85
Z1	$C_{17}H_{12}ClN_5O_6S$	4-[3-chloro-2-(5-nitro-furan-2-yl)-4-oxo-azetidin-1-yl]-N-pyrimidin-2-yl-benzenesulfonamide	241-243	64
Z2	$C_{13}H_{10}ClN_3O_6S$	4-[3-chloro-2-(5-nitro-furan-2-yl)-4-oxo-azetidin-1-yl]-benzenesulfonamide	223-225	76

2.4 In vitro Antimicrobial activity

Antimicrobial activity of the synthesized compounds was performed by using the disc diffusion method. The antibacterial activity was screened against two pathogens such as Escherichia coli and Staphylococcus aureus. The anifungal activity was carried out against the fungal species Aspergilus niger and Aspergilus flavus. Circular paper discs (6 mm diameter) were impregnated with the specific amount of the test sample (1000 ug/ml) DMSO and placed on Muller Hinton agar medium(antibacterial activity) and sabouroud dextrose agar medium for antifungal activity. Control experiment was carried out under similar conditions by using amoxicillin and fluconazole as standard drugs. After incubation, the plates were observed for growth inhibition zones around discs. The diameter of the zone (in mm) of inhibition is proportional to the antimicrobial activity of the compound[7, 11, 12].

3. Results and discussion

The mechanism of the synthesis of azetidinone compounds from the reaction of Schiff bases and chloroacetyl chloride in the presence triethylamine can be illustrated in Scheme 2.



X= pyrimidine (Z1) and H (Z2)

Scheme 2: The reaction mechanism of the synthesis of azetidinones

The C. H. N. S. analysis data of the compounds are presented in Table 2. The measured values are in good agreement with the calculated values.

Table 2: Elemental analysis data.

Compound	Found				Calculated			
	C%	H%	N%	S%	C%	H%	N%	S%
S1	48.57	3.01	18.98	8.44	48.26	2.97	18.76	8.59
S2	45.02	2.99	14.10	10.94	44.74	3.07	14.23	10.86
Z1	45.70	2.22	15.68	7.62	45.39	2.69	15.57	7.13
Z2	42.35	2.66	11.23	8.50	42.00	2.71	11.30	8.63

3.1 FT-IR spectra

The IR spectra for all Z1and Z2 compounds were performed by the KBr disc method. The Table 3 represents the data of the important bands of the IR spectra of Z1 and Z2 compounds. The IR data of the compounds showed bands at 3425 and 3357 cm^{-1} for Z1 and 3317cm^{-1} for Z2 which is characteristic of the N-H stretching[13,14] of these compounds, as shown in Figures 1 and 2. The two IR spectra of the compounds showed strong-medium bands at 1655-1671cm^{-1} and 1591-1606 cm^{-1} which are characteristic of the C=O of lactam ring and C=C of aromatic ring stretching, respectively[15-17]. Strong bands at 1259-1292 cm^{-1} and 1153-1251 cm^{-1} which are characteristic for C-O/C-N asymmetric and symmetric stretching, respectively. Strong absorption bands at 1543 and 1325cm^{-1} and for Z1 and 1325 and 1344 cm^{-1} for Z2 attributed to stretching vibration of N=O for the nitro group. Strong bands at 1078 and 1091cm^{-1} which are characteristic of S=O stretching of sulfonyl group of Z1 and Z2, respectively. Compound Z1 shows a strong band at 1622cm^{-1} reflected to stretching vibration of C=N of pyrimidine ring[18, 19].

Table 3: Spectral FT-IR data of synthesized compounds

Compound		Assignment
Z1	Z2	
3317 m	3425 m 3357 m	N-H stretching
3145 m 3101 m	3120 w 3090 w	C-H stretching aromatic
2947 w	2937 w 2871 w	C-H stretching aliphatic
1671 s	1680 s	C=O stretching lactam group
1622 s	-----	C=N stretching
1606 s 1496 m	1591 m 1494 s	C=C stretching of aromatic rings
1442 m	1438 m	C-H bending aliphatic
1560 s 1344 s	1543 s 1325 s	N=O stretching
1292 s	1259 s	C-O and C-N asymmetric stretching
1251 s	1153 s	C-O and C-N symmetric stretching
1078 s	1091 s	S=O stretching
962 m	941 s	C-H bending aromatic

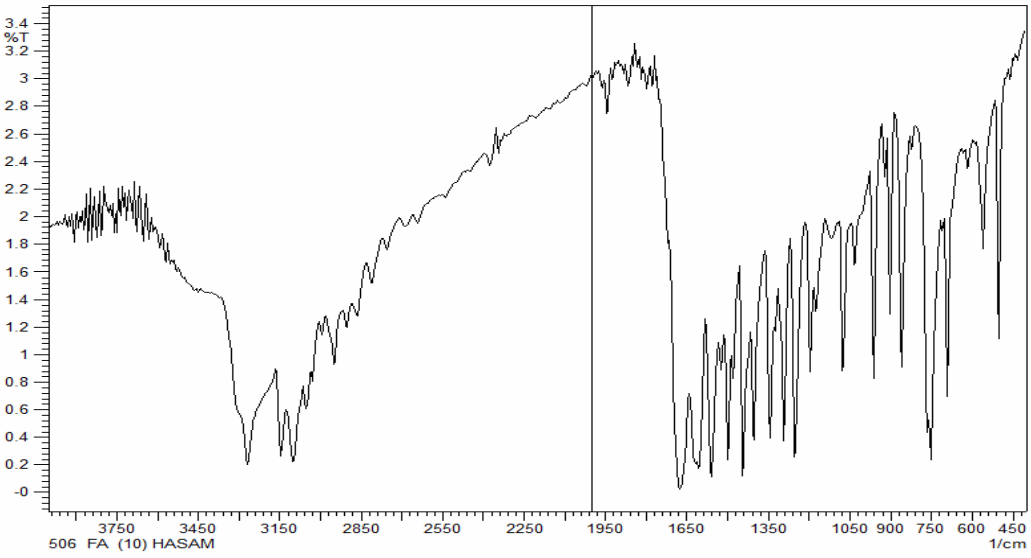


Figure 1: FT-IR spectrum of azetidinone Z1

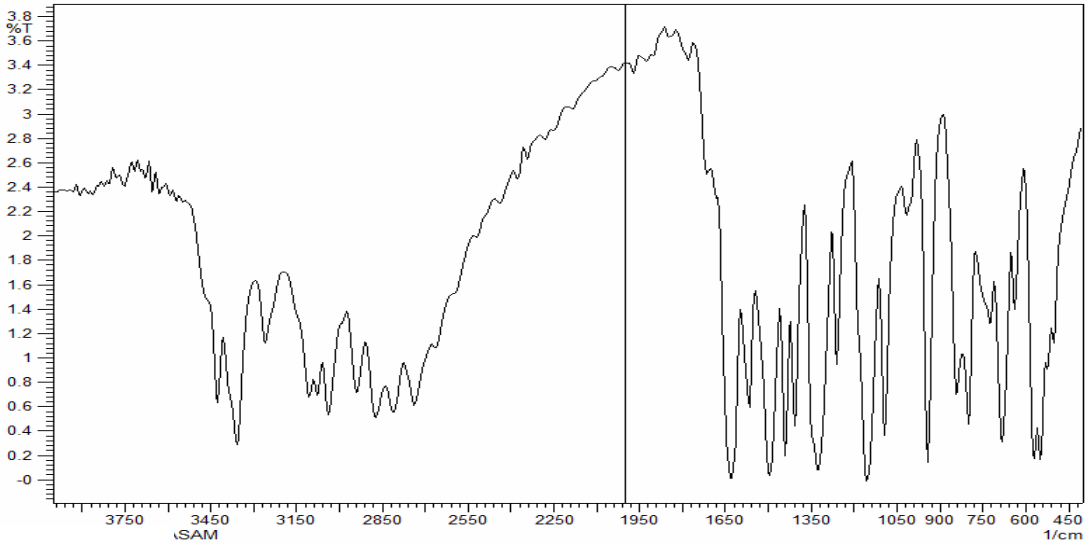


Figure 2: FT-IR spectrum of azetidinone Z2

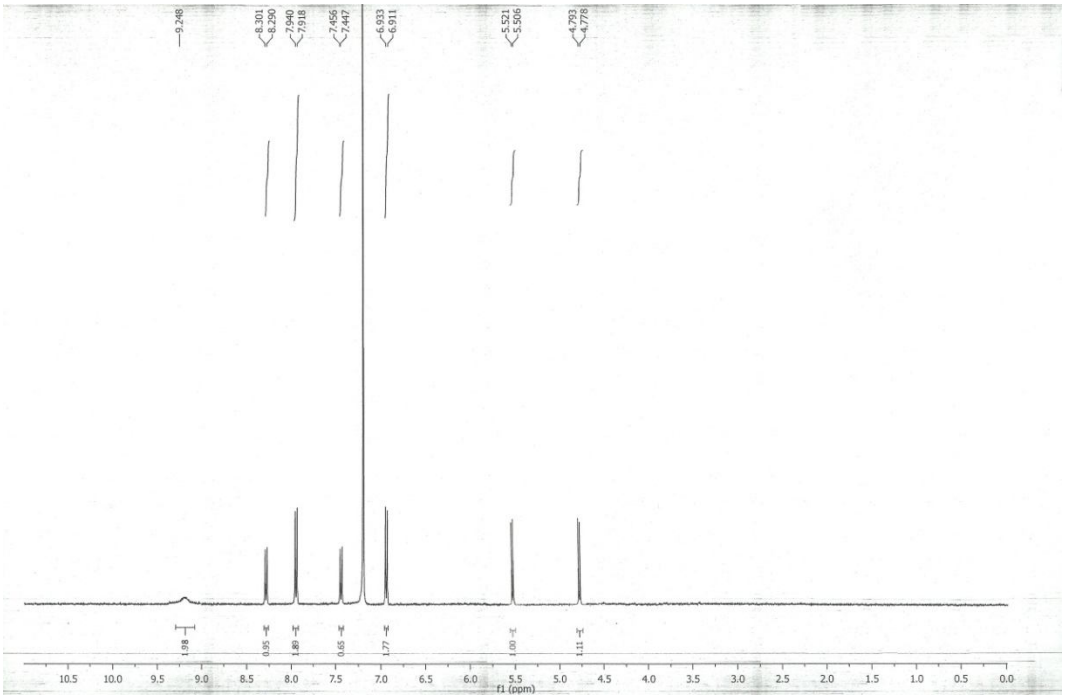
3.2 ^{1}H-NMR spectra

^{1}H-NMR spectra of the synthesized azetidinones were shown in Figures 3 and 4. Table 4 lists the chemical shifts (ppm) of protons of the compounds in $CDCl_3$. The ^{1}H-NMR spectra show signals which are characteristics of the anticipated structure of compounds. The N-CH proton signal (4.790ppm, J=5.6 Hz) and CH-Cl (4.785, J=5.6 Hz) in the NMR spectra confirmed the formation of 3-chloro-2-azetidinone nucleus[20-22]. The ^{1}H-NMR spectra of displayed broad singlet at 9.900 ppm (-NH-) and 9.248 ppm (-NH2-) for compounds Z1 and Z2, respectively[4,23]. The spectra of the two compounds show two doublet signals, the first signal for the proton H5 which appeared at 7.139 ppm($J_{5,6}$= 4.4 Hz) and 7.451 ppm ($J_{5,6}$= 3.6 Hz) for Z1 and Z2, respectively. The second signal is attributed to the proton H6 which appeared at 8.043 ppm ($J_{5,6}$= 4.4 Hz) and 8.295 ppm ($J_{5,6}$= 4.4 Hz) for Z1 and Z2, respstively. The protons of the phenyl signals of the para substituents moiety were assigned at 7.743, 7.929 ppm(H1,4) and 6.495, 6.922 ppm (H2,3) for Z1 and Z2,respectively, with J-coupling 8.8 Hz. The ^{1}H-NMR spectrum of compound Z1 showed triplet signal at 6.773 ppm and doublet signal at 8.411 ppm which can be attributed to the protons of the pyrimidine ring[24, 25].

Table 4: ^{1}H-NMR spectral data [δ (ppm), J (Hz)] of azetidinones Z1 and Z2

Symbol	CH-Cl	CH-N	H1,4	H2,3	$-NH_2$	-NH-	H5	H6	pyrimidine
Z1	5.447 d J= 5.6	4.790 d J=5.6	7.743 d $J_{1,2}$=8.8	6.495 d $J_{1,4}$=8.8	------	9.900 b	7.139 d $J_{5,6}$=4.4	8.043 d $J_{5,6}$=4.4	6.773 t 8.411d
Z2	5.513 d J= 6	4.785 d J= 6	7.929 d $J_{1,2}$=8.8	6.922 d $J_{1,2}$=8.8	9.248 b	------	7.451 d $J_{5,6}$=3.6	8.295 d $J_{5,6}$=4.4	------

Figure 3: ^{1}H-NMR spectrum of azetidinone Z1



3.3 Antimicrobial activity

The titled compounds showed promising antimicrobial activity, as shown in Figures 5 and 6, and the observed zone of inhibition was presented in Table 5. The activity studies suggest that the novel azetidinone compounds had showed good antibacterial and antifungal activity in comparison to that of the standard drugs amoxicillin, fluconazole, sulfadiazine and sulfanilamide. Compound Z1 showed higher activity as compared with Z2 and these two compounds have higher activity as compared with the synthesized Schiff bases(S1 and S2). The good activity was greatly concerned with presence β-lactam ring.

Table 5: Data of in vitro antimicrobial activity of synthesized compounds in DMSO

Figure 4: ^{1}H-NMR spectrum of azetidinone Z2

Compound	In vitro activity zone of inhibition (mm)			
	Antibacterial activity		Antifungal activity	
	S. aureus	E. coli	A. nieger	A. flavus
Z1	24	21	22	16
Z2	22	19	20	17
S1	21	18	11	11
S2	18	14	12	12
Sulfadiazine	18	14	------	------
Sulfanilamide	15	13	------	------
Amoxicillin	28	21	------	------
Fluconazole	------	------	------	------
DMSO	------	------	------	------

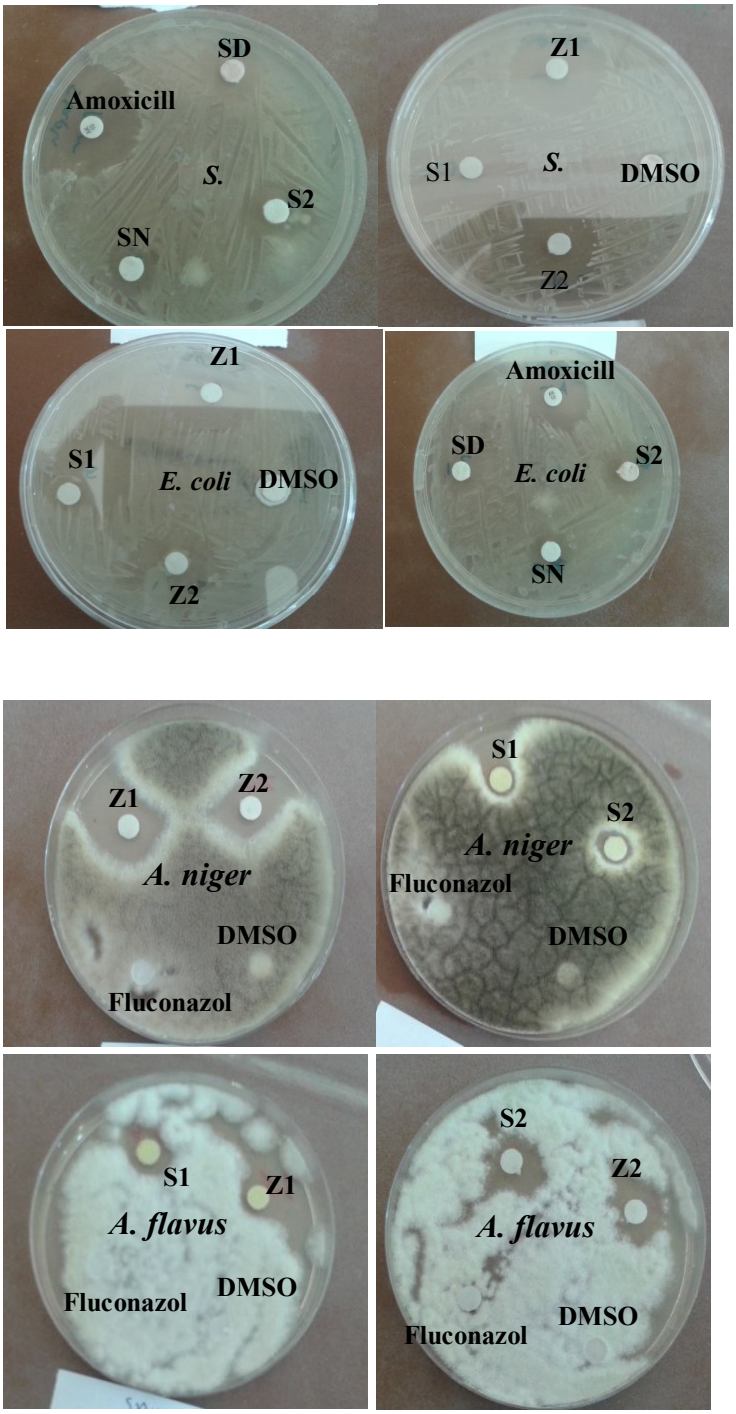


Figure 5: Antifungal activity of Synthesized compounds against S. aureus and E. coli

Figure 6: Antifungal activity of the synthesized compounds against A. niger and A. flavus

4. Conclusions

The synthesized compounds were evaluated for antibacterial activity against E. coli and S. aureus and antifungal activity against A. niger and A. flavus by using disc diffusion method. The antimicrobial activity of the newly synthesized compounds Z1 and Z2 bearing a 2-azetidinone moiety revealed that all the tested compounds showed good antibacterial and antifungal activities against the selected microbial strains.

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