AL-ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

Al-Zahraa Journal for Health and Medical Sciences 2025; 3:9-35 Vol. 3, No.2, 2025
www.zjhms.alzahraa.edu.iq

Synthesis of Novel 2-Azetidinones as Antibacterial Agents

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Received: 16 May (2025), Accepted: 14 June. 2025. Published: 30 June. 2025

ABSTRACT

Amino function of 5-(4-aminophenyl)-1,3,4-oxadiazole-2-thiol (1) has been diazotized by hydrochloric acid and sodium nitrite. The generating oxadiazolyldiazonium chloride was directly reacted with sodium phenoxide of salicylaldehyde affording azo compound (2) that containing aldehyde function. Compound (2) has been condensed with aromatic amines (o-methoxyaniline, 2,4-dimethylaniline, *p*-aminophenol, *o*-chloroaniline, 2,4-dichloroaniline, and *m*-nitroaniline) utilizing microwave method in ethyl alcohol to give the imine compounds (3a-f), correspondingly. Cyclization reaction of Schiff bases and Chloroacetyl chloride usage microwave method in dimethylformamide gave easy Azetidin-2-one derivatives (4a-f), both Gram-negative and Gram-positive bacteria were used to assess the antibacterial properties of azetidinone molecules. The findings indicated that azetidinone derivatives (4a, 4b, and 4d) have more efficacy against Gram-negative bacteria than the common antibiotic amoxicillin-clavulanate, whereas all derivatives (4a-f) appeared less impact than the control drug athwart Gram-positive bacteria. Due to the growing resistance of bacteria to conventional antibiotics, there is a pressing need to develop new antimicrobial agents with novel structural features. In this context, the objective of this research was to synthesize and characterize a series of novel 2-azetidinone derivatives bearing 1,3,4-oxadiazole rings and evaluate their antibacterial activity. The compounds were synthesized using microwave-assisted methods, which is solvent-free, green processes that align with sustainable and eco-friendly chemistry principles.

Keywords: Azetidin-2-ones, Microwave technique, Cycloadditions, Chloroacetyl chloride, Antibacterial test.

1. Introduction

The current ineffectiveness of some well-known antimicrobial medications against microorganisms is due to the developing germ resistance caused by antibiotic overuse [1]. Thus, it is imperative to develop new and improved antimicrobial medications. It has been discovered that the synthesis of novel pharmacologically and physiologically active compounds requires heterocyclic structures, which comprise the majority of naturally existing bioactive chemicals [2]. According to this perspective, our research focuses on the synthesis of novel β-lactam (2-azetidinone) derivatives incorporating 1,3,4-oxadiazole moieties, leveraging the known antibacterial potential of both scaffolds. Microwave technique was employed to assist the synthesis process[3], which offer notable advantages over conventional heating, including faster reaction times, higher yields, better selectivity, and improved energy efficiency [4-8]. Unlike conventional methods that suffer from slow heating, uneven temperatures, and high energy loss [9], microwave technique enables solvent-free, green processes that align with sustainable and eco-friendly chemistry principles [10,11]. Organic chemical substances characterized by a cyclic structure that includes one or more heteroatoms, predominantly nitrogen, sulfur, or oxygen, are referred to as heterocyclic compounds [12]. Among their numerous benefits are the antibacterial, anti-inflammatory, and antioxidant qualities of heterocycles [13,14]. There is at least one heterocycle ring present in several newly identified antibacterial drugs [15-17]. The most important heterocyclic compounds are 1,3,4-oxadiazoles and their derivatives [18]. 1,3,4-Oxadiazole have found extensive usage in medicinal chemistry and drug development such as antibacterial [19], anti-diabetic [20], antifungal [21], anticonvulsant [22], antioxidant [23], and anti-cancer [24]. Due to the wide range of therapeutic benefits of 1,3,4-oxadiazole, the majority of research on the drug in the literature concentrated on this isomer rather than the less well-known 1,2,3-, 1,2,4-, and 1,2,5-oxadiazole isomers [25]. Because of its ability to form hydrogen bonds with receptor sites and its metabolic properties. The oxadiazole core's integration with the azole (-N--C-O-) motif improves lipophilicity, making these compounds good bio isosteres of amides and esters, this characteristic is essential for allowing medications to diffuse across membranes and efficiently reach their targets. [26]. Azetidine is one of the most significant four-membered heterocycles used in chemical synthesis and medicinal chemistry [27]. It is known as monocyclic β-lactams or monobactams [28]. Beta-lactams are organic compounds with a heterogeneous quaternary ring, in which the heteroatom is the nitrogen atom. The ring's properties are different from those of ternary, pentagonal, and hexagonal rings because it is a tetrahedron.[29] . The primary classes of β-lactam antibiotics that are taken orally are cephalosporins and penicillins, while monolactams and carbapenems are used by injection. Since peptidoglycan is an essential part of the cell wall of bacteria which keeps it mechanically stable, all β-lactam antibiotics prevent the final stage in its synthesis [30,31]. With a wide range of clinical indications, β-lactams continue to be the most prescribed antibiotic class globally Owing to their wide range of action against bacteria that are Gram-positive and Gram-negative, oral administration alternatives, Outpatient therapy accessibility, excellent tolerability, and a generally favourable safety profile [32]. The usage of β-lactam antibiotics is under danger due to the increase in bacterial resistance and the quick dissemination the mechanisms of resistance. Resistance to β-lactams has a complex underlying mechanism. Bacteria can manufacture the β-lactamases, which alter target-site penicillin-binding proteins (PBPs) to break down β-lactam medications, pump the antibiotic out of the periplasmic region, or reduce their affinity for antibiotics [30-32]. The permeability of Gram-negative bacteria's outer membranes (OMs) can also be decreased by altering the amount, kind, or structure of OM proteins, or porins [33]. The enzymatic transpeptidation pathway is disrupted by β-lactams, which have bactericidal properties by preventing the formation of bacterial cell walls. The β-lactam nucleus may function as a structural analog of the terminal D-alanyl-D-alanine dipeptide of the developing peptidoglycan, attaching irreversibly to the Ser403 residue of the penicillin binding protein (PBP) active site. This has been suggested as the mechanism of action. This procedure breaks down the cell wall by preventing the peptidoglycan layer's final crosslinking, or transpeptidation [34,35]. This work aims to synthesis of series of novel 2-azetidinone derivatives using microwave methods as sustainable and eco-friendly, and then study their antibacterial activity.

2. Materials and Methods

All chemicals and solvents were purchased by Sigma Aldrich, BDH, Fluka, J.T.Baker, Scharlau, Merck. Domestic microwave oven was used for preparing imines and the target azetidinones. utilizing iodine vapor as a developer and a silica gel 60 F254 plate, thin layer chromatography was performed. Using an Electrotherm Stuart SMP 30 capillary melting point device, melting points were determined. SHIMADZU FTIR–8400S Infrared Spectrophotometer were used for recording infrared spectra as (KBr) disc. DMSO-d6 was used as the solvent and TMS as an internal reference to record 1H NMR spectra using a Varian-INOVA USA spectrometer, 500 MHz NMR spectrometer. Mass spectra were measured using the MS Agilent Technologies-5975c, which was equipped with (ESI-MS 70eV).

2.1 Preparation of 2-hydroxy-5-((4-(5-mercapto-1,3,4- oxadiazol-2 yl) phenyl) diazenyl) benzaldehyde (2) [36]

5-(4-aminophenyl)-2-thiol-1,3,4-oxadiazole (1) (1.93 g, 10 mmol) was dissolved in 3.2 mL of strong hydrochloric acid and 10 mL of purified water. A sodium nitrite solution (0.69 g, 10 mmol) diluted in 5 mL of distilled water was added to the mixture drop-wise while stirring after it had been chilled to 0°C in an ice bath. During the addition, the ice bath's temperature was maintained between 0 and 5 °C. After making a solution of 2-Hydroxybenzaldehyde (1.22 g, 10 mmol) in 15 mL of 10%w/v sodium hydroxide solution, it was cooled to 5 °C by submersion in an ice bath and rapidly agitated. After adding the diazozonium salt solution very gradually to the phenoxide solution, a red precipitate quickly separated. After adding all of the diazonoim salt solution, the mixture was left in an ice bath for 30 minutes, stirring occasionally. After that, the solution was filtered, and the dark red precipitate was thoroughly cleaned with distilled water, dried on filter paper, then placed in an oven to recrystallize from ethanol., yield (2.534g , 70 %), mp 245- 247ºC. IR (KBr,cm^{-1}): 3074br (νO-H, νN-H thione form, νC-H benzene, overlapped), 2939 and 2760 (νC-H aldehyde), 2582 (νS–H), 1658 (νC=O aldehyde), 1599 (νC=N oxadiazol), 1498 (νN=N overlapped with νC=C benzene), 1417 (νC=C benzene), 1247 (νC=S thione form).

2.2 preparation of Schiff bases (3a-f) [37]

A mixture of (0.326 g, 1 mmol) of aldehyde derivative (2) and (1 mmol) of aniline derivatives were dissolved in (1 mL) of ethyl alcohol has been irradiated in microwave oven around (300W) for (1-3 min). End of reaction was monitored by TLC (n-hexane: EtOAc, 1:2). Ethyle alcohol was used to recrystallized crude yields.

2.2.1 preparation of 4-((E))-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)-2-((E))-((2-methoxyphenyl)imino)methyl)phenol (3a):

Red solid, mp 256–258 °C, Yield 57%. IR (KBr, cm^{-1}): 3275 (νO-H), 3105 (νN-H thione form), 3070 (νC-H benzene), 2943 (νC-H, OCH3), 2766 (νC-H imine), 2553 (νS=H), 1608 (νC=N imine and νC=N oxadiazol, overlapped), 1519 and 1419 (νC=C benzene), 1465 (νN=N), 1257 (νC=S thione form), 840 (Ar. C-H ben).

2.2.2 preparation of 2-((E)-((2,4-dimethylphenyl)imino)methyl)-4-((E)-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenol (3b)

Red solid, mp 263–265 °C, Yield 47%. IR (KBr, cm^{-1}): 3267 (νO-H), 3136 (ν N-H thion form), 3090 (νC-H benzene), 2943 (ν CH, CH_3), 2758 (νC-H imine), 2576(ν S-H),1608 (ν C=N imine and νC=N oxadiazol, overlapped), 1516 and 1415 (ν C=C benzene), 1481 (νN=N), 1253 (νC=S thione form), 837 (Ar.C-H ben).

2.2.3 preparation of 2-((E)-((4-hydroxyphenyl)imino)methyl)-4-((E)-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenol(3c)

Red solid, mp 225–227 °C,Yield 65%. IR(KBr,cm^{-1}): 3275 (νO-H), 3090 (ν N-H thion form and ν C-H benzene, overlapped), 2762 (ν C-H imine), 2573 (νS-H), 1608 (νC=N imine and νC=N oxadiazol, overlapped), 1516 and 1415 (ν C=C, benzene), 1481 (ν N=N), 1253 (ν C=S thion form), 840 (Ar.C-H ben).

2.2.4 preparation of 2-((E)-((2-chlorophenyl)imino)methyl)-4-((E)-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenol (3d)

Red solid, mp 258–260 C, Yield 55%). IR (KBr, cm^{-1}): 3267 (νO-H), 3190 (νV-H thione form),3093 νC-H benzene), 2762 (νC-H imine), 2557 (νS-H), 1604 (νC=N imine and νC=N oxadiazol, overlapped), 1516 and 1419 (νC=C benzene), 1516 (νN=N, overlapped), 1253 (νC=S thione form), 837 (Ar. C-H ben).

2.2.5 preparation of 2-((E)-((2,4-dichlorophenyl)imino)methyl)-4-((E)-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenol (3e)

Red solid, mp 153–155 °C,Yield 58%. IR (KBr, cm^{-1}): 3267 (νO-H), 3194 (νN-H thione form),3093 (ν C-H benzene), 2766 (ν C-H imine), 2557 (νS-H), 1604 (ν C=N imine and ν C=N oxadiazol, overlapped), 1516 and 1419 (ν C=C, benzene), 1516 (ν N=N, overlapped), 1253 (ν C=S thion form), 840 (Ar C-H ben).

2.2.6 preparation of 4-((E)-(4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)-2-((E)-((3-nitrophenyl)imino)methyl)phenol (3f)

Red solid, mp 161–163°C, Yield 0.25 g, 45%. IR (KBr, cm^{-1}): 3271 (νO-H), 3198 (νN-H thione form), 3097 and 3066 νC-H benzene), 2766 (ν C-H imine), 2553(ν S-H), 1608(νC=N imine), 1585(ν C=N oxadiazol)), 1512 (νas.NO_2), 1481(ν N=N), 1458 and 1415(ν C=C benzene), 1319 (νs.NO2), 1257 (ν C=S thion form), 840 (Ar.C-H ben).

2.3 Preparation of azetidin-2-ones (4a-f) [38]

A mixture of compounds (3a-f),(1 mmol), Chloroacetyl chloride (0.113 g,1 mmol) and DMF (1 mL) has been heated for three to five minutes in a microwave oven with a power of about 300W.End of reactions was monitored by TLC (n- hexane: EtOAc, 1:1). Recrystallization of crude yields has been carried out using ethyl alcohol.

2.3.1 preparation of (E)-3-chloro-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl) diazenyl)phenyl)-1-(2-methoxyphenyl)azetidin-2-one (4a)

Brown solid, mp 197–199 °C, 93%. IR (KBr, cm^{-1}): 3066(ν O-H,ν N-H thion form,ν C-H benzene, overlapped), 2928and2746 (ν C-H aliph.), 2550 (νS-H), 1708 and 1639 (νC=O lactam), 1597 (νC=N oxadiazol), 1504 and 1415 (ν C=C benzene),1480 (ν N=N), 1260(ν C=S thion form), 840(Ar.C-H ben). 1H NMR (500 MHz, DMSO-d_6)δ (ppm):2.50 (DMSO), 2.72 (s,1H, HC-N, lactam), 2.88 (s, 1H, HC-Cl, lactam). 3.36 (HDO), 3.88 (s, 3H, OCH3), 4.27 (s, 1H, N–H, thione form) [40], 7.06–8.15 (m,11H,Ar–H), 8.89 (s, 1H, O–H), 11.46 (s, 1H, S–H). MS (ESI): m/z [M + H]+ calculated :507.08, found: 507.100.

2.3.2 preparation of (E)-3-chloro-1-(2,4-dimethylphenyl)-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)azetidin-2-one (4b)

Brown solid, mp 145–147 °C,Yield 62%. IR(KBr, cm^{-1}): 3055 (ν O-H,ν N-H thion form,ν C-H benzene, overlapped), 2924 and2739 (νC-H aliph.), 2569 (νS-H), 1643 (νC=O lactam), 1600 (νC=N oxadiazol), 1504 and 1415 (ν C=C benzene), 1485 (ν N=N), 1253 (ν C=S thion form), 840 (Ar.C-H ben). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 2.23 and 2.32 (ss, 6H, 2CH3), 2.50 (DMSO), 2.73 (s,1H, HC-N, lactam), 2.89 (s,1H, HC-Cl, lactam), 3.37 (HDO), 4.28 (s, 1H, N–H, thione form), 7.01–8.14 (m, 10H, Ar–H), 8.68 (s, 1H, O–H) , 11.34 (s, 1H, S–H). MS (ESI): m/z [M + H]+ calculated :505.10, found: 505.800.

2.3.3 preparation of (E)-3-chloro-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl) phenyl)diazenyl)phenyl)-1-(4-hydroxyphenyl)azetidin-2-one (4c)

Brown solid, mp 240–242 °C, Yield 95%. IR (KBr, cm^{-1}): 3055 (νO-H, νN-H thione form, νC-H benzene, overlapped), 2978 and2770 (νC-H aliph.), 2592 (νS-H), 1685 (νC=O lactam), 1600 (νC=N oxadiazol), 1504 (νC=C benzene), 1480 (νN=N), 1250 (νC=S thione form), 848 (Ar. C-H ben). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 2.49 (DMSO), 2.72 (s, 1H, HC-N, lactam), 2.87 (s, 1H, HC-Cl, lactam), 3.35 (HDO), 4.33 (s, 1H, N–H, thione form), 7.09–8.08 (m, 11H, Ar–H), 8.88 (s, 2H, 2O–H) , 10.80 (s, 1H, S–H). MS (ESI): m/z [M + H]+ calculated :493.06, found: 493.100.

2.3.4 preparation of (E)-3-chloro-1-(2-chlorophenyl)-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)azetidin-2-one (4d)

Brown solid, mp 221–223 °C, Yield 77%. IR (KBr, cm^{-1}): 3267 (νO-H), 3174 (νN-H thione form), 3043 (νC-H benzene), 2928 and 2746 (νC-H aliph.), 2569 (νS-H), 1708 and 1639 (νC=O lactam), 1597 (νC=N oxadiazol), 1508 and 1415 (νC=C benzene), 1481 (νN=N), 1257 (νC=S thione form), 840 (Ar. C-H ben). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 2.50 (DMSO), 2.73 (s, 1H, HC-N, lactam), 2.89 (s, 1H, HC-Cl, lactam), 3.43 (HDO), 4.23 (s, 1H, N–H, thione form), 7.10–8.11 (m, 11H, Ar–H), 10.80 (s, 1H, O–H) , 11.50 (s, 1H, S–H). MS (ESI): m/z [M + H]+ calculated :511.03, found: 510.900.

2.3.5 preparation of (E)-3-chloro-1-(2,4-dichlorophenyl)-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)azetidin-2-one (4e)

Brown solid, mp 237–239 °C, Yield 97%. IR (KBr, cm^{-1}): 3080 (νO-H, νN-H thione form, νC-H benzene, overlapped), 2924 and 2746 (νC-H aliph.), 2570 (νS-H), 1708 and 1640 (νC=O lactam), 1597 (νC=N oxadiazol), 1512 and 1415 (νC=C benzene), 1480 (νN=N), 1260 (νC=S thione form), 840 (Ar. C-H ben). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 2.50 (DMSO), 2.72 (s, 1H, HC-N, lactam), 2.88 (s, 1H, HC-Cl, lactam), 3.36 (HDO), 4.32 (s, 1H, N–H, thione form), 6.98–8.14 (m, 10H, Ar–H), 8.82 (s, 1H, O–H) , 10.24 (s, 1H, S–H). MS (ESI): m/z [M + H]+ calculated :544.99, found: 544.950.

2.3.6 preparation of (E)-3-chloro-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl) phenyl)diazenyl)phenyl)-1-(3-nitrophenyl)azetidin-2-one (4f)

Brown solid, mp 227–229 °C, Yield 96%. IR (KBr, cm^{-1}): 3080 (νO-H, νN-H thione form, νC -H benzene, overlapped), 2924 and 2742 (νC-H aliph.), 2570 (νS-H), 1705 and 1639 (νC=O lactam), 1597 (νC=N oxadiazol), 1512 and 1415 (νC=C benzene), 1481 (νN=N), 1260 (νC=S thione form), 840 (Ar. C-H ben). 1H NMR (500 MHz, DMSO-d6) δ (ppm): 2.50 (DMSO), 2.73 (s, 1H, HC-N, lactam), 2.88 (s, 1H, HC-Cl, lactam), 3.43 (HDO), 4.23 (s, 1H, N–H, thione form), 7.10–

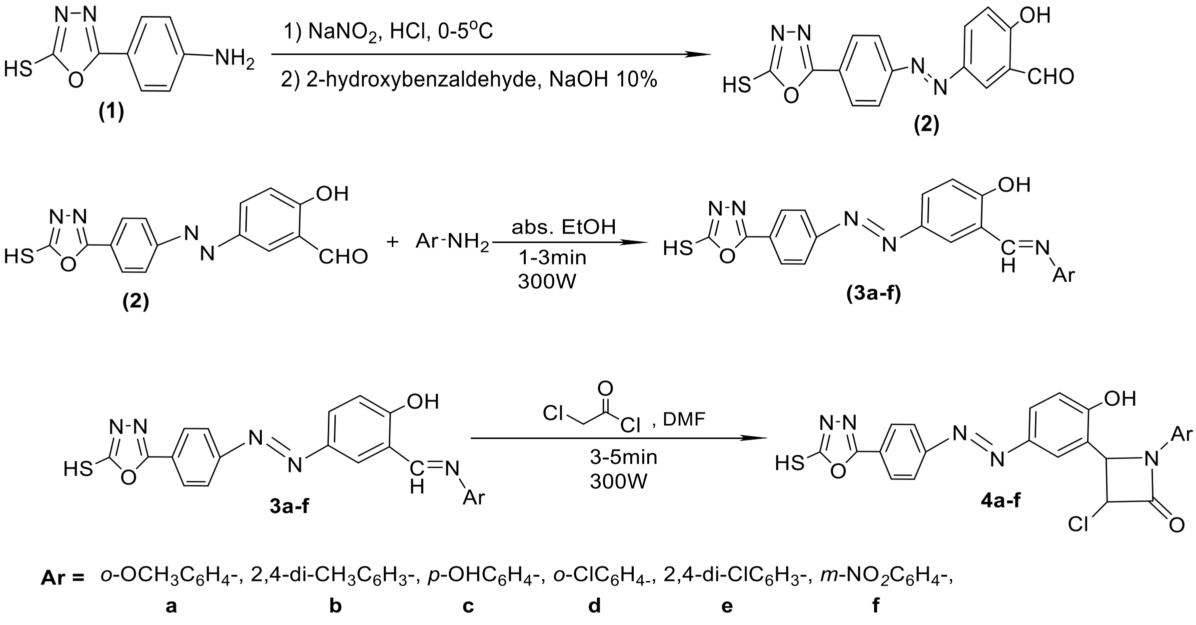
8.11 (m, 11H, Ar–H), 8.55 (s, 1H, O–H) , 11.52 (s, 1H, S–H). MS (ESI): m/z [M + H]+ calculated :522.05, found: 522.000.

2.4 Antibacterial test

Evaluation of antibacterial effects of the target azetidin-2-one derivatives (4a-f), athwart Staphylococcus aurous and Escherichia coli bacteria has been done by the agar diffusion method [43] on tryptic soya agar media. The concentration of (20 mg/mL) for each compound has been prepared in (DMSO) as solvent. Amoxicillin-clavulanate has been used as a common antibiotic, and the activities were displayed as inhibition zones for each component.

3. Results and Discussion

Diazotization of 5-(4-aminophenyl)-1,3,4-oxadiazole-2-thiol(1) using sodium nitrite and hydrochloric acid afforded diazonium chloride salt that was reacted with phenoxide salt of 2-hydroxybenzaldehyde giving azoaldehyde (2). Carbonyl function in compound (2) was condensed with (*o*-methoxyaniline, 2,4-dimethylaniline, *p*-aminophenol, *o*-chloroaniline, 2,4-dichloroaniline, and *m*-nitroaniline) utilizing microwave method in ethyl alcohol yielding Schiff bases (3a-f) correspondingly, as intermediates. Using microwave irradiation in dimethylformamide, chloroacetylchloride was added to imines (3a–f) to create 2-azetidinone derivatives (4a–f)., respectively (Scheme 1).



Scheme 1. ***Azetidin-2-ones synthesis***

IR, ^{1}H NMR, and Mass spectral methods have been employed to infer the chemical structures of the produced molecules of concern.

Infrared of compound (2) showed the absence of peaks around 3456cm^{-1} and 3352cm^{-1} assigned to(NH_2) stretching in the initial compound (1), and appearing broad peak around 3074cm^{-1} for(O-H) stretching, peak around 1658 cm^{-1} recorded for aldehyde (C=O) stretching, the peak around 1498 cm^{-1} for (N=N) stretching, the stretching of oxadiazolic (C=N) was pointed at 1658 cm^{-1}[39], as shown in Figures (1-2).

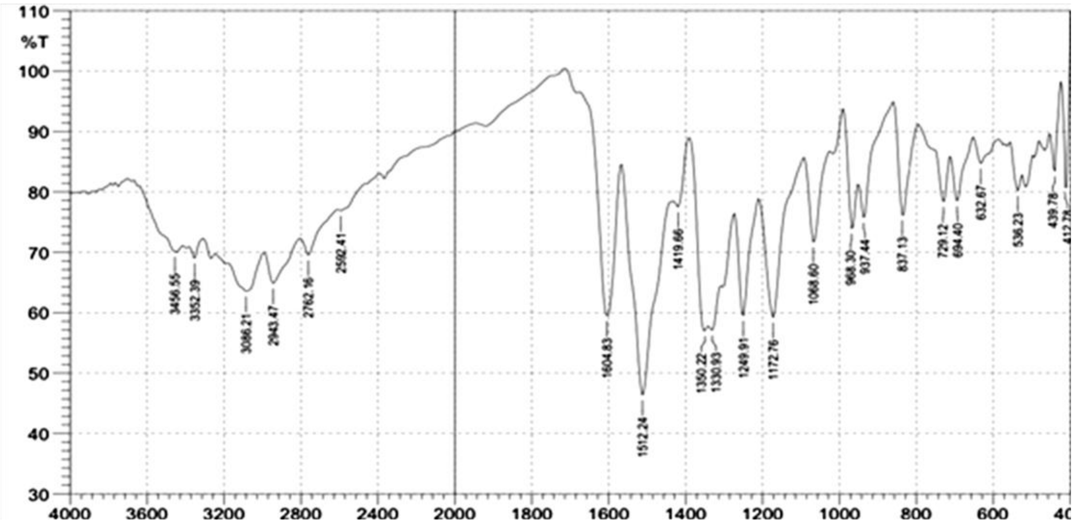


Figure1. *FT-IR spectrum of compound 1*

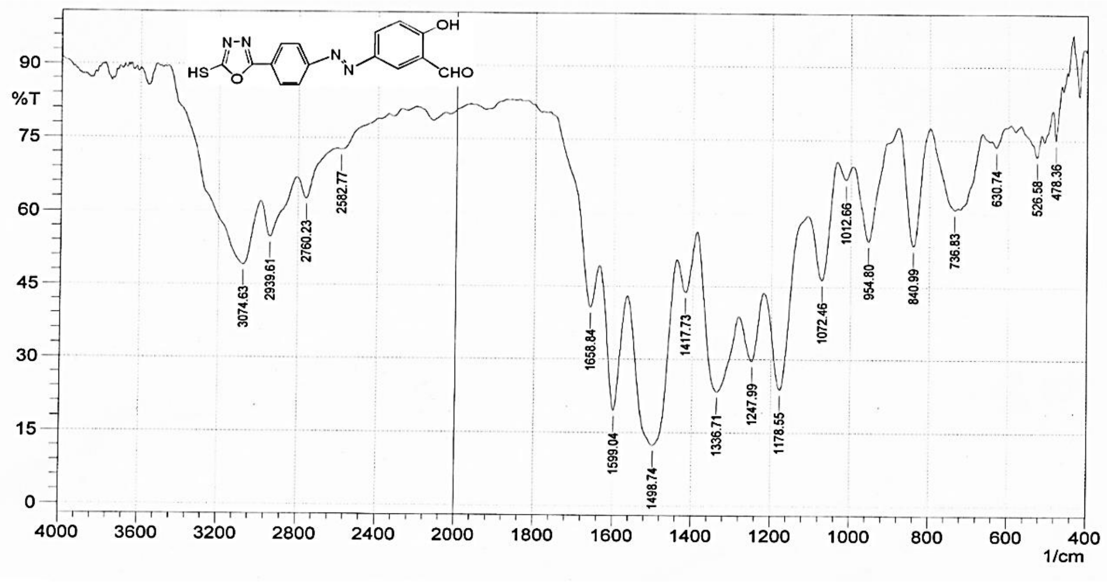


Figure2. *FT-IR spectrum of compound 2*

Infrared of Schiff bases (3a-f) indicated the absence of peak for aldehydic (C=O) str. around 1658 cm^{-1}, also absence of absorption for (NH_2) stretching of amines around scope (3400-3250) cm^{-1} and pointing peak around scope (1604-1608) cm^{-1} for stretching of imine function (C=N)[39], as shown in Figures (3-6).

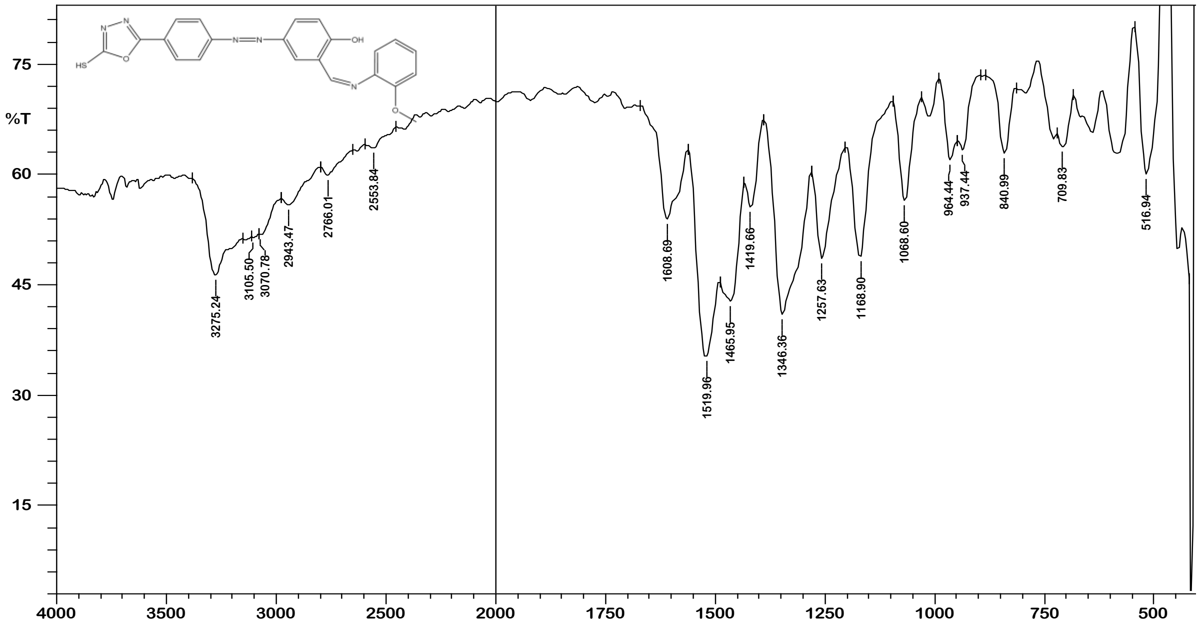


Figure3. *FT-IR spectrum of compound 3a*

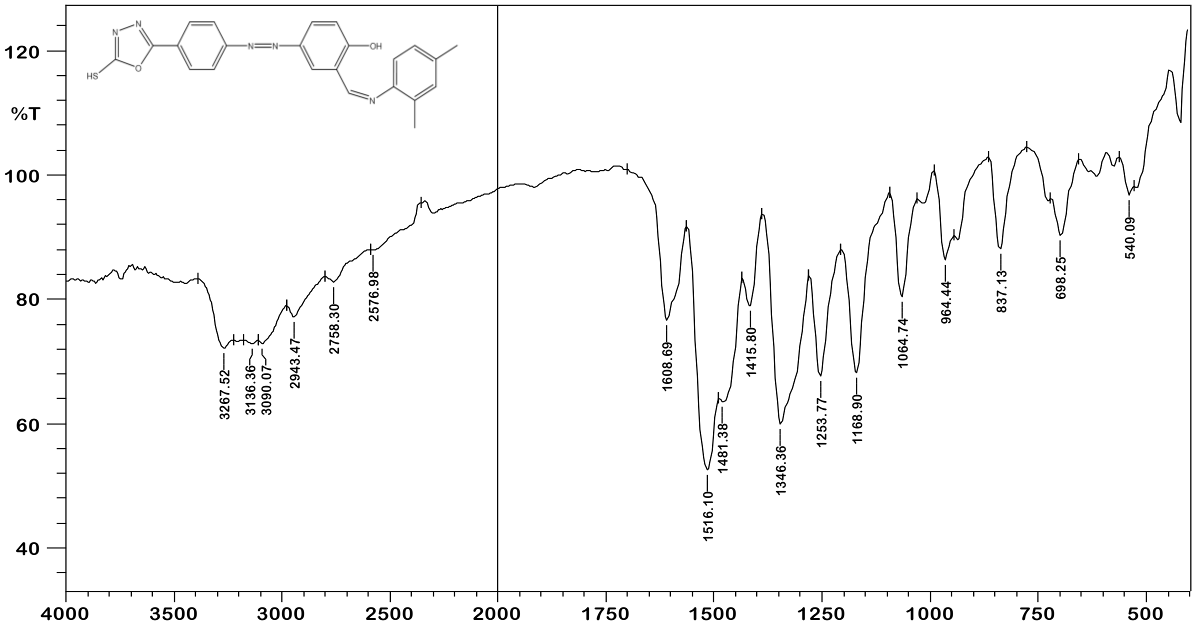


Figure4. *FT-IR spectrum of compound 3b*

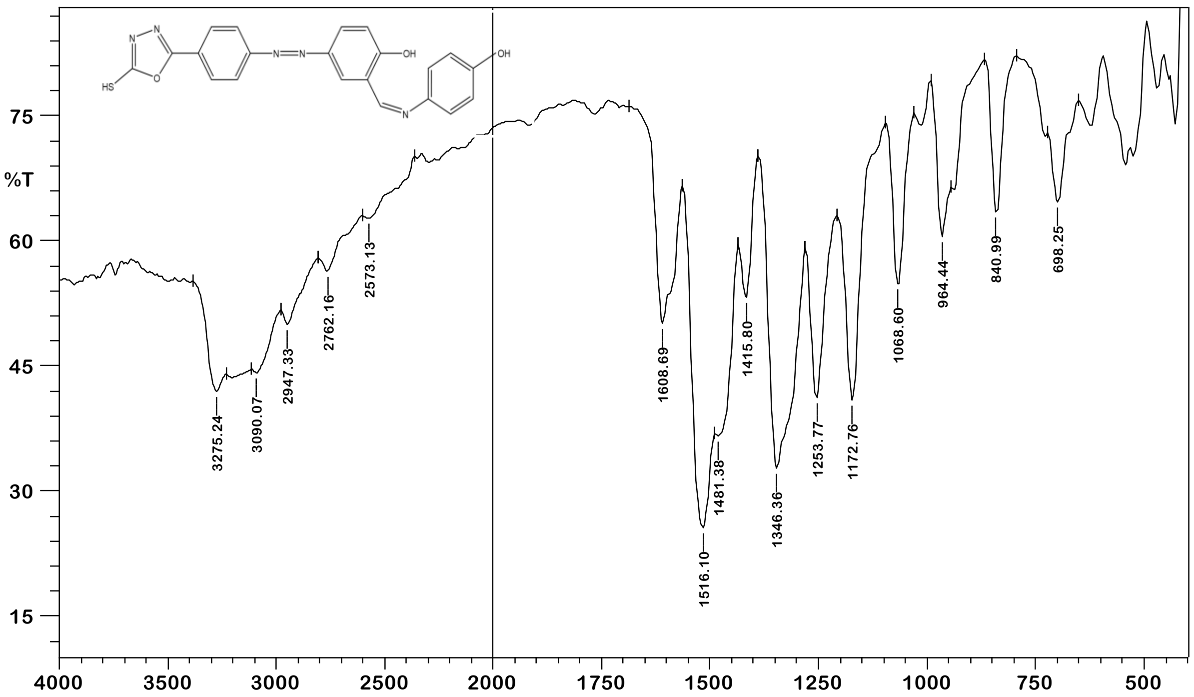


Figure 5. *FT-IR spectrum of compound 3c*

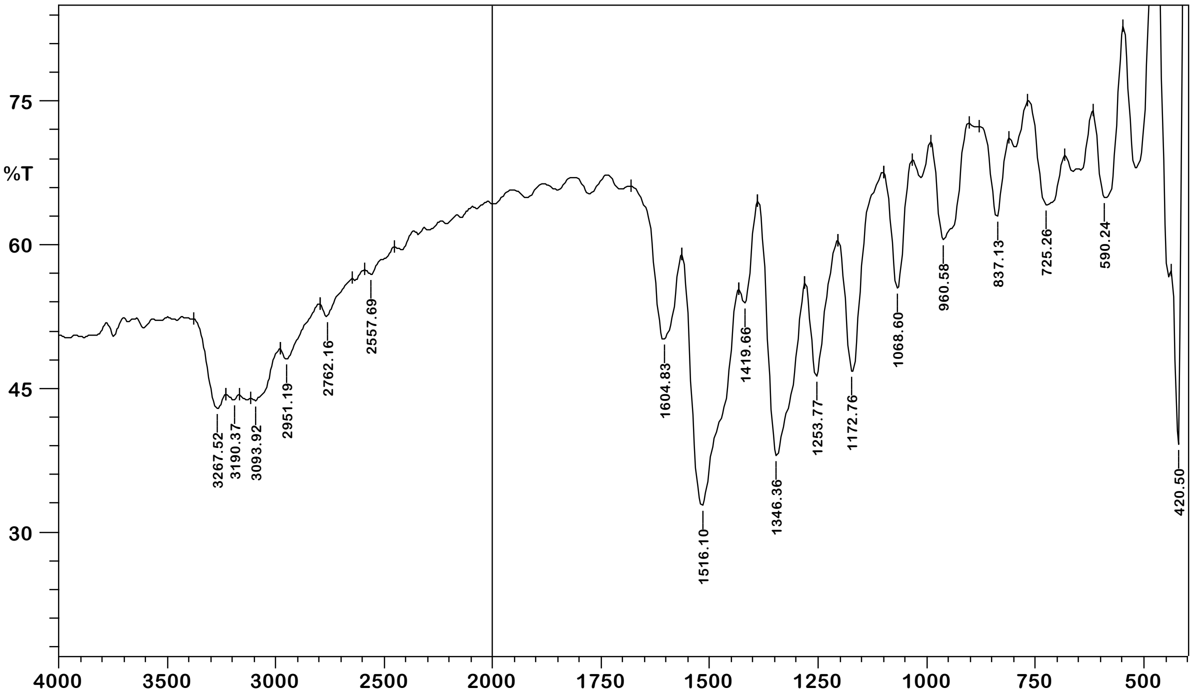


Figure 6. *FT-IR spectrum of compound 3d*

Infrared of azetidin-2-ones (4af) showed the (C=N) stretching of oxadiazole around the scope (1600-1597 cm^{-1}) and the (C=O) stretching of the lactam ring (1708-1639 cm-1), while imine (C=N) stretching disappeared[39]., Figures (7-12).

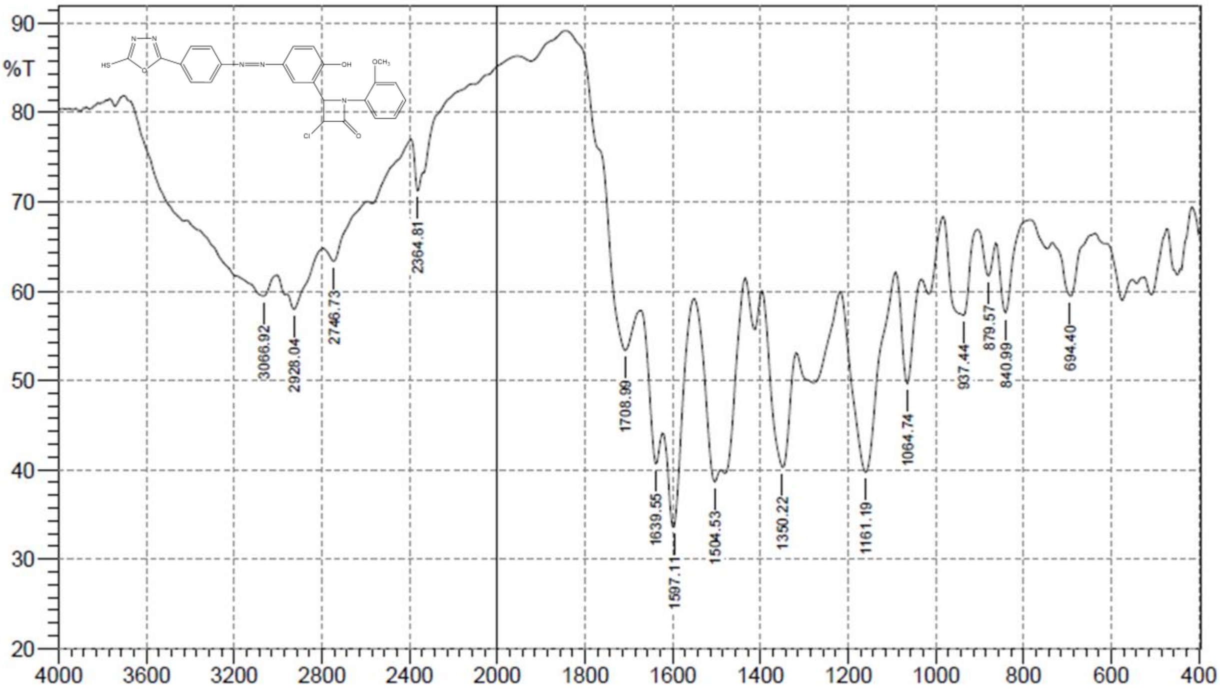


Figure 7. *FT-IR spectrum of compound 4a*

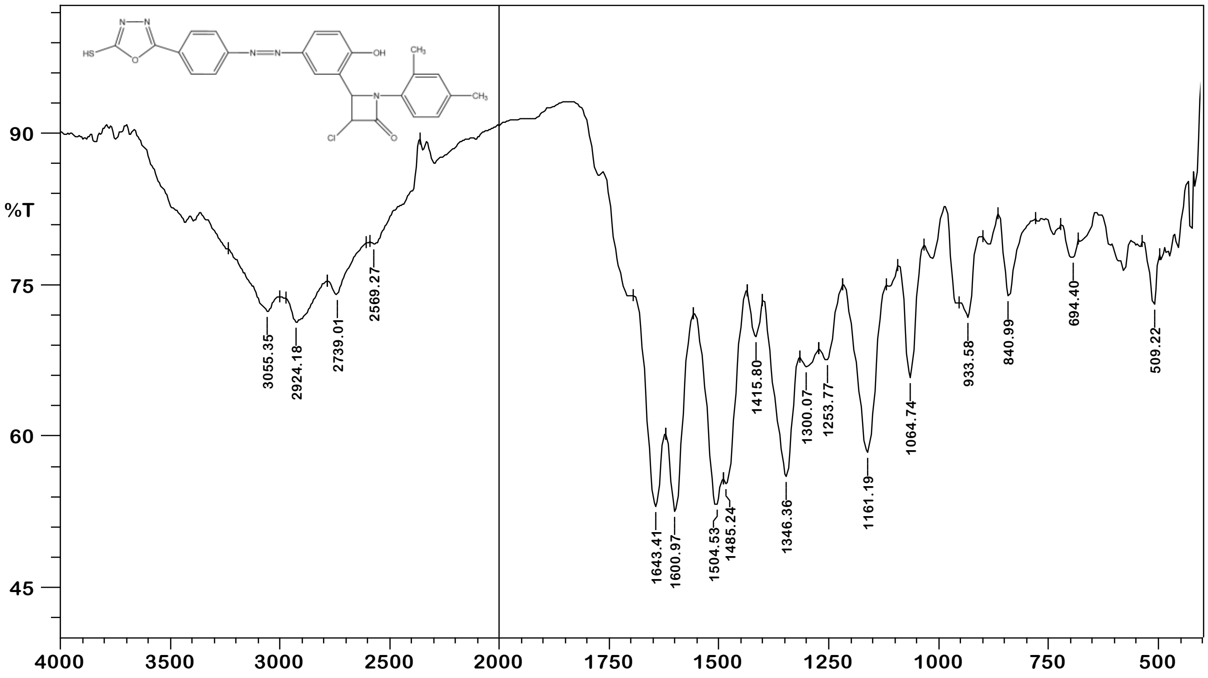


Figure 8. *FT-IR spectrum of compound 4b*

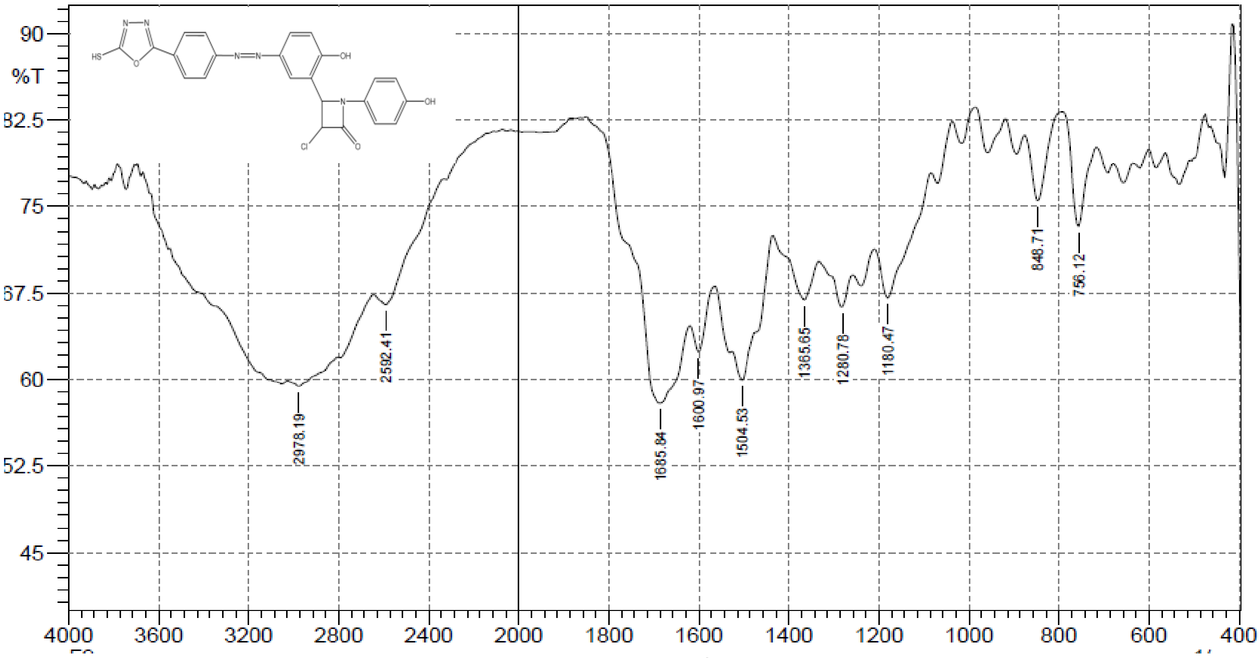


Figure 9. *FT-IR spectrum of compound 4c*

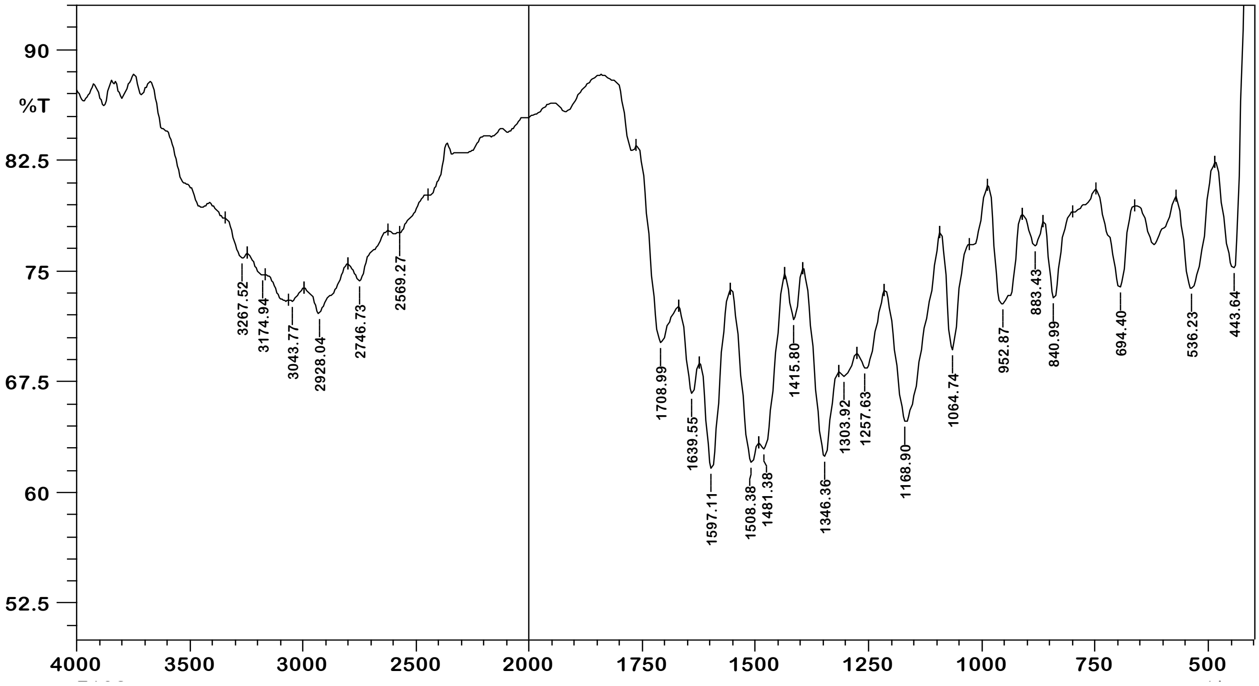


Figure 10. *FT-IR spectrum of compound 4d*

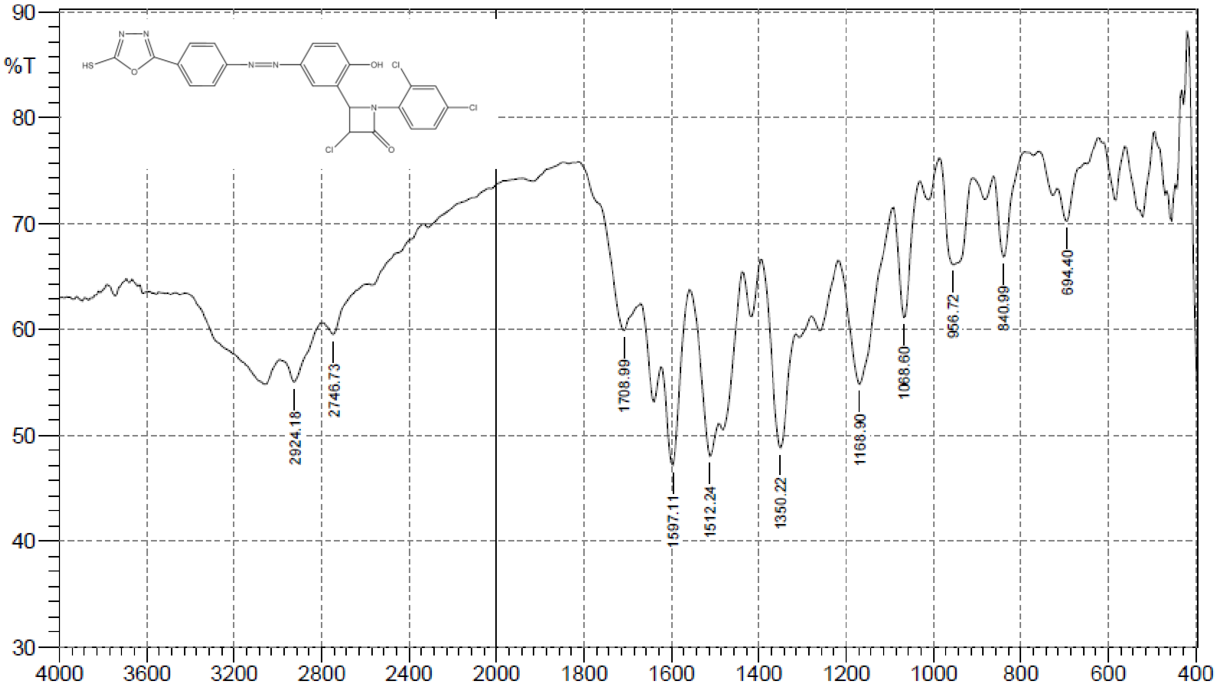


Figure 11. *FT-IR spectrum of compound 4e*

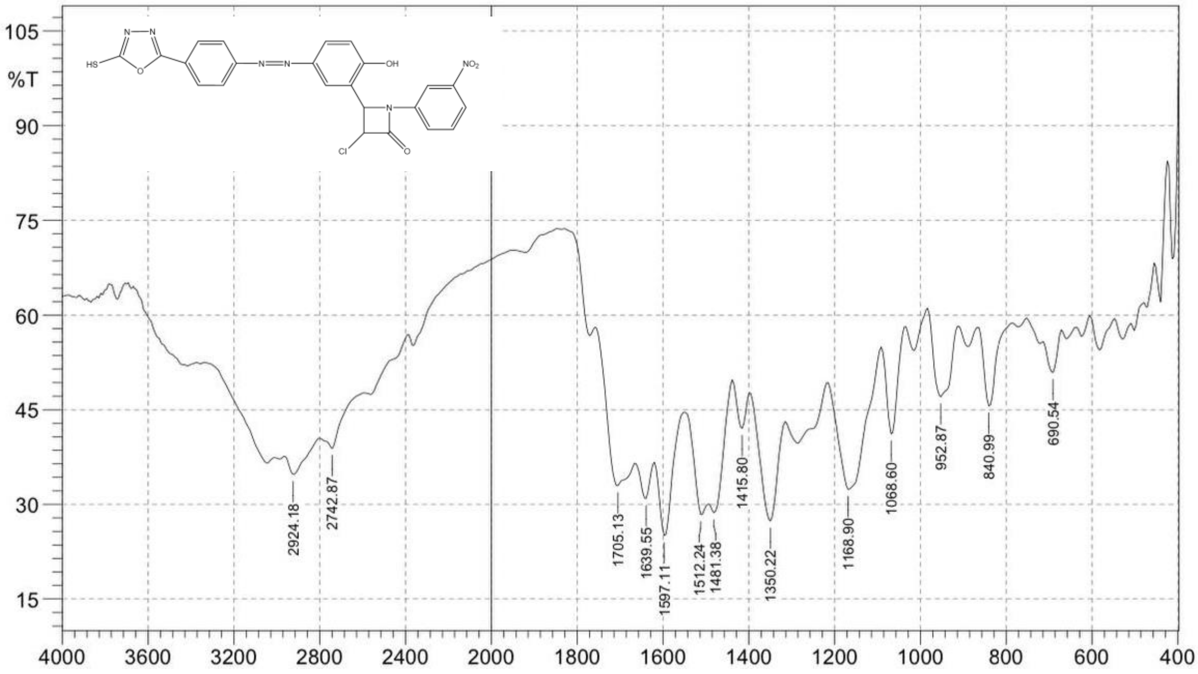


Figure 12. *FT-IR spectrum of compound 4f*

The structures of azetidin-2-ones (**4a-f**) have been proven by ^1H NMR (500MHz) in DMSO-d_6 as solvent in addition to Mass spectra, as shown in Figures (13-24).

^1H NMR of compound (4a) recorded singlet peak for azetidine ring (HC-N) hydrogen around δ 2.72ppm,while the signal of (HC-Cl) hydrogen for the same ring was recorded as singlet at δ 2.88 ppm. The singlet peak for (OCH_3) protons appeared at δ 3.88 ppm. The proton of (N–H, thione form) pointed singlet δ 4.27 ppm [40]. Aromatic (Ar-H) signals were pointed about δ 7.06–8.15ppm. The proton of the hydroxyl group (O-H) is identified by the singlet signal at about δ 8.89 ppm. At δ 11.46 ppm, the sulfhydryl proton (S-H) pointed.

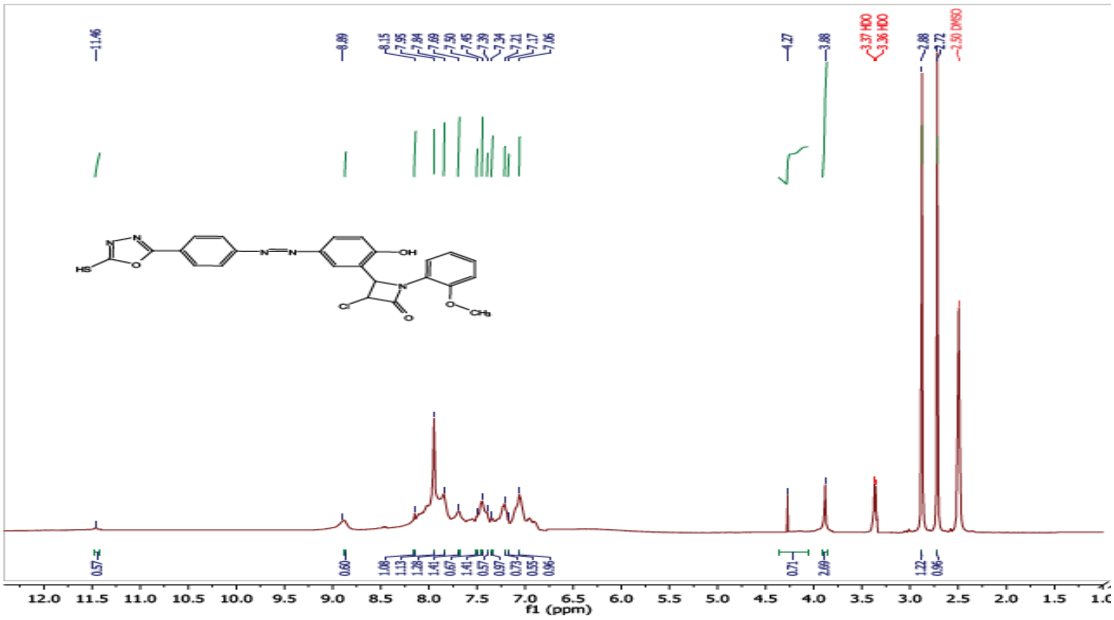


Figure 13. *^1H NMR spectrum of compound 4a*

^1H NMR spectrum of compound(4b) appeared signals of (CH_3) protons around δ 2.23 ppm and 2.32 ppm. (HC-N) proton of azetidine unit recorded singlet peak around δ 2.73 ppm. The singlet peak at δ 2.89 ppm for (HC-Cl) proton of azetidine ring. The proton of (N–H, thione form) pointed singlet at δ 4.28 ppm. The aromatic (Ar-H) signals were appears around δ 7.01–8.14 ppm. The singlet peak at δ 8.68 ppm attributed to proton of(O-H). The proton of (S-H) recorded at δ 11.34ppm[40].

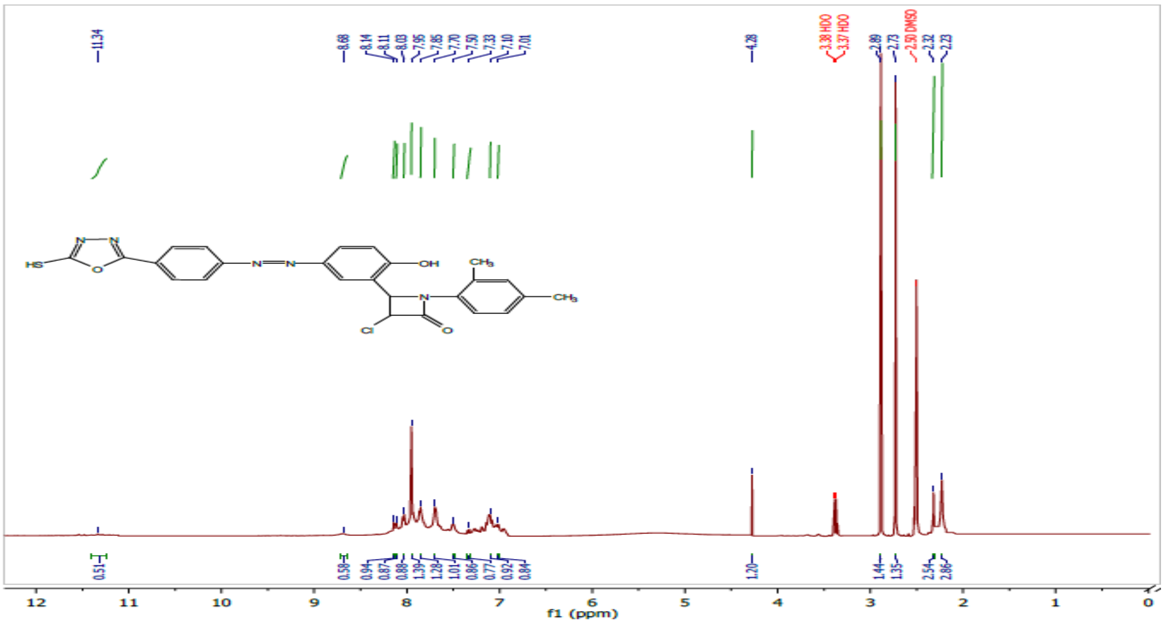


Figure 14. *^{1}H NMR spectrum of compound 4b*

^{1}H NMR spectrum of compound (4c) showed singlet peak around δ 2.72 ppm assigned to (HC-N, lactam). The signal of (HC-Cl, lactam) proton pointed singlet around δ 2.87 ppm. The proton of (N–H, thione form) appeared as singlet at δ 4.33 ppm. The multiple signals around δ 7.09–8.08 ppm assigned to (Ar-H) hydrogens. The peak around δ 8.88 ppm assigned to protons of (O-H) groups. The peak around δ 10.80 ppm attributed to (S-H) proton[40].

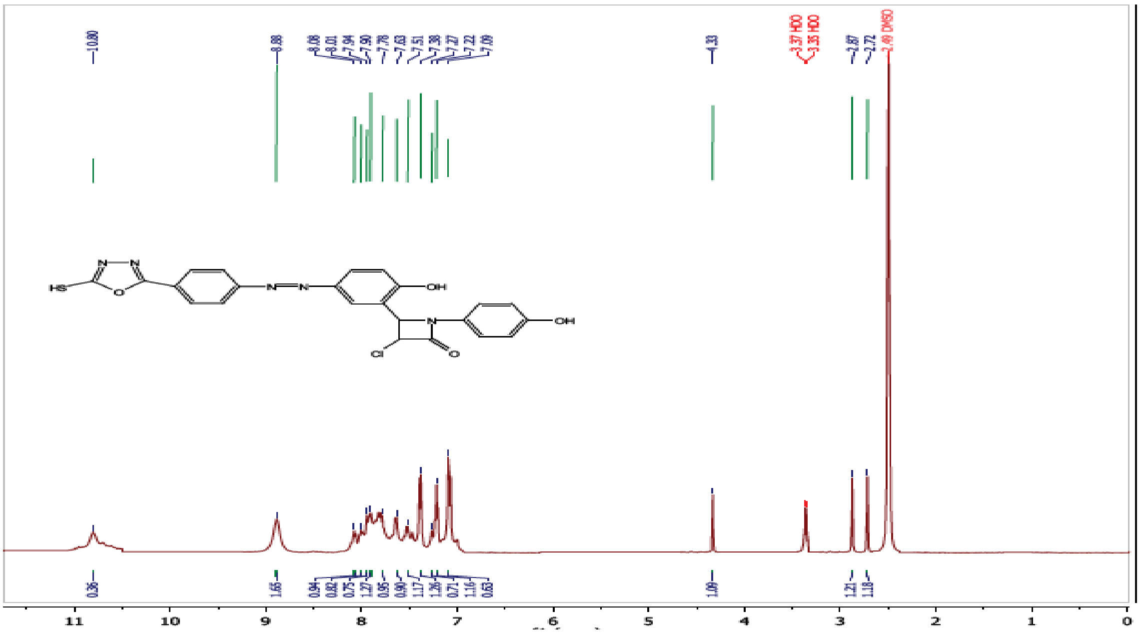


Figure 15. *^{1}H NMR spectrum of compound 4c*

The ^{1}H NMR spectrum of compound (4d) spectrum showed a signal for the lactam ring (HC-N) proton at about 2.73 ppm. Around 2.89 ppm, the lactam ring (HC-Cl) proton peak manifested as a singlet. At δ 4.23 ppm, the proton of (N–H, thione form) was visible as a singlet. Around δ 7.10–8.11, the (Ar-H) hydrogens showed up as a multiplet. The (O-H) group hydrogen signals were detected at δ 10.80 ppm and 9.81 ppm. S-H proton was measured at δ 11.50 ppm[40].

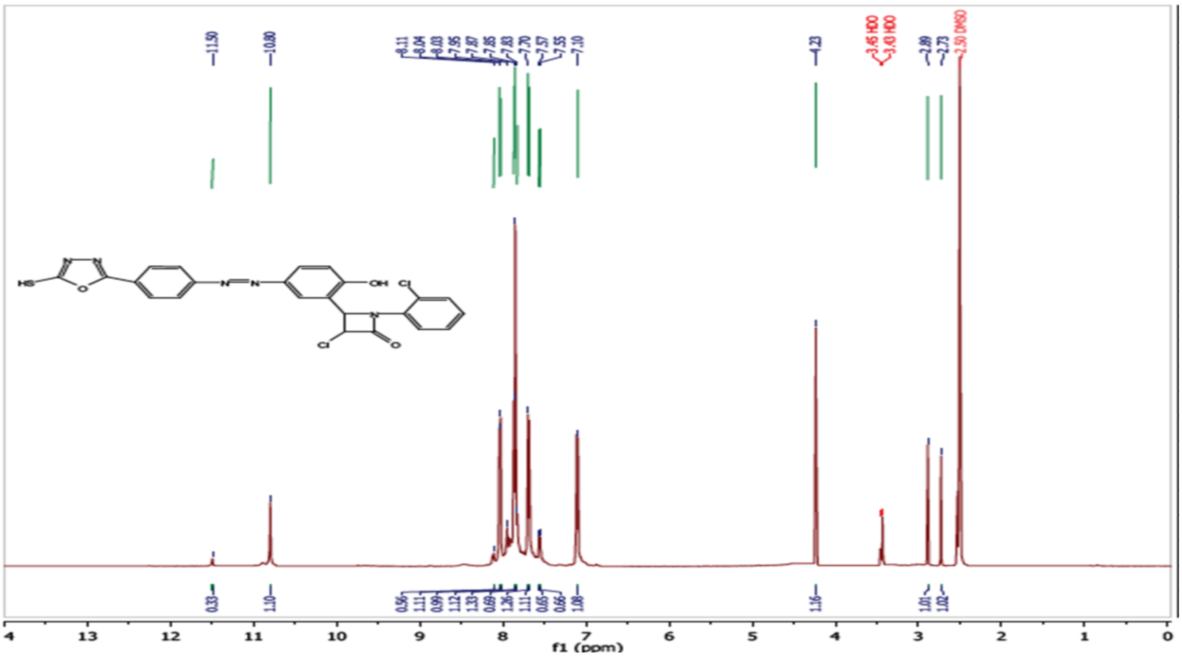


Figure 16. *^{1}H NMR spectrum of compound 4d*

^{1}H NMR of derivative (4e) appeared the signal of ((HC-N, lactam)) proton around δ 2.72 ppm, the (HC-Cl) hydrogen appeared around δ 2.88 ppm. The proton of (N–H, thione form) appeared as singlet at δ 4.32 ppm. (Ar-H) hydrogens as multiplet at δ 6.98–8.14 ppm. The hydroxyl proton signal around δ 8.82 ppm. The peak around 10.24ppm for(S-H) hydrogen[40].

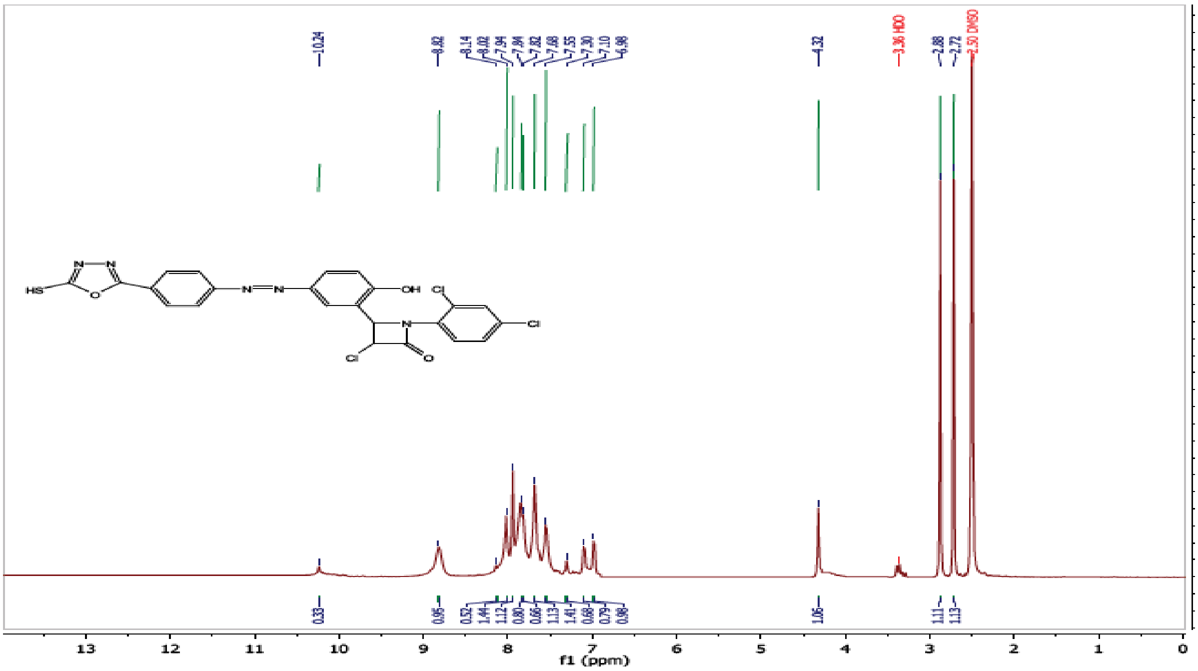


Figure 17. ^{1}*H NMR spectrum of compound 4e*

^{1}H NMR of derivative (4f) appeared the signal of ((HC-N, lactam)) proton around δ 2.73 ppm, the (HC-Cl) hydrogen appeared around δ 2.88 ppm. The proton of (N–H, thione form) appeared as singlet at δ 4.23 ppm. (Ar-H) hydrogens as multiplet at δ 7.10–8.11 ppm. The signal about δ 8.55ppm due to(O-H) proton. While peak around 11.52 ppm for (S-H) proton [40].

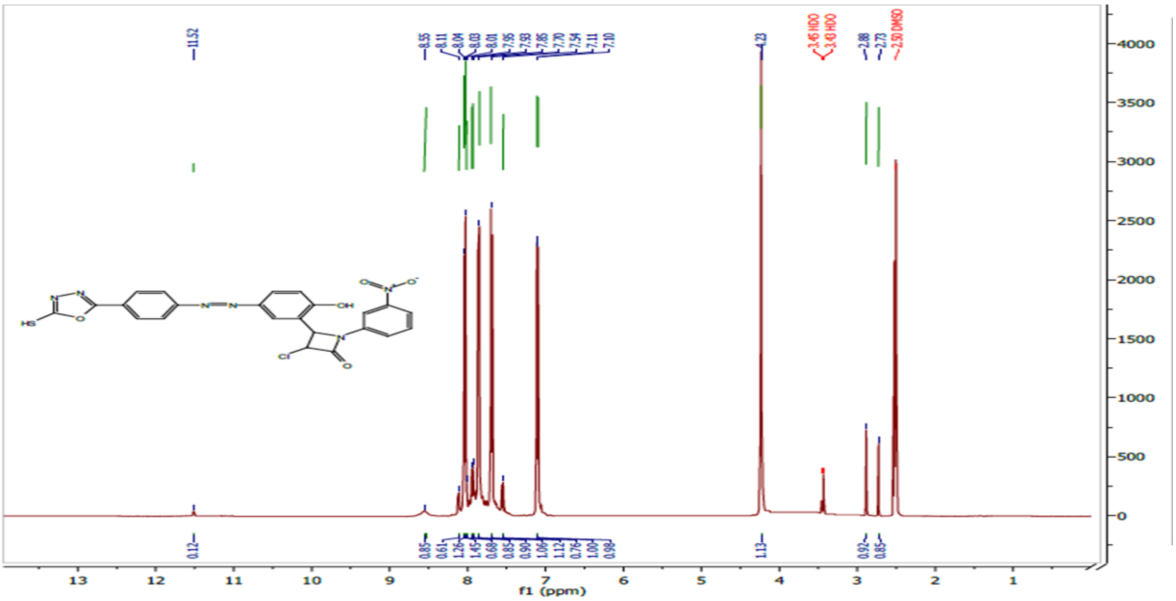


Figure 18. ^{1}*H NMR spectrum of compound 4f*

The mass spectra of the synthesized 2-azetidinones appeared signal at (m/z) relative to the molecular ion, the value close to the calculated molecular weight (g/mole).

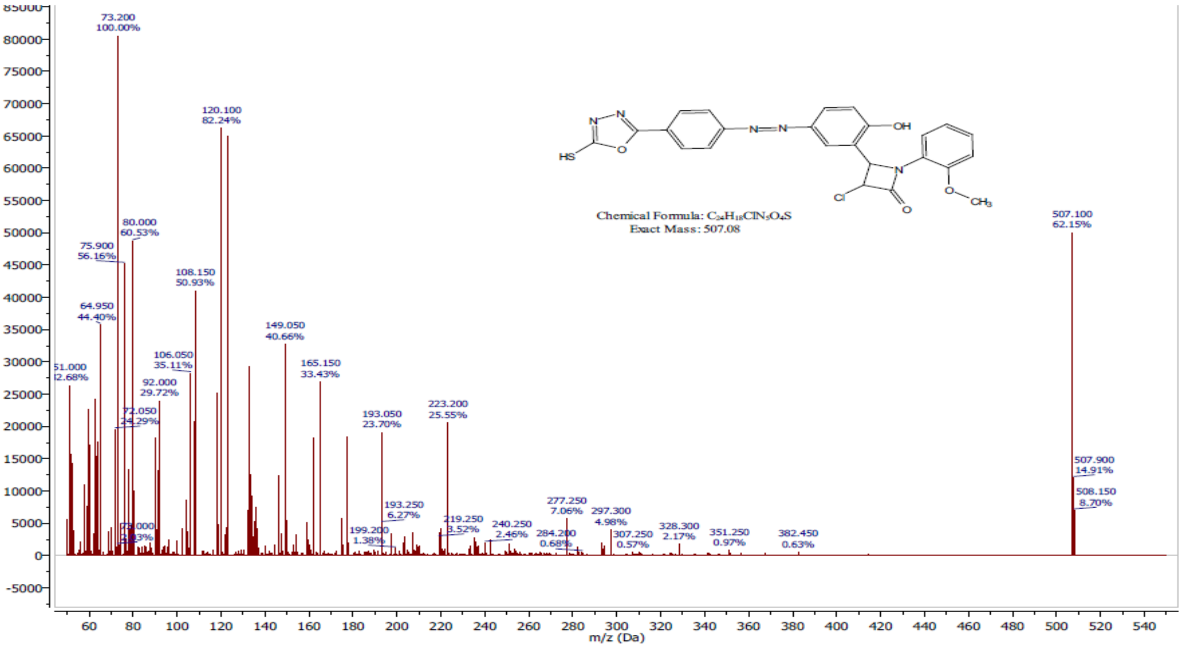


Figure 19. *Mass spectrum of compound 4a*

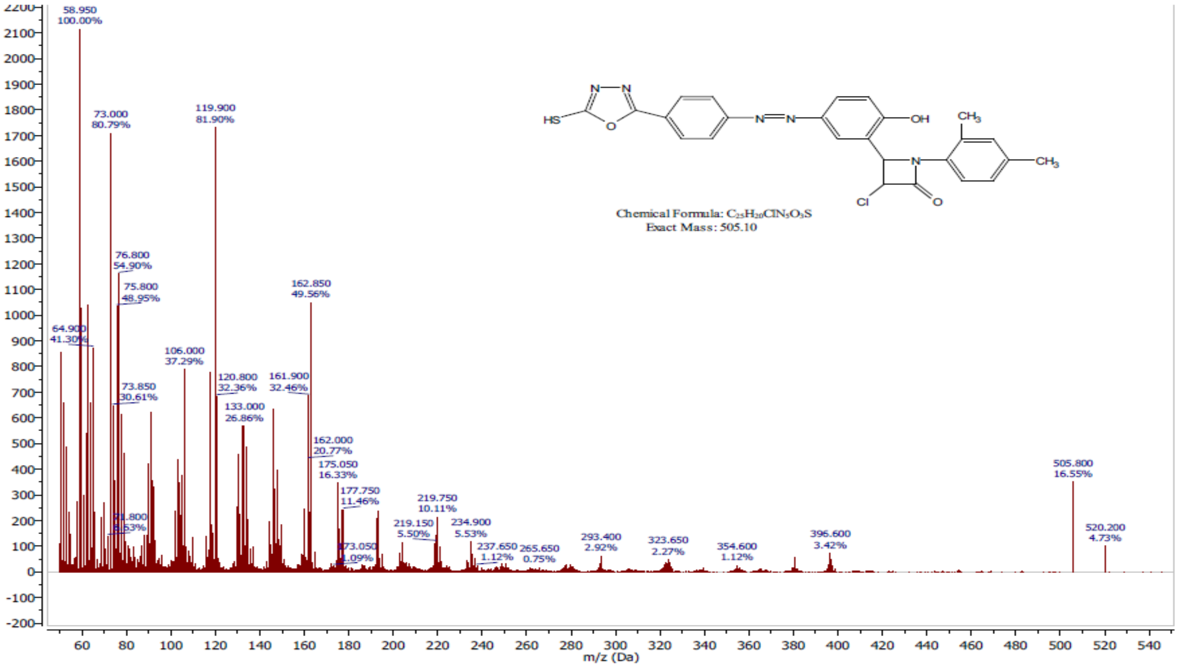


Figure 20. *Mass spectrum of compound 4b*

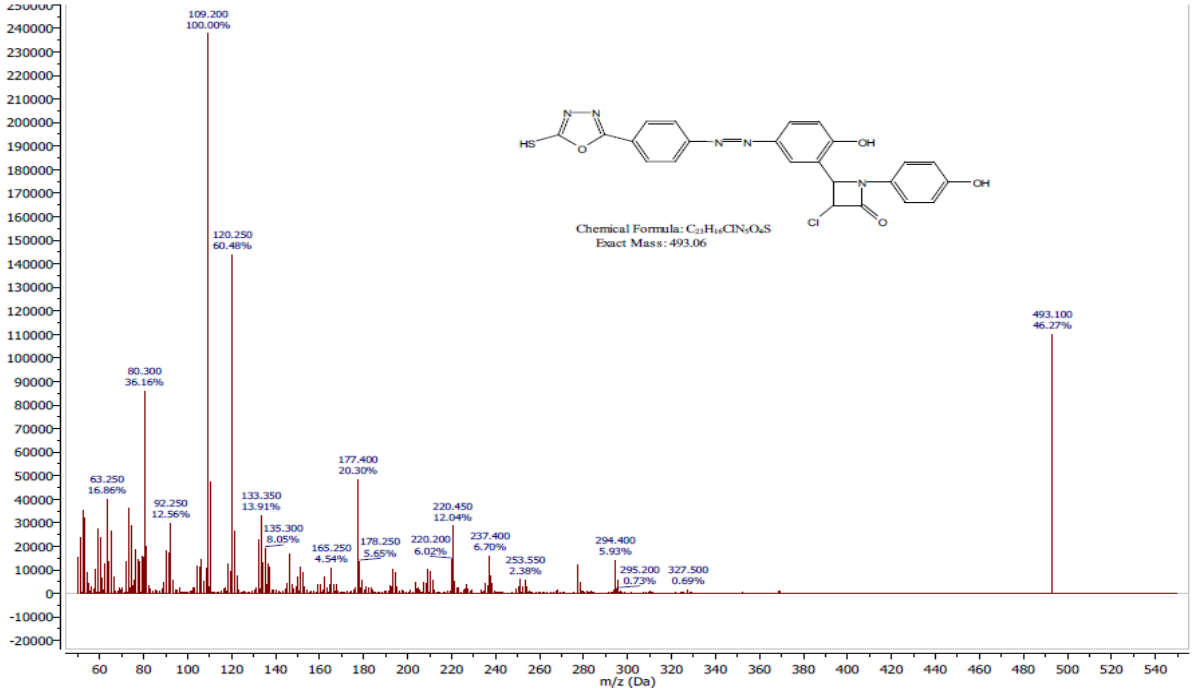


Figure 21. *Mass spectrum of compound 4c*

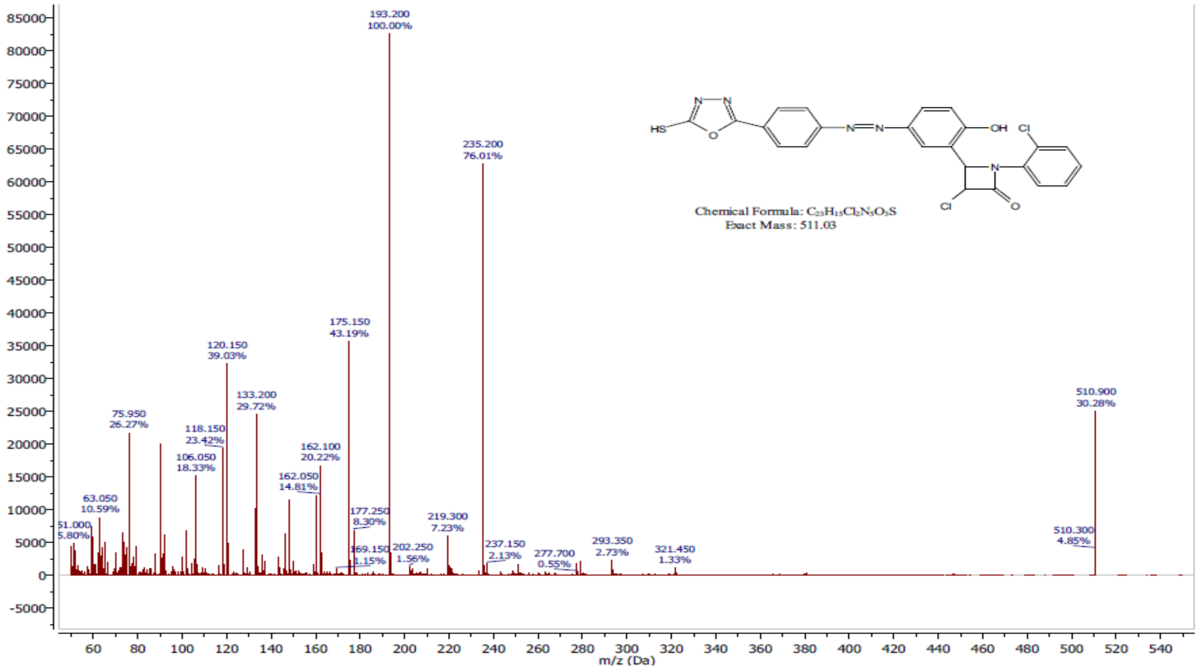


Figure 22. *Mass spectrum of compound 4d*

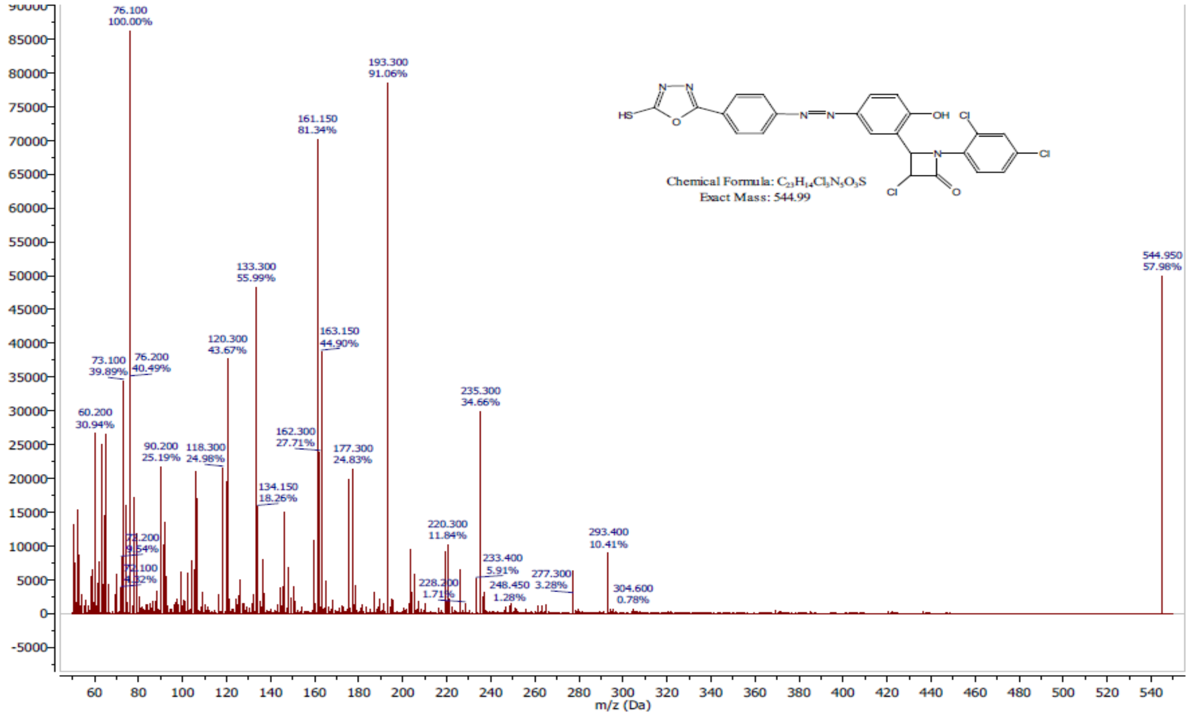


Figure 23. *Mass spectrum of compound 4e*

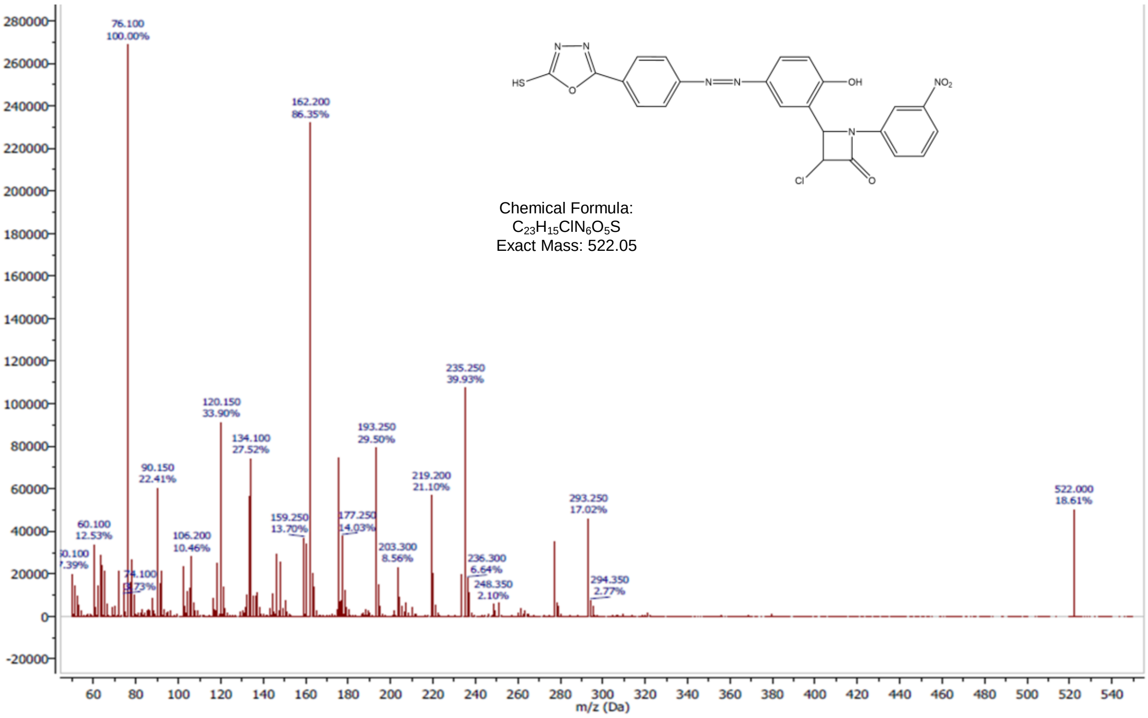


Figure 24. *Mass spectrum of compound 4f*

3.1 Antibacterial activities

Using the agar diffusion method, the targeted azetidinone compounds (4a–f) were tested for antibacterial activity against two types of Gram-positive and Gram-negative bacteria. (29on soy agar tryptic medium. The solvent used is (DMSO) using concentration (20 mg/ mL) for the test compounds. The results pointed that compound (4a, 4b, and 4d) showed greater activity athwart Gram-negative *Escherichia coli* than reference drug (amoxicillin-clavulanate), on the other hand, all derivatives pointed less activity than the reference antibiotic athwart Gram-positive *Staphylococcus aurous* as indicated in Table 1 and figure 25.

Table 1. **(The activities of azetidin-2-ones (4a-f) against bacteria).**

Entry	*Staphylococcus aurous*	*Escherichia coli*
4a	15.5	21
4b	9.5	19
4c	16	16
4d	16.5	20
4e	12	15
4f	14.5	16.5
[DMSO]	0	0
[Amoxicillin-Clavulanate]	18	18

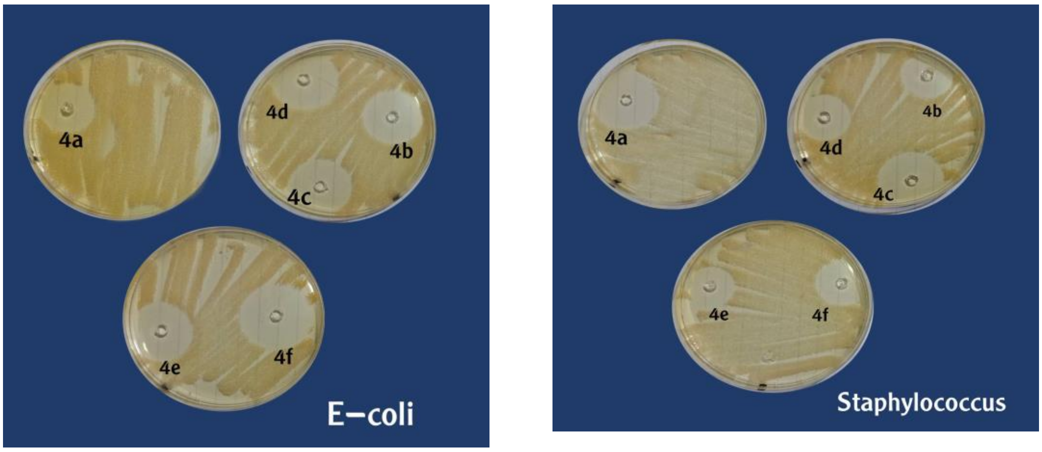


Figure 25: *Antibacterial photographs of 2-azetidinone derivatives (4a-f) against Gram-positive and Gram-negative bacteria.*

4. Conclusions

Microwave irradiation method gave excellent yields from the target β-lactams compounds in very short reaction timeThe synthesized azetidin-2-ones demonstrated encouraging antibacterial activity against Gram-negative bacteria. Most compounds demonstrated activity greater than that of conventional antibiotics.

5. Acknowledgement

We offer our gratitude to personnel at central laboratory, Tehran University, Iran for measuring mnuclear magnetic resonance and mass spectra for new azetidinone.

6. References

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