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THE INTERNATIONAL ARABIC JOURNAL OF ANTIMICROBIAL AGENTS

2013
Vol. 3 No. 3:1
doi: 10.3823/733

Antimicrobial efficacy of n-[3-chloro-(substituted aryl)-4-oxoazetidin-1-yl] pyridine-4-carboxamides against resistant bacterial strains obtained from clinical isolates

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Abstract

Background: the treatment of infectious diseases is still an important and challenging problem due to the emergence of numerous infectious diseases and increasing number of multi-drug resistant microbial pathogens which cause a variety of illnesses ranging from hospital-acquired pneumonia, bloodstream infections and urinary tract infections from catheters, abdominal infections and even meningitis.

Material and Methods: the main objective of the present study was to evaluate the antimicrobial efficacy of the synthesized 2-azetidinones against standard and resistant bacterial strains, assayed *in vitro* by the two-fold broth dilution technique.

Results: the tested 2-azetidinones exhibited antimicrobial efficacy comparable to the standard drugs Ampicillin and Griseofelvin. Among the tested compounds, N-[3-chloro-2-(2,5-dimethoxyphenyl)-4-oxoazetidin-1-yl] pyridine-4-carboxamide (5o) exhibited the highest activity with MIC of 6.25 µg/mL (tested gram +ve and gram -ve bacteria), 1.56 µg/mL (*Aspergillus niger*) and 3.12 µg/mL (*Aspergillus terrus* and *penicillum chrysogenum*) respectively. Also the screened compounds, 5d, 5f, 5h, 5j and 5o exhibited pronounced activity against resistant strains of *E. coli*, *S. aureus* and *K. pneumonia* which were resistant to the standard antibiotic amoxycillin. The 2-azetidinones can thereby prove to be beneficial towards the development of anti-infectives for the treatment of infections caused by drug resistant microorganisms.

Keywords: 2-Azetidinone, Antimicrobial, Multi-drug resistant strains.

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Introduction

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram positive bacteria [1, 2]. Bacterial resistance has currently become a grave concern for physicians. Resistant bacteria, particularly *Staphylococci*, *Enterococci*, *Klebsiella pneumoniae* and *Pseudomonas* species are becoming common in healthcare institutions [3-5]. Bacterial resistance often results in treatment failure, which can have serious consequences, especially in critically ill patients. Inadequate empiric antibacterial therapy has been associated

with increased mortality rates in patients with bloodstream infections due to these resistant microorganisms [6, 7].

Multidrug-resistant Gram positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA) have become a serious problem in the medical community [8-10]. A study found that the rate of developed mutations in *Escherichia coli* is of the order of 10^{-5} per genome per generation, which is 1000 times as high as previous estimates, a finding which may be significant for the study and management of bacterial antibiotic resistance [11]. Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *S. aureus*, through a process called horizontal gene transfer. *E. coli* bacteria often carry multiple drug-resistance plasmids, and under stress, readily transfer these plasmids to other species. Thus, *E. coli* and the other enteric bacteria are important reservoirs of transferable antibiotic resistance [12]. Extended-spectrum beta-lactamase producing *E. coli* is also highly resistant to an array of antibiotics, and infections by these strains are difficult to treat [13]. Infection with carbapenem-resistant *Enterobacteriaceae* (CRE), especially carbapenem-resistant *Klebsiella pneumonia* (CRKP) is also emerging as an important challenge in healthcare settings [14, 15]. Antibacterial drug resistance also places an added burden on healthcare costs, although its full economic impact remains to be determined.

The 2-azetidinone (β-lactam) ring system is the common structural feature of a number of broad spectrum β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, nocardicins, monobactams, clavulanic acid, sulbactam and tazobactam; which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases [16]. The success of these antibiotics is due to their bactericidal activity, good pharmacokinetics and low host toxicity. Research efforts in this class led to preparation of semi-synthetic and synthetic monobactam analogues (2-azetidinones) like aztreonam and carumonam, active mostly against Gram-negative bacteria.

Currently several 2-azetidinones are being extensively studied as promising anti-bacterial agents. Recently Singh et al. described the synthesis of new 1-alkyl/cyclohexyl-3, 3-diaryl-1'-methylspiro [azetidine-2, 3'-indoline]-2', 4-diones with promising antibacterial activity [17]. Halve et al. developed a series of 3-chloro-4-(3-methoxy-4-acetyloxyphenyl)-1-[3-oxo-3-(phenylamino) propanamido] azetidin-2-ones, and 3-chloro-4-[2-hydroxy-5-(nitro substituted phenylazo)phenyl]-1-phenylazetidin-2-ones and assayed there *in vitro* growth inhibitory activity against pathogenic micro-organisms [18], 2-{2-[3-chloro-2-(aryl)-4-oxoazetidin-1-ylamino]-acetylamino} benzothiazole-6-carboxylic acid derivatives were synthesized by Chavan et al. with good to moderate antibacterial activity [19]. Various 4-alkylidenazetidin-2-ones [20], 1'-[(Benzimidazol-2-yl)thioacetamidyl-3-(phenylamino)propanamido]azetidin-2-ones [21], 1-substituted-3, 3- diaryl- 4- [2'-(o-diarylacyl) hydroxyphenyl]-2-azetidinones [22], were evaluated for their antibacterial activity with potent activity against multi-drug resistant pathogens. The 2-azetidinone-based heterocyclics are also reported to exhibit β-lactamase inhibitory activity [23, 24]. Due to their potential activity, the 2-azetidinone scaffold represents an attractive target for contemporary organic synthesis [25].

On the basis of the above findings, a series of N-[3-chloro-2-(substituted aromatic)-4-oxoazetidin-1-yl] pyridine-4-carboxamide analogues containing the 2-azetidinone ring were synthesized by our previously reported green route method of sonication from Schiff's bases of Isoniazid (INH).

Looking to the promising antimicrobial activity of some 2-azetidinones and in continuation of our earlier studies (Thomas, AB, Nanda, RK, Kothapalli, LP and Hamane, SC. Synthesis and biological evaluation of Schiff's bases and 2-azetidinones of isonicotiniyl hydrazone as potential antidepressant and nootropic agents. *Arabian Journal Chemistry* [In Press]), we were stimulated to explore the synthesized 2-azetidinone analogues for their antibacterial activity against resistant bacterial strains obtained from clinical isolates across hospitals in India.

Materials and Methods

Bacterial strains

The standard microbial strains selected for the study include *Bacillus subtilis* (ATCC 9372, NCIM 2951), *Bacillus anthracis* (ATCC 14579, NCIM B9373), *Staphylococcus aureus* (ATCC 6538P, NCIM 2079), *Escherichia coli*(ATCC 9637,NCIM2563), *Enterobacter aerogenus* (NCIM 5139), *Pseudomonas aeroginosa* (ATCC 19429, NCIM 2036), *Aspergillus niger* (ATCC 10864, NCIM 616), *Penicillium chrysogenum* (ATCC 10002, NCIM 738) and *Aspergillus terrus* ATCC10020,NCIM657) which were procured from National Collection of Industrial Microorganisms (NCIM), Pune, India.

Resistant strains of *E. coli*, *S. aureus* and *K. pneumonia* were clinical isolates collected from hospitals across India (Obtained from MTCC Chandigarh, India; *Escherichia coli*: ATCC no: 25922, *Klebsiellia pneumoniae*: 29665, *Staphylococcus aureus*: 12598). The resistant strains received in lyophilized form, were revived and then sub cultured on Blood Agar medium. The colonies were initially identified by presumptive identification tests which included gram staining, motil-

ity, catalase, oxidase and slide coagulase testing, and they were further subjected to biochemical tests to confirm their identity. All bacterial strains were preserved in 15% glycerol broth at -80°C, thawed and sub cultured as test required.

Drugs

Ampicillin (Neiss Labs Ltd., Mumbai, India), amoxycillin (Hindustan Antibiotics Ltd., Pune, India) and griseofulvin (Merit Organics Ltd., Mumbai, India) were employed as the standard drugs for anti-microbial screening. N'-[(1Z)-(substituted aromatic) methylidene] pyridine-4-carbohydrazide (3a-q) and N-[3-chloro-2-(substituted aromatic)-4-oxoazetidin-1-yl] pyridine-4-carboxamides (5b-j, l-q) were synthesized in our laboratory and characterised by suitable spectroscopic and chromatographic tools.

Chemistry

N'-[(1Z)-(aryl/substituted aryl)methylidene] pyridine-4-carbohydrazides (Schiff's base)

The synthesis of compounds (3a-q) was performed according to our previously reported procedure [Scheme 1] [26]. The crude products upon recrystallisation from alcohol gave the pure Schiff's bases (3a–q). The synthesised compounds were characterised on the basis of their spectral and analytical data (UV, IR, ^{1}H NMR, MS, CHN).

N-[3-chloro-2-(substitutedaryl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide(2 azetidinones) using Schiff's bases

The 2-azetidinones (5b-j,l-q) were synthesized by our reported green route method of sonication [Scheme 2] [27]. The crude products on recrystallisation from suitable solvent yielded the pure 2-azetidinones of isonicotinoyl hydrazones. The synthesized compounds were characterized on the basis of their spectral and analytical data (UV, IR, ^{1}H NMR, MS, and CNH).

Evaluation of antimicrobial efficacy of compounds against standard anti-microbial strains

The antimicrobial susceptibility testing of the synthesized compounds (3a-q; 5b-j, l-q) were assayed *in vitro* by the two-fold broth dilution technique. Test drug solutions were dissolved in dimethylsulfoxide, diluted in culture media (Mueller–Hinton broth for bacteria and Sabouraud Liquid medium for fungi) to obtain final concentrations in the range of 0.78-1000 µg /mL and then assayed to determine the minimal inhibitory concentration (MIC, µg/mL). The MIC was determined as the lowest concentration that prevented visible growth of microorganisms after incubation for a specified period at a fixed temperature. The amount of inocula employed was 5 x 10^4 bacteria/mL and 1 x 10^3 fungi/mL. The MIC's were read after incubation at 37± 0.5° for antibacterial activity for 24 h and 48 h at 28 ± 0.5° for antifungal activity [28].

Table 1. Antibiotic sensitivity pattern of resistant bacterial strains obtained from clinical isolates.

Antibiotic	*E.coli*	*K. pneumoniae*	*S. aureus*
Amoxycillin	R	R	R
Erythromycin	R	R	S
Penicillin	R	R	R
Amoxycillin +	R	R	R
Cephalexin	R	R	R
Ciprofloxacin	R	R	R
Gentamicin	S	S	S
Cefotaxime	R	R	R
Cefuroxime	S	S	R
Linizolid	S	S	S
Ofloxacin	R	R	S
Levofloxacin	S	S	S
Netilmicin	S	S	S

S: sensitive; R: resistant.

Evaluation of antibacterial potency against resistant bacterial strains obtained from clinical isolates

The most potent compounds (5d,f,h,j,o) of the 2-azetidinone series were further evaluated for their activity against resistant strains of *E. coli, S. aureus* and *K. pneumonia* obtained from clinical isolates across India. The antibiotic sensitivity pattern for the bacterial strains are summarised in **Table 1.**

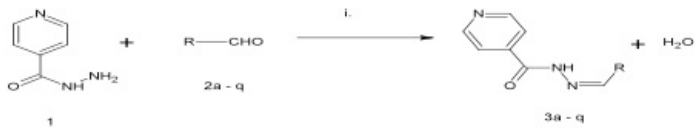
The antibiotic sensitivity testing was performed according to the standard Kirby Bauer method using Hi Media antibiotic discs. Sensitivity was analyzed by the presence/absence of zone of inhibition and comparison of the diameter of zone of inhibition with reference to the standard values. The antimicrobial testing was carried out by the broth dilution technique, and the minimum inhibitory concentrations were determined [29)].

Results and Discussion

Chemistry

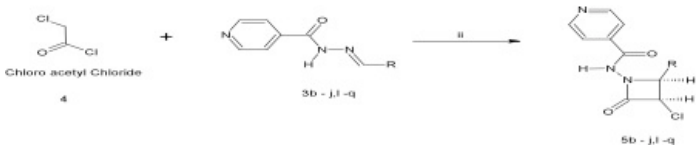
The synthetic strategies adopted to obtain the intermediate and target compounds are illustrated in **Schemes 1** and **2** respectively.

Scheme 1. Microwave assisted synthesis of intermediate Schiff's bases of INH (3a-q)



Reagents and conditions: i. H_2O, MWI, Power level 3 (240 W, 35% irradiation).

Scheme 2. Synthesis of 2-azetidinones (5b-j,l-q) using Schiff's bases



Reagents and conditions: ii. MS3A, Et_3N, CH_2Cl_2, sonication.

Table 2. Synthesized Schiff's bases of INH (3a-q).

Compound Code	Imines(3a-q)	Nature of R
3a	*N*′-[(1*Z*)-phenylmethylidene]pyridine-4-carbohydrazide	C_6H_5
3b	*N*′-[(1*Z*)-(2-hydroxyphenyl)methylidene]pyridine-4-carbohydrazide	C_6H_5O
3c	*N*′-[(1*Z*)-(4-hydroxyphenyl)methylidene]pyridine-4-carbohydrazide	C_6H_5O
3d	*N*′-[(1*Z*)-(4-chlorophenyl)methylidene]pyridine-4-carbohydrazide	C_6H_4Cl
3e	*N*′-[(1*Z*)-(3-chlorophenyl)methylidene]pyridine-4-carbohydrazide	C_6H_4Cl
3f	*N*′-[(1*Z*)-(4-nitrophenyl)methylidene]pyridine-4-carbohydrazide	$C_6H_4NO_2$
3g	*N*′-[(1*Z*)-(3-nitrophenyl)methylidene]pyridine-4-carbohydrazide	$C_6H_4NO_2$
3h	*N*′-[(1*Z*)-(2-nitrophenyl)methylidene]pyridine-4-carbohydrazide	$C_6H_4NO_2$
3i	*N*′-[(1*Z*)-(4-fluorophenyl)methylidene]pyridine-4- carbohydrazide	C_6H_4F
3j	*N*′-[(1*Z*)-(4-methoxyphenyl)methylidene]pyridine-4-carbohydrazide	C_7H_7O
3k	*N*′-[(1*Z*,2*E*)-3-phenylprop-2-en-1-ylidene]pyridine-4-carbohydrazide	C_8H_7
3l	*N*′-[(1*Z*)-(4-hydroxy-3-methoxyphenyl)methylidene]pyridine-4-carbohydrazide	$C_7H_7O_2$
3m	*N*′-[(1*Z*)-furan-2-ylmethylidene]pyridine-4-carbohydrazide	C_4H_3O
3n	*N*′-{(1*Z*)-[4-(dimethylamino)phenyl]methylidene}pyridine-4-carbohydrazide	$C_8H_{10}N$
3o	*N*′-[(1*Z*)-(2,5-dimethoxyphenyl)methylidene]pyridine-4-carbohydrazide	$C_8H_9O_2$
3p	*N*′-[(1*Z*)-(5-nitrothiophen-2-yl)methylidene]pyridine-4-carbohydrazide	$C_4H_2NO_2S$
3q	*N*′-[(1*Z*)-(2-hydroxynaphthalen-1-yl)methylidene]pyridine-4-carbohydrazide	C_9H_7O

Table 3. Synthesized 2-azetidinones (5b-j,l-q).

Compound Code	2-Azetidinones	Nature of R
5b	*N*-[3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_6H_5O
5c	*N*-[3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_6H_5O
5d	*N*-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_6H_4Cl
5e	*N*-[3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_6H_4Cl
5f	*N*-[3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	$C_6H_4NO_2$
5g	*N*-[3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	$C_6H_4NO_2$
5h	*N*-[3-chloro-2-(2-nitrophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	$C_6H_4NO_2$
5i	*N*-[3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_6H_4F
5j	*N*-[3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_7H_7O
5l	*N*-{3-chloro-2-oxo-4-[(*E*)-2-phenylethenyl]azetidin-1-yl}pyridine-4-carboxamide	C_8H_7
5m	*N*-[3-chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_4H_3O
5n	*N*-(3-chloro-2-furan-2-yl-4-oxoazetidin-1-yl)pyridine-4-carboxamide	$C_8H_{10}N$
5o	*N*-[3-chloro-2-(2,5-dimethoxyphenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	$C_8H_9O_2$
5p	*N*-[3-chloro-2-(5-nitrothiophen-2-yl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	$C_4H_2NO_2S$
5q	*N*-[3-chloro-2-(2-hydroxynaphthalen-1-yl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide	C_9H_7O

Substitution at R: 3a:C_6H_5; 3b/5b: 2-OHC_6H_4; 3c/5c: 4-OHC_6H_4; 3d/5d:4-ClC_6H_4; 3e/5e: 4-ClC_6H_4;3f/5f:4-$NO_2C_6H_4$;3g/5g:3-$NO_2C_6H_4$;3h/5h:2-$NO_2C_6H_4$;3i/5i:4-FC_6H_4;3j/5j:4-$OCH_3C_6H_4$;3k:C_8H_7;3l/5l:4-OH,3-$OCH_3C_6H_3$;3m/5m:2-C_4H_3O;3n/5n:4-$(CH_3)_2NC_6H_4$; 3o/5o: 2,5$(OCH_3)_2C_6H_3$; 3p/5p: $C_4H_2NO_2S$; 3q/5q:C_9H_7O

The N'-[(1Z)-(substituted aromatic) methylidene] pyridine-4-carbohydrazide(3a-q) were prepared in excellent yields in a one step reaction (Scheme 1) of isonicotinyl hydrazone (INH,1) with various substituted aryl/hetero aryl aldehydes (2a-q) in water using our previously reported microwave irradiation method in shorter reaction times (6-9 mins) with improved yields (86.7-99.2%). The synthesized intermediates were utilised for the synthesis of N-[3-chloro-2-(substitutedaromatic)-4-oxoazetidin-1-yl]pyridine-4-carboxamides(5b-j,l-q) containing 2-azetidinone nucleus by sonication (Scheme 2) with high yields (81.0-95.2%) in shorter reaction times (20-45 min). The compounds synthesized are enlisted in **Tables 2** and **3** respectively. Structures of the obtained compounds have been ascertained on the basis of their IR, ^{1}H NMR and Mass spectral assignments.

Evaluation of antimicrobial efficacy of compounds against standard anti-microbial strains

The antimicrobial activity of all compounds (3a-q; 5b-j,l-q) determined in terms of MIC, µg/mL are presented in **Tables 4** and **5** respectively. As reported earlier [30], the investigated intermediate Schiff's bases displayed very weak inhibition of growth of both bacteria and fungi with MIC in the range of 125-1000µg/mL.

The 2-azetidinones however exhibited moderate anti-microbial activity as compared to their respective Schiff's bases with MIC in the range of 6.25-200µg/mL against the tested bacterial strains and 1.56-200µg/mL against the tested fungal strains.

Among the N-[3-chloro-2-(substitutedaromatic)-4-oxoazetidin-1-yl]pyridine-4-carboxamide derivatives tested, compound 5o with a 2,5 dimethoxy substituted phenyl group at C-4 position of the azetidinone ring exhibited highest activity with MIC of 6.25 µg/mL against the tested bacterial strains (both gram +ve and gram –ve bacteria). However compound 5o exhibited more pronounced antifungal activity with MIC of 1.56 µg/mL against *A. niger* and 3.12 µg/mL against both *A. terrus* and *P. chrysogenum*.

Table 4. Antimicrobial activity of Schiff's bases of INH.

IMINES	Minimum Inhibitory Concentration (MIC)[a] in µg/mL								
	Gram positive bacteria			Gram negative bacteria			Fungi		
	Bacillus subtilis	Staphylococcus aureus	Bacillus anthracis	Escherichia coli	Klebsiella pneumonia	Enterobactor aerogenes	Aspergillus niger	Aspergillus terrus	Penicillium chrysogenum
3a-i	>250[b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]
3j	250	>250 [b]	>250 [b]	250	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]
3k-l	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]
3m	>250 [b]	>250 [b]	250	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]
3n	>250	250	250	250	>250 [b]	250	>250 [b]	250	>250
3o	250	125	250	125	250	250	250	125	125
3p	250 [b]	250	250	250	250	250	250	250	250
3q	>250[b]	>250	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]	>250 [b]
Amp[c]	3.13	3.13	3.13	3.13	3.13	3.13	--	--	--
Gre[d]	--	--	--	--	--	--	3.13	3.13	3.13

[a]MIC: Lowest concentration of an antimicrobial agent that significantly inhibits the visible growth of microorganism after a period of incubation ;[b] No inhibition up to a highest concentration of 250 µg/ml; [c]Amp: Ampicillin; [d]Gre: Greseofulvin.

Table 5. Antimicrobial activity of synthesized 2-azetidinones.

2-AZETIDINONES	Minimum Inhibitory Concentration (MIC)[a] in µg/mL								
	Gram positive bacteria			Gram negative bacteria			Fungi		
	Bacillus subtilis	Staphylococcus aureus	Bacillus anthracis	Escherichia coli	Klebsiella pneumonia	Enterobactor aerogenes	Aspergillus niger	Aspergillus terrus	Penicillium chrysogenum
5b	25	12.5	25	25	25	25	12.5	12.5	25
5c	12.5	25	25	12.5	12.5	12.5	25	12.5	12.5
5d	12.5	12.5	25	12.5	25	25	12.5	12.5	12.5
5e	25	25	100	100	200	100	200	100	100
5f	25	12.5	25	25	25	25	12.5	25	12.5
5g	100	50	25	200	100	50	200	100	100
5h	6.25	12.5	12.5	12.5	25	12.5	25	12.5	12.5
5i	12.5	25	25	12.5	12.5	25	12.5	25	25
5j	12.5	6.25	12.5	12.5	12.5	12.5	6.25	25	6.25
5l	12.5	12.5	12.5	25	12.5	25	12.5	25	12.5
5m	25	25	25	25	12.5	25	12.5	25	25
5n	25	12.5	25	25	25	25	12.5	12.5	12.5
5o	6.25	6.25	6.25	6.25	6.25	6.25	1.56	3.12	3.12
5p	25	50	25	100	200	100	200	50	50
5q	100	100	25	100	200	200	200	100	25
Amp[c]	3.13	3.13	3.13	3.13	3.13	3.13	--	--	--
Gre[d]	--	--	--	--	--	--	3.13	3.13	3.13

[a]MIC: Lowest concentration of an antimicrobial agent that significantly inhibits the visible growth of microorganism after a period of incubation ;[b] No inhibition up to a highest concentration of 250 µg/ml; [c]Amp: Ampicillin; [d]Gre: Greseofulvin.

It was observed that compound 5h, N-[3-chloro-2-(2-nitrophenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide exhibited good inhibitory activity with MIC of 6.25 µg/ml against the gram-positive bacteria strain *B. subtilis*.

Evaluation of antibacterial potency against resistant bacterial strains obtained from clinical isolates

The most potent compounds of the 2-azetidinone series (5d,f,h,j,o) were further evaluated against drug resistant bacterial strains *E. coli*, *S. aureus* and *K. pneumonia* obtained from clinical isolates. The standard antibiotic Amoxycillin was found to be resistant to the tested bacterial strains, *E. coli*, *S. aureus* and *K. pneumonia*. In comparison, the screened 2-azetidinones exhibited moderate inhibitory potency against the resistant strains with MIC ranging from 62.5-500 µg/mL (**Table 6**). Compound 5d, exhibited inhibitory activity with MIC of 62.5 µg/ml against resistant bacterial strain, *E. coli*.

Table 6. timicrobial activity of 2-azetidinones against resistant bacterial strains.

Minimum Inhibitory Concentration (MIC) in µg/mL			
2-Azetidinones	***S.aureus****	***E. coli****	***K. pneumonia****
5d	62.5	125	125
5f	125	125	125
5h	250	500	125
5j	125	125	125
5o	125	125	125
Am	R	R	R

* Resistant strains obtained from clinical isolates; am: Amoxycillin; R: Resistant.

Conclusions

In the present study, the antimicrobial efficacies of 2-azetidinones of isoniazid have been described. The synthesis of the N'-[(1Z)-(substituted aromatic) methylidene] pyridine-4-carbohydrazides and N-[3-chloro-2-(substituted aromatic)-4-oxoazetidin-1-yl] pyridine-4-carboxamides were carried out by us using green route methods of microwave irradiation and sonication resulting in improved yield with shorter reaction times. N-[3-chloro-2-(2,5-dimethoxyphenyl)-4-oxoazetidin-1-yl]pyridine-4-carboxamide (5o) exhibited the highest activity against the tested standard bacterial and fungal strains. The 2-azetidinones (5d,f,j,o) containing electron withdrawing (chloro, nitro, methoxy) substituent's on phenyl ring, exhibited pronounced inhibitory activity against amoxycillin resistant bacterial strains, *E. coli*, *S. aureus* and *K. pneumonia*. The 2-azetidinones thereby can prove to be beneficial towards the development of anti-infectives for the treatment of infections caused by drug resistant microorganisms.

Acknowledgements

The authors thank the Management and Principal Padm. Dr. D. Y. Patil Institute of Pharmaceutical Science and Research, Pune (India) for providing the necessary infrastructural facilities to carry out this work. The authors would like to thank Dr. Kishore Bhat, Professor and Head, Microbiology, Maratha Mandal's NGH institute of Dental Sciences, Karnataka, India for helping with the antibacterial screening against resistant microorganisms.

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