Acta Pharm. Sci. Vol 57 No: 4. 2019
DOI: 10.23893/1307-2080.APS.05727

A Novel Bioactive Compounds of 2-Azetidinone Derived from Pyrazin Dicarboxylic Acid: Synthesis and Antmicrobial Screening

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ABSTRACT

A series of novel 2-azetidinons entitled N, N'-bis[3-chloro-2-oxo-4-(substituted pyridine-2-yl)-azetidin-1-yl] pyrazine-2,3-dicarboxamide and N,N'-bis[3,3-dichloro-2-oxo-4-(substitutedpyridine-2-yl)-azetidin-1-yl] pyrazine-2,3-dicarboxamide have been synthesized from the relating newly Schiff bases by [2+2] cycloaddition reaction in good yields. Starting with pyrazine-2,3-dicarboxylic acid which was converted to the corresponding diester in absolute ethanol and glacial acetic acid as a catalyst. After that, the hydazinolysis of resulted diester with hydrazine hydrate afforded dicarbohydrazide which further treated with different substituted pyridine-2-carbaldehyde to give new Schiff bases. These new Schiff bases were reacted with chloroacetochloride and (or) dichloroacetochloride in presence of trimethylamine in DMF solvent under reflux and stirring to yield new derivatives of titled compounds. The structural assignments were estimated from their spectroscopic analysis such as IR,^{1}H NMR, ^{13}C NMR, and C, H, N elemental analysis. The newly prepared 2-azetidinones have been screened for their antimicrobial activity and some of them revealed excellent antibacterial and antifungal activities.

Keywords: 2-Azetidinones, Antimicrobial, Pyrazine-2,3-dicarboxylic acid, Schiff bases, Synthesis.

INTRODUCTION

2-Azetidinones, also called (β-lactam) compounds are classified as an important class of heterocyclic compounds whish are consist the most structural feature of β-lactams antibiotics such as; penicillin, cephalosporin and clavu-

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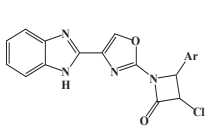
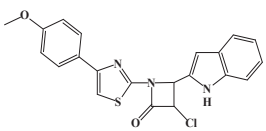
(Received 15 July 2019, accepted 22 August 2019)

lanic acid.[1,2] Because of the biological properties of 2-azetidinone compounds as antibiotics, antimicrobial, and anti-inflammatory,[3-7] as well as the chemical importance as an intermediate for synthesis of many bioactive compounds,[8] a remarkable attraction and the main framework for all interested researchers toward synthesis and discover of new 2-azetidinone derivatives. Many of the current antimicrobial drugs has becoming less effective versus microbe resistance. Herein, it was necessary to synthesis and development of new antimicrobial agents which may be possess more activity against these strains resistant.[9] However, by the literature survey, many various procedures were reported to prepare a new derivative of substituted azetidin-2-one with powerful biological activity. For example, benzimidazolyloxazolyl 2-azetidinone derivatives has been prepared with good yields.[10] Also, a series of 2-azetidinone derivatives have been prepared from their corresponding azomethine compounds and triethyl amine in presence of chloroacetyl chloride.[11] Moreover, the cyclocondensation of azolylindol Schiff bases and chloroacetyl chloride afforded new derivatives of 3-chloro-azetidin-2-one.[12] More recently, other new 2-azetidinones derivatives have been prepared by cycloaddition reaction of some Schiff bases with triethylamine and chloroacetyl chloride, the newly products exhibited good antibacterial activity.[13] (Fig. 1).

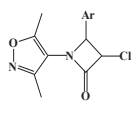
So, based on the facts above and as a part of our interesting, this present work aims to design and development of new 2-azetidinone derivatives and investigation of their antimicrobial activities.



benzimidazolyloxazolyl 2-azetidinones [10]



3-chloro-4-(1H-indol-2-yl)-1-(4-(4-methoxyphenyl)thiazol-2-yl)azetidin-2-one [12]



4-aryl-3-chloro-1-(3,5-dimethyl-4-isoxazol-2-yl)-azeidine-2-one [13]

Figure 1. Some of the recently prepared of 2-azetidinone derivatives.

METHODOLOGY

Chemicals and all reagents and solvents have been used as received from their suppliers BDH, Fluka, Merck, and Sigma-Aldrich companies without more purification. The reactions progress was monitored on pre-coated aluminum plates by thin layer chromatography (TLC) technique. Melting points were measured in Celsius degree on open-capillary Electro thermal apparatus and are uncorrected. IR spectra were recorded on FTIR Shimadzu (8400s) spec-

trophotometer using KBr disc. The spectra of ^{1}H NMR and ^{13}C NMR have been outlined with Bruker spectrometer in DMSO-d_6 solvent, (400 and 100 MHz), respectively, and expressed as part per million (δ ppm) downfield from tetramethylsilane (TMS as an internal standard reference). Elemental analysis of the (C, H, N) percentages were found with Elemental Analyzer Model (Fison EA1108). For chemical structures drawing, Chem Draw Ultra (6.0) software application was operated.

***General procedure for the preparation of dimethyl pyrazine-2,3-dicarboxylate,* 2:** The standard esterification procedure was applied to convert pyrazine-2,3-dicarboxylic acid **1** into corresponding diester **2.**[14]

***General procedure for the preparation of pyrazine-2,3-dicarbohydrazide,* 3:** According to the Smith procedure,[15] compound **3** was prepared by hydrazinolysis of **2.**

***General procedure for the synthesis of N'², N'³-bis[(E)(substituted-pyridine-2-yl) methylidene] pyrazine-2,3-dicarbohydrazide,* 4a-e:** A solution of (2 mmol) of appropriate pyridine-2-carbaldehyde in 20 mL of absolute ethanol was added to (1 mmol) of compound **3** and 3-4 drops of glacial acetic acid (GAA). The mixture was refluxed under stirring for 3h. After cooling at room temperature for 24h., the precipitated solid was filtered, washed with water, dried, and purified by recrystallization from ethanol.

General procedure for the synthesis of N,N'-bis[3-chloro-2-oxo-4-(substitutedpyridine-2-yl)-azetidin-1-yl] pyrazine-2,3-dicarboxamide, 5a-e: A solution (2 mmol) of chloroacetyl chloride and (2 mmol) of trimethylamine (TEA) in 10 mL of DMF was added with stirring to (1 mmol) of the suitable newly prepared Schiff base **4a-e** and refluxed for 4-5 h. under stirring, then the reaction flask contents were kept to cool and poured onto crushed ice. The solid was separated off by filtration, dried and recrystallized from dimethylsufoxide (DMSO).

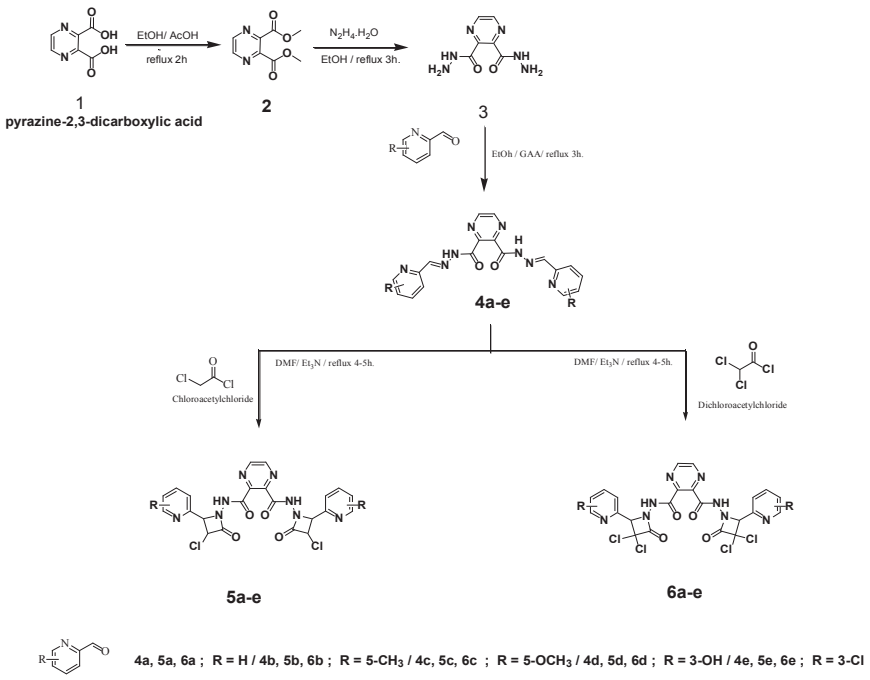
General procedure for the synthesis of N,N'-bis[3,3-dichloro-2-oxo-4-(substitutedpyridine-2-yl)-azetidin-1-yl] pyrazine-2,3-dicarboxamide, 6a-e: The same procedure mentioned above for compounds **5a-e** was applied for compounds **6a-e** except the dichloroacetyl chloride was used instead of chloroacetyl chloride.

Table 1. Physicochemical properties of all synthesized compounds

Compound	R	Yield (%)	m.p (°C)	M.wt. (g/mol.)	Empirical Formula	Anal. (calcd.) / found %
2		87	155 (dec.)	196.16	$C_8H_8N_2O_4$	(C, 48.98; H, 4.11; N, 14.28) / C, 48.92; H, 4.08; N, 14.25
3		82	117-118	196.17	$C_6H_8N_6O_2$	(C, 36.74; H, 4.11; N, 42.84) / C, 36.80; H, 4.14; N, 42.89
4a	H	69	188-189	374.36	$C_{18}H1_4N_8O_2$	(C, 57.75; H, 3.77; N, 29.93) / C, 57.70; H, 3.73; N, 29.88
4b	5-CH_3	73	203-205	402.41	$C_{20}H_{18}N_8O_2$	(C, 59.69; H, 4.51; N, 27.85) / C, 59.62; H, 4.48; N, 27.82
4c	5-OCH_3	66	212-214	434.41	$C_{20}H_{18}N_8O_4$	(C, 55.30; H, 4.18; N, 25.79) / C, 55.24; H, 4.15; N, 25.75
4d	3-OH	81	241-242	406.36	$C_{18}H_{14}N_8O_4$	(C, 53.20; H, 3.47; N, 27.58) / C, 53.17; H, 3.45; N, 27.54
4e	3-Cl	68	198-200	443.25	$C_{18}H_{12}Cl_2N_8O_2$	(C, 48.77; H, 2.73; N, 25.28) / C, 48.72; H, 2.70; N, 25.24
5a	H	72	188-189	527.32	$C_{22}H_{16}Cl_2N_8O_4$	(C, 50.11; H, 3.06; N, 21.25) / C, 50.08; H, 3.04; N, 21.22
5b	5-CH_3	74	191-194	555.37	$C_{24}H_{20}Cl_2N_8O_4$	(C, 51.90; H, 3.63; N, 20.18) / C, 51.87; H, 3.61; N, 20.14
5c	5-OCH_3	65	199-201	587.37	$C_{24}H_{20}Cl_2N_8O_6$	(C, 49.08; H, 3.43; N, 19.08) / C, 49.03; H, 3.40; N, 19.05
5d	3-OH	79	256-259	559.32	$C_{22}H_{16}Cl_2N_8O_6$	(C, 47.24; H, 2.88; N, 20.03) / C, 47.21; H, 2.86; N, 20.00
5e	3-Cl	69	190-192	596.21	$C_{22}H_{14}Cl_4N_8O_4$	(C, 44.32; H, 2.37; N, 18.79) / C, 44.27; H, 2.34; N, 18.74
6a	H	78	203-204	596.21	$C_{22}H_{14}Cl_4N_8O_4$	(C, 44.32; H, 2.37; N, 18.79) / C, 44.28; H, 2.37; N, 18.75
6b	5-CH_3	82	218-220	624.26	$C_{24}H_{18}Cl_4N_8O_4$	(C, 46.18; H, 2.91; N, 17.95) / C, 46.13; H, 2.88; N, 17.91
6c	5-OCH_3	72	227-228	656.26	$C_{24}H_{18}Cl_4N_8O_6$	(C, 43.92; H, 2.76; N, 17.07) / C, 43.88; H, 2.73; N, 17.05
6d	3-OH	85	259-262	628.21	$C_{22}H_{14}Cl_4N_8O_6$	(C, 42.06; H, 2.25; N, 17.84) / C, 42.01; H, 2.22; N, 17.81
6e	3-Cl	70	187-189	665.10	$C_{22}H_{12}C_{l6}N_8O_4$	(C, 39.73; H, 1.82; N, 16.85) / C, 39.69; H, 1.80; N, 16.82

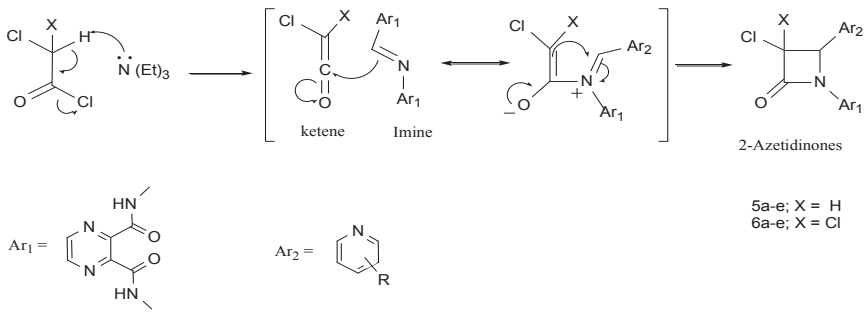
RESULTS AND DISSCUSION

1,3,4-Trisubstituted-2-Azetidinone derivatives **5a-e** and **6a-e**, as well as their Schiff bases **4a-e** have been synthesized according to the synthetic route that predicted in Scheme 1.



Scheme 1. General synthetic pathway of the target compounds.

At the beginning, a standard esterification method was applied to convert pyrazine-2,3-dicarboxilic acid **1** to the corresponding diester compound **2** with good yield. Then the hyrazinolysis of 2 in refluxing ethanol afforded diacarbohydrazide **3** which further condensed with some derivatives of pyridine-2-carbaldehyde in acidic medium of absolute ethanol solution under reflux and stirring to yield new azomethine (Schiff bases) derivatives **4a-e**. Finally, the new derivatives of target 2-azetidinone compounds **5a-e** were synthesized by [2+2] cycloaddition reaction between chloroacetochloride and newly prepared Schiff bases in presence of triethylamine (TEA) in DMF under reflux. By the same procedure, the other designed 2-azitidinones **6a-e** have been prepared in presence of dichloroacetochloride instead of chloroacetochloride as a ketene compound according to an imine-ketene mechanism, Scheme 3. [16]



Scheme 2. Proposed mechanism for 2-azetidinones derivatives 5a-e and 6a-e.

The chemical structures of all newly synthesized compounds have been established from their IR, ^{1}H NMR, and ^{13}C NMR which gave the comfortable results for the desired structures. FTIR spectrum of **2**, (Fig. 2) showed the absence the bands due to hydroxyl group stretching vibration of raw material while the new bands were observed at 2955 cm^{-1} and 1687 cm^{-1} which belonging to the stretching vibrations of aliphatic C-H and ester carbonyl C=O groups, respectively. On the other hand, the more significant peak due to carboxylic acid protons of raw material was disappeared in the ^{1}H NMR spectrum of compound **2**, by contrast the methoxy protons as a singlet signal was noted at 3.98 ppm, (Fig. 3).

Concerning compound 3, the FTIR spectrum showed stretching absorption bands due to NH_2 and NH group in the region 3441-3283 cm^{-1} and another band at 1682 cm^{-1} corresponded to amidic C=O group, (Fig. 4). Furthermore, ^{1}H NMR spectrum of 3 confirmed the presence of NH and NH_2 protons when new signals were recorded at 7.52 ppm and 2.50 ppm as shown in (Fig. 5).

As for newly Schiff bases **4a-e**, ^{1}H NMR spectrum of **4a** and **4c**, (Fig. 6 and Fig. 7) revealed the more characteristic peak of azomethine proton (CH=N) at 8.04 and 7.84 ppm, respectively. In addition to the microanalysis, IR and ^{1}H NMR spectra, the ^{13}C NMR spectra of newly synthesized 2-azetidinones **5a-e** and **6a-d** as shown in (Fig. 9) and (Fig. 10), respectively**,** displayed several signals with full coincidence of the proposed structures beside the other spectral data which are given in Table 2.

Biological Studies

Antimicrobial activities of the newly compounds **5a-e** and **6a-e** have been screened against two bacterial strains*; Staphylococcus Sciuri* as gram positive *and Escherichia Coli* as gram negative, as well as against fungal strains; *Candida Albicans* and *Aspergillus Flavus*, by diffusion method.[17] The tested compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare 100 µg/mL concentration. The plates of bacterial culture were incubated at 37 °C for 24h. while for fungal culture were incubated at 25 °C and tested after 72 h. Cefuroxime and fluconazole were used (as antibacterial and antifungal drugs), respectively. The growth inhibition ability of the tested compounds was measured as inhibition zone diameter in milliliters. The results are listed in Table 3, showed good to excellent activities of the examined newly 2-azetidinones.

Table 2. Spectral data of all synthesized compounds

Spectral data
2) ***dimethyl pyrazine-2,3-dicarboxylate*** IR (KBr, cm^{-1}): 3086 (Ar. C-H), 2955 (Ali. C-H), 1687 (ester C=O),1645 (C=N), 1290 (C-O-C). ^{1}H NMR (DMSO-d_6, δ, ppm): 9.52-9.28 (s, 2H, 2-pyrazine), 3.52 (s, 6H, OCH_3).**3)** ***pyrazine-2,3-dicarbohydrazide*** IR (KBr, cm^{-1}): 3441-3283 (NH), 3088 (Ar. CH), 1682 (C=O), 1654 (C=N), 1280 (C-N). ^{1}H NMR, (DMSO-d_6, δ, ppm): 9.43 (s, 2H, 2- Pyrazine), 7.52 (s, 2H, NH), 2.50 (s, 4H, NH_2).
4a) ***N'²,N'³-bis[(E)-(pyridine-2-yl)methylidene]pyrazine-2,3-dicarbohydrazide*** IR (KBr) (v, cm^{-1}): 3386-3182 (NH), 3085 (Ar. C-H), 1679 (C=O), 1642 (C=N), 1594 (C=C), 1238-1108 (C-N). ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 6.95 (s, 2H, NH), 7.47 (s, 2H, N=CH), 7.87-8.82 (m, 8H, pyridine Ring), 9.37 (s, 2H, 2-pyrazine).
4b) ***N'²,N'³-bis[(E)-(5-methylpyridine-2-yl)methylidene]pyrazine-2,3-dicarbohydrazide*** IR (KBr) (*υ*, cm^{-1}): 3349-3162 (NH), 3095 (Ar. C-H), 2988 (Ali. C-H), 1672 (C=O), 1642 (C=N), 1595 (C=C), 1230-1110 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.49 (s, 6H, Ar-CH_3), 6.25 (s, 2H, NH), 7.35 (s, 2H, N=CH), 7.99-8.86 (m, 6H, pyridine Ring), 9.46 (s, 2H, 2-pyrazine).
4c) ***N'²,N'³-bis[(E)-(5-methoxypyridine-2-yl)methylidene]pyrazine-2,3-dicarbohydrazide*** IR (KBr) (*υ*, cm^{-1}): 3404-3210 (NH), 3092 (Ar. C-H), 2995 (Ali. C-H), 1685 (C=O), 1650 (C=N), 1587 (C=C), 1232-1110 (C-N). ^{1}H NMR

(400 MHz, DMSO-d_6) δ (ppm): 3.42 (s, 6H, Ar-OCH_3), 7.04 (s, 2H, NH), 7.21 -7.84 (m, 6H, pyridine Ring and s, 2H, N=CH), 8.54 (s, 2H, 2-pyrazine).

4d) ***N'²,N'³-bis[(E)-(3-hydroxypyridine-2-yl)methylidene]pyrazine-2,3-dicarbohydrazide*** IR (KBr) (*υ*, cm^{-1}): 4487 (OH), 3349-3210 (NH), 3082 (Ar. C-H), 1678 (C=O), 1640 (C=N), 1595 (C=C), 1222-1108 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.88 (s, 2H, Ar. OH), 6.83 (s, 2H, NH), 7.72 (s, 2H, N=CH), 7.80-8.85 (m, 6H, pyridine Ring), 9.60 (s, 2H, 2-pyrazine).

4e) ***N'²,N'³-bis[(E)-(3-chloropyridine-2-yl)methylidene]pyrazine-2,3-dicarbohydrazide*** IR (KBr) (*υ*, cm^{-1}): 3382-3195 (NH), 3088 (Ar. C-H), 1680 (C=O), 1656 (C=N), 1590 (C=C), 1248-1115 (C-N), 721 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.71 (s, 2H, NH), 7.04 (s, 2H, N=CH), 7.97-8.85 (m, 6H, pyridine Ring), 9.52 (s, 2H, 2-pyrazine).

5a) ***N,N'-bis[3-chloro-2-oxo-4-(pyridine-2-yl)-azetidin-1-yl] pyrazine-2,3-dicarboxamide*** IR (KBr) (*υ*, cm^{-1}): 3249-3181 (NH), 3095 (Ar. CH), 1722 (C=O, β-lactam), 1668 (C=O, sec. amide), 1642 (C=N), 1598 (C=C), 721 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.40 (s, 2H, NC_3H, β-lactam), 6.72 (s, 2H, C_3H-Cl, β-lactam), 7.38-7.62 (m, 8H, Ar. H, and 2H, NH), 9.35 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 62.8 (N-CH-C-Cl, azetidine ring), 65.8 (C-Cl azetidine ring), 120.2-137.4 (aromatic carbons), 143.2 (C-N pyridine ring), 152.5 (C=N, pyridine ring), 163.2 (N-C=O, azetidine ring), 169.5 (HNCO-Ar).

5b) ***N,N'-bis[3-chloro-2-oxo-4-(5-methylpyridine-2-yl)-azetidin-1-yl]pyrazine-2,3-dicarboxamide*** IR (KBr) (*υ*, cm^{-1}): 3345-3210 (NH), 3080 (Ar. CH), 2995 (Ali. CH), 1710 (C=O, β-lactam), 1665 (C=O, sec. amide), 1638 (C=N), 1588 (C=C), 724 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.48 (s, 6H, Ar. CH_3), 4.85 (s, 2H, NC_3H, β-lactam), 5.80 (s, 2H, C_3H-Cl, β-lactam), 7.24-8.72 (m, 6H, Ar. H, and 2H, NH), 8.86 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.2 (Ar. CH_3), 62.5 (N-CH-C-Cl, azetidine ring), 66.2 (C-Cl azetidine ring), 120.2-138.7 (aromatic carbons), 141.8 (C-N pyridine ring), 155.0 (C=N, pyridine ring), 163.4 (N-C=O, azetidine ring), 169.8 (HNCO-Ar).

5c) ***N,N'-bis[3-chloro-2-oxo-4-(5-methoxypyridine-2-yl)-azetidin-1-yl]pyrazine-2,3-dicarboxamide*** IR (KBr) (*υ*, cm^{-1}): 3249-3181 (NH), 3097 (Ar. CH), 2988 (Ali. CH), 1698 (C=O, β-lactam), 1675 (C=O, sec. amide), 1648 (C=N), 1590 (C=C), 719 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.71 (s, 6H, Ar. OCH_3), 5.02 (s, 2H, NC_3H, β-lactam), 5.48 (s, 2H, C_3H-Cl, β-lactam), 7.38-8.28 (m, 6H, Ar. H, and 2H, NH), 9.08 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 54.8 (Ar. OCH_3), 62.8 (N-CH-C-Cl, azetidine ring),

64.0 (C-Cl azetidine ring), 121.0-137.9 (aromatic carbons), 149.4 (C-N pyridine ring), 157.5 (C=N, pyridine ring), 164.2 (N-C=O, azetidine ring), 170.3 (HNCO-Ar).

5d) ***N,N'-bis[3-chloro-2-oxo-4-(3-hydroxypyridine-2-yl)-azetidin-1-yl]pyrazine-2,3-dicarboxamide*** IR (KBr) (υ, cm^{-1}): 3442 (OH), 3342-3152 (NH), 3087 (Ar. CH), 1712 (C=O, β-lactam), 1664 (C=O, sec. amide), 1644 (C=N), 1598 (C=C), 722 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.04 (s, 2H, Ar. OH), 4.96 (s, 2H, NC_3H, β-lactam), 5.30 (s, 2H, C_3H-Cl, β-lactam), 7.25-8.15 (m, 6H, Ar. H, and 2H, NH), 8.82 (s, 2H, 2-pyrazine). ^{13}C

NMR (100 MHz, DMSO-d_6) δ (ppm): 62.6 (N-CH-C-Cl, azetidine ring), 64.5 (C-Cl azetidine ring), 120.5-138.8 (aromatic carbons), 151.2 (C-N pyridine ring), 163.2 (C=N, pyridine ring), 164.2 (N-C=O, azetidine ring), 170.8 (HNCO-Ar).

5e) ***N,N'-bis[3-chloro-2-oxo-4-(3-chloropyridine)-azetidin-1-yl] pyrazine-2,3-dicarboxamide*** IR (KBr) (υ, cm^{-1}): 3395-3252 (NH), 3010 (Ar. CH), 1708 (C=O, β-lactam), 1671 (C=O, sec. amide), 1653 (C=N), 1591 (C=C), 721 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.84 (s, 2H, NC_3H, β-lactam), 5.52 (s, 2H, C_3H-Cl, β-lactam), 7.11-7.98 (m, 6H, Ar. H, and 2H, NH), 8.92 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 63.2 (N-CH-C-Cl, azetidine ring), 63.8 (C-Cl azetidine ring), 119.8-136.8 (aromatic carbons), 152.8 (C-N pyridine ring), 161.8 (C=N, pyridine ring), 164.4 (N-C=O, azetidine ring), 170.2 (HNCO-Ar).

6a) ***N,N'-bis[3,3-dichloro-2-oxo-4-(pyridine-2-yl)-azetidin-1-yl] pyrazine-2,3-dicarboxamide*** IR (KBr) (υ, cm^{-1}): 3385-3168 (NH), 1712 (C=O, β-lactam), 3098 (Ar. CH), 1674 (C=O, sec. amide), 1661 (C=N), 1601 (C=C), 721 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.80 (s, 2H, NC_3H, β-lactam), 7.35-8.62 (m, 8H, Ar. H, and 2H, NH), 9.45 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 72.3 (N-CH-C-Cl_2, azetidine ring), 92.3 (C-Cl_2 azetidine ring), 120.8-137.8 (aromatic carbons), 148.4 (CH=C-N pyridine ring), 154.6 (N-C=O, azetidine ring), 163.2 (C-C=N-C, pyridine ring), 169.8 (HNCO-Ar).

6b) ***N,N'-bis[3,3-dichloro-2-oxo-4-(5-methylpyridine-2-yl)-azetidin-1-yl]pyrazine-2,3-dicarboxamide*** IR (KBr) (υ, cm^{-1}): 3282-3144 (NH), 3083 (Ar. CH), 2992 (Ali. CH), 1704 (C=O, β-lactam), 1666 (C=O, sec. amide), 1639 (C=N), 1596 (C=C), 722 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.26 (s, 6H, Ar. CH_3), 4.79 (s, 2H, NC_3H, β-lactam), 7.38-8.82 (m, 6H, Ar. H, and 2H, NH), 9.22 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.8 (Ar. CH_3), 72.7 (N-CH-C-Cl_2, azetidine ring), 97.8 (C-Cl_2 azetidine ring), 120.4-136.2 (aromatic carbons), 151.0 (CH=C-N pyridine ring), 160.3 (N-C=O,

azetidine ring), 163.7 (C-C=N-C, pyridine ring), 169.2 (HNCO-Ar).

6c) ***N,N'-bis[3,3-dichloro-2-oxo-4-(5-methoxypyridine-2-yl)-azetidin-1-yl]pyrazine-2,3-dicarboxamide*** IR (KBr) (υ, cm^{-1}): 3393-3172 (NH), 3010 (Ar. CH), 2998 (Ali. CH), 1712 (C=O, β-lactam), 1672 (C=O, sec. amide), 1648 (C=N), 1590 (C=C), 719 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.03 (s, 6H, Ar. OCH_3), 4. 90 (s, 2H, NC_3H, β-lactam), 7.25-8.90 (m, 6H, Ar. H, and 2H, NH), 9.02 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 55.4 (Ar. OCH_3), 72.0 (N-CH-C-Cl_2, azetidine ring), 96.5 (C-Cl_2 azetidine ring), 119.8-137.8 (aromatic carbons), 151.4 (CH=C-N pyridine ring), 161.7 (N-C=O, azetidine ring), 162.8 (C-C=N-C, pyridine ring), 170.8 (HNCO-Ar).

6d) ***N,N'-bis[3,3-dichloro-2-oxo-4-(3-hydroxypyridine)-azetidin-1-yl] pyrazine-2,3-dicarboxamide*** IR (KBr) (υ, cm^{-1}): 3455 (OH), 3297-3185 (NH), 3010 (Ar. CH), 1721 (C=O, β-lactam), 1676 (C=O, sec. amide), 1648 (C=N), 1596 (C=C), 721 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.19 (s, 2H, Ar. OH), 5.48 (s, 2H, NC_3H, β-lactam), 7.02-7.70 (m, 6H, Ar. H, and 2H, NH), 8.92 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 73.4 (N-CH-C-Cl_2, azetidine ring), 97.0 (C-Cl_2 azetidine ring), 120.8-136.0 (aromatic carbons), 151.8 (CH=C-N pyridine ring), 161.6 (N-C=O, azetidine ring), 163.5 (C-C=N-C, pyridine ring), 169.8 (HNCO-Ar).

6e) ***N,N'-bis[3,3-dichloro-2-oxo-4-(3-chloropyridine)-azetidin-1-yl] pyrazine-2,3-dicarboxamide*** IR (KBr) (υ, cm^{-1}): 3284-3180 (NH), 3080 (Ar. CH), 1706 (C=O, β-lactam), 1667 (C=O, sec. amide), 1642 (C=N), 1599 (C=C), 723 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.93 (s, 2H, NC_3H, β-lactam), 6.98-8.02 (m, 6H, Ar. H, and 2H, NH), 8.82 (s, 2H, 2-pyrazine). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 74.2 (N-CH-C-Cl_2, azetidine ring), 98.3 (C-Cl_2 azetidine ring), 122.2-137.4 (aromatic carbons), 151.6 (CH=C-N pyridine ring), 161.5 (N-C=O, azetidine ring), 164.2 (C-C=N-C, pyridine ring), 169.8 (HNCO-Ar).

Table 3. Antibacterial and antifungal activities of newly synthesized compounds

	Zone of inhibition (mm) bacterial strains		fungal strains	
compd.	**Staph. Sciuri**	**Escherichia Coli**	**Aspergillus Flavus**	**Candida Albicans**
5a	14	14	17	21
5b	18	17	17	16
5c	13	11	21	21
5d	22	20	20	17
5e	16	18	18	18
6a	16	13	15	14
6b	19	16	19	16
6c	22	21	13	20
6d	17	20	19	19
6e	15	19	18	22
cefuroxime	18	22		
fluconazole			20	24

*concentration (100 µg/mL), diameter (mm); milliliter.

Variety derivatives of 2-azetidinone and related Schiff bases have been synthesized in good yields and cost-effective procedures. A coincidence for the proposed structures was achieved as deduced from their physiochemical and spectroscopic data. The newly 2-azetidinone compounds were screened for their antibacterial and antifungal properties. The results showed that most of these compounds have a good to excellent antimicrobial activities.

ACKNOWLEDGEMENT

Authors are thankful to the ministry of science and technology, as well as to the university of Al-Mustansiriayah, Bagdad-Iraq for operating most of this work.

REFERENCES

1. Fleming, A. On the Antibacterial Action of Cultures of Apenicillium, With Special Reference to Their Use in The Isolation of B. Influenza. *Br. J. Exp. Pathol.* **1929**, *10*, 226-236.

2. Knowles, J. R. Penicillin Resistance: The Chemistry of Beta-Lactamase Inhibition. *Acc. Chem. Res.* **1985**, *18*, 97-104.

3. Patel, N. B.; Patel, J. C. Synthesis and Antimicrobial Activity of Schiff Bases and 2-Azetidinones Derived from Quinazolin-4(3H)-One. *Arabian J. Chem.* **2011**, *4*, 403-411.

4. Gurupadayya, B. M.; Gopal, M.; Padmashaly, B.; Manohara, Y. Synthesis and Pharmacological Evaluation of Azetidin-2-ones and Thiazolidin-4-ones Encompassing Benzothiazole. *Indian J. Pharm. Sci.* **2008**, *70*, 572-577.

5. Ishwar, B. K.; Mubeen, M.; Kalluraya, B. Synthesis and Pharmacological Studies of Some Azetidinones bearing Ibuprofen Moiety. *Ind. J. Heterocycl. Chem.* **2003**, *13*, 183-184.

6. Gilani, S. J.; Alam, O.; Khan, S. A.; Siddiqui, N.; Kumar, H. Synthesis of Some Derived Thiazolidin-4-one, Azetidin-2-one and 1, 3, 4-Oxadiazole Ring System from Isonicotinic Acidhydrazide: A Novel Class of Potential Anticonvulsant Agent. *Der. Pharm. Lett.* **2009**, *1*, 1-8.

7. Mohan, J.; Kumar, A. Condensed Bridgehead Nitrogen Heterocyclic Systems: Synthesis, Bioactivity and Stereochemistry of Pyrazolo [3′,4′:4,5] Thiazolo[3,2-b]-s-Triazoles. *Ind. J. Heterocycl. Chem.* **2003**, *13*, 97–100.

8. Halve, A. K.; Bhadauria, D.; Dubey, R. N/C-4 Substituted Azetidin-2-ones: Synthesis and Preliminary Evaluation as New Class of Antimicrobial Agents. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 341-345.

9. Jin, X.; Zheng, C. J.; Song, M. X.; Wu, Y.; Sun, L.; Piao, H. Synthesis and Antimicrobial Evaluation of L-Phenylalanine-Derived C5-Substituted Rhodanine and Chalcone Derivatives Containing Thiobarbituric Acid or 2-Thioxo-4-Thiazolidinone. *Eur. J. Med. Chem.* **2012**, *56*, 203-209.

10. Kishore, B.; Brahameshwari, G. Synthesis and Antimicrobial Activity of Benzimidazolyl Oxazolyl Thiazolidine-4-ones and Azetidine-2-ones. *Indian J. Chem. Sec. B.* **2018**, *57B*, 1042-1050.

11. Jaberi, H. R.; Fattahi, H.; Ahmannasrollahi, A.; Yarandpour, M.; Sedaghatizadeh, S. Synthesis of New 2-Azetidinone Derivatives and Related Schiff Bases from 3-Phenyl-2,3,6,7-tetrahydroimidazo [2,1-b] Thiazolo [5,4-d] Isoxazole. *Org. Chem. Res.* **2019**, *5*, 42-50.

12. Sankar, P. S.; Divya, K.; Dinneswara, R. G.; Padmavathi, V.; Grigory, V. Z. Synthesis, Characterization and Antimicrobial Activity of Azetidinone and Thiazolidinone Derivatives. *AIP Conf. Proc.* [Online] **2019**, *2063*, 040047-1.

13. Marri, S.; Kakkerla, R.; Krishna, M. P. S. Synthesis, QSRT Studies and Antibacterial Activity of 4-Aryl-3-chloro-1-(3,5-dimethyl isoxazol-4-yl)-azetidine-2-ones. *Indian J. Chem. Sec. B.* **2019**, *58B*, 381-386.

14. Nikpour, F.; Kazemi, S.; Sheikh, D. A Facile and Convenient Synthesis of 1H-Isoindole-1,3(2H)-diones. *Heterocycles.* **2006**, *68*, 1559-1568.

15. Brian, S. F.; Antony, J. H.; Peter, W. G. S.; Austin, R. T. *In Practical Organic Chemistry,* 5th ed.; Vogel's, Eds.; Longman Scientific Technical: New York, 1989; pp. 1077-1079.

16. Gupta, A.; Halve, A. K. Synthesis & Antifungal Screening of Novel Azetidin-2-ones. *Open Chemistry Journal.* **2015**, *2*, 1-6.

17. Arthington-Skaggs, B.; Motley, M.; Morrison, C. J. Comparative Evaluation of PASCO and National Committee for Clinical Laboratory Standards M-27-A Broth Microdilution Methods for Antifungal Drug Susceptibility Testing of Yeast. *J. Clin. Microbiol.* **2000**, *38*, 2254-2260.