Biological evaluation of 2-azetidinones from 2-hydroxy-1-naphthaldehyde and amino-acid Schiff bases

S. Ramakrishna Rao [±], V. Selvamani [± ±] and S. Mahalakshmi [± ±]
[±] Govt. Degrees College, Srungavarapukota Kota, Vizianagaram District, Andhra Pradesh, INDIA.
[± ±] Post Graduate and Research Department of Chemistry, Pachaiyappa's College,
Chennai – 600030, Tamilnadu, INDIA.

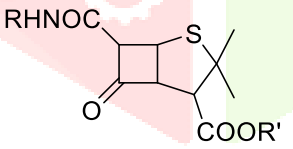
Abstract

The *in vitro* antimicrobial and antioxidant activities of 2-azetidinones synthesized from Schiff bases of 2-hydroxy-1-naphthaldehyde and amino acids were evaluated. The bacterial stains of *Staphyloccus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and the fungal stains of *Aspergillus niger*, *Aspergillus fumigates* and *Candida albicans* were used for the evaluation.

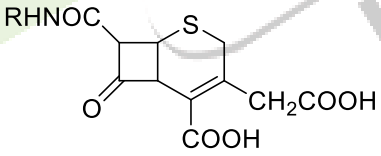
1. Introduction

The massive range of structures which organic compounds adopt, have ring system as a component and in particular, most of the rings are heterocyclic in nature. Many of the drugs contain heterocycles as the building blocks. The heterocycles have conquered the area of pharmaceutical chemistry over the course of many years.

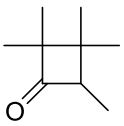
Extensively used antibiotic drugs, including the penicillins (i) and cephalosporins (ii), have their antibacterial activity because of the presence of a β-lactam structure in it. The simplest possible β-lactam is 2-azetidinone (iii). Besides its antibacterial activity, some other types of biological activities like antifungal, antitubercular and anticancer, have also been reported in compounds containing 2-azetidinone ring system [1].



(i)

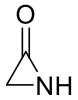


(ii)

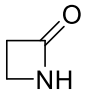


(iii)

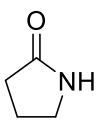
In general cyclic amides are called as lactams. Depending upon the size of the cyclic ring present in lactams, they are named as α-lactams (iv), β-lactams (v), γ-lactams (vi) and δ-lactams (vii).



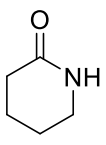
(iv)



(v)



(vi)



(vii)

The carbonyl derivatives of azetidines having carbonyl group at the position-2 are called 2-azetidinones. The nomenclature of 2-azetidinones simply the same as lactones. Lactams are azacycloalkanones, these are also known as 2-azetidinones or more generally β-lactams.

Simple and facile synthesis has been employed for the synthesis of the titled derivatives and it was previously reported by us (*J. Chem. and Chemical Sci.*, **7(5)**, 376 - 380, 2017) [2]. It includes, elimination of simple molecules from the substrates by condensation and uncomplicated cyclocondensation to afford the 4-membered heterocyclic derivatives.

Since 2-azetidinones are the key pharmacophore of most vital β-lactam antibiotics, many research articles and investigations were magnificently carried out in decades by the chemists and scholars. Beside its typical biological properties such as antimicrobial,[3] antibacterial,[4] antifungal,[5] anti-inflammatory,[6] antitubercular,[7] anticancer [8] and anticonvulsant [9]. It also functions as enzyme inhibitors and are active on the central nervous system [10]. Looking to the biological importance of 2-azetidinones, we report here the evaluation of the antimicrobial properties of the synthesized derivatives.

2. Experimental

2.1. Screening of antimicrobial activity

Two screening methods were employed to analyze and compare the antimicrobial properties of the synthesized compounds. They are,

i) Disk diffusion method, and

ii) Serial dilution method

Disk diffusion method [11]

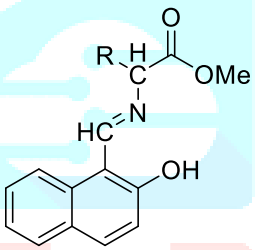
The culture medium was prepared by dissolving agar in 250 ml distilled water with warming and stirring to get uniform media. The medium was sterilized in autoclave at 15 psi pressure and 120 ^{o}C for 20 min. After the completion of the sterilization process, the culture media is taken out and immediately poured into petri dishes to form a uniform layer of thickness 2 mm to 5 mm. In order to use the microorganism-free petri dishes for the screening, the petri dishes were stored in incubator. Equal concentration of solutions (50 mg/ml) of the synthesized compounds and the reference substances were prepared. 10 µl of the solution of the synthesized compounds and reference standard were applied on the surface of the medium in triplicate. 50 mg/ml concentration of tetracycline, streptomycin, ampicillin trihydrate and clotrimazole were used as reference standards to observe the sensitivity of each tested microbial species. DMSO was used to prepare negative controls and dissolve the test compounds. The inoculated plates were incubated for 24 to 48 hrs. at 38 ^{o}C. After that, microorganism growth around each plate was observed. The clear zone around the sample and reference that has no growth is referred to as zone of inhibition. The inhibition zone is measured in mm, and the zones of the inhibition of the test sample and the reference are compared quantitatively to measure the antimicrobial activity of the samples.

Serial dilution method [12]

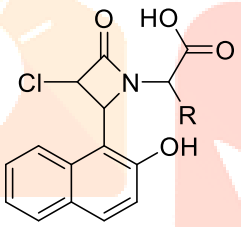
The *in vitro* antimicrobial properties of the synthesized compounds were screened against the microorganisms *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus fumigates* and

Candida albicans. Both the antibacterial and antifungal properties were assessed by Minimum Inhibitory Concentration (MIC) by this serial dilution method.

By using DMSO, various concentrations (6.25, 12.5, 25, 50, 100, 200, 500, and 1000 µg/ml) of each compound were prepared from their stock solution. Muller Hilton broth for bacteria and Sabouraud Dextrose broth for fungi were used as culture media. For evaluating Minimum Inhibitory Concentration, a standard drop of the microbial culture was added to the different concentration of compounds in their respective broths. The solutions were then incubated at 38 ^{o}C for 24 hrs. for bacteria and 30 - 32 hrs. for fungi. MIC is the lowest concentration of the test solution that will inhibit the visible growth of the microorganism after incubation. The zone of inhibition was determined by pouring the inoculated Muller Hilton agar and Sabouraud agar separately into the petri dishes. The poured material was allowed to set and after that 8 mm CUPS were prepared by punching into the agar surface with a sterile borer and scooping out the punched part of the agar. Using a sterile syringe, the test solution of the compounds was added in to these cups. The cups were incubated 38 ^{o}C for 24 hrs. for bacteria and 30 - 32 hrs. Noroxin and Miconazole were used as positive control.



3a-3d



4a-4d

R = $(CH_2)_3$, CH_2, -CH-$(CH_3)_2$-CH-, C_6H_5-CH-

3. Results & Discussion

3.1. Antimicrobial activity of compounds by Disc diffusion method

The synthesized derivatives were evaluated for their active *in vitro* antimicrobial properties against Gram-positive bacterial stains: *Staphylococcus aureus*, *Bacillus subtilis*, Gram-negative stains: *Pseudomonas aeruginosa*, *Escherichia coli*, and the Fungal stains: *Aspergillus niger*, *Aspergillus fumigates*, *Candida albicans* by disk diffusion method. The results were documented for each compound in terms of average diameter of inhibition zones (IZ) in mm. The reference drug ampicillin trihydrate (50 mg) dissolved in 1 ml of DMSO was used in each plate as reference standard.

The results given in Table-1 showed that, the compounds 3a-3d have only feeble inhibitory activity against the microorganisms. The fact is that, the electron withdrawing groups cause the increased inhibitory activity and the electron withdrawing groups lead to the decrease in inhibitory action. The compounds 3a-3d having electron donating alkyl groups, and it results decrease in the antimicrobial activity of the compounds considerably. Whereas, the titled derivatives 4a-4d, compared to the free Schiff bases, have showed good to moderate antimicrobial activity. The weak electron withdrawing effect of the chlorine atom increases the inhibitory action of these compounds profoundly.

Table - 1

Antimicrobial activity of compounds 3a-3d and 4a-4d (DDM)

Entry	Zone of inhibition (mm)						
	Bacterial stains				Fungal stains		
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*A. niger*	*A. fumigates*	*C. albicans*
3a	12	14	15	11	13	11	12
3b	11	13	13	10	11	12	13
3c	09	09	10	11	09	10	11
3d	10	11	13	10	12	11	10
4a	13	14	16	13	14	15	16
4b	15	14	12	15	16	15	15
4c	13	12	11	15	14	14	13
4d	23	24	23	18	21	19	20
Ampicillin trihydrate	29	26	28	28	26	21	25
DMSO	00	00	00	00	00	00	00

3.2. Antimicrobial activity of compounds by Serial dilution method

The antimicrobial activity of the synthesized derivatives was evaluated by using serial dilution method. The bacterial stains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and the fungal stains *Aspergillus niger*, *Aspergillus fumigates* and *Candida albicans* were used to evaluate the inhibitory activity. The microbial activities were assessed by Minimum Inhibitory Concentration (MIC). Noroxin was used as reference for evaluating antibacterial properties and Miconazole was used as reference for evaluating antifungal properties of the synthesized compounds.

The results given in Table-2 showed that, the Schiff base 3d exhibit moderate antibacterial activity with MIC 12.5 µg/ml against the bacterial stain *E. coli* and the fungal stain *A. niger*. The remaining Schiff bases (3a-3c) were exhibited a mild activity. While, the synthesized derivative 4d exhibit good antibacterial activity with MIC 6.25 µg/ml against the bacterial stains *S. aureus*, *P. aeruginosa* and the fungal stain *A. niger*.

Table - 2

Antimicrobial activity of compounds 3a-3d and 4a-4d (SDM)

Entry	MIC (µg/ml)					
	Bacterial stains			Fungal stains		
	S. aureus	***P. aeruginosa***	***E. coli***	***A. niger***	***A. fumigates***	***C. albicans***

3a	25	25	25	50	25	50
3b	50	25	50	50	25	50
3c	25	50	50	50	100	100
3d	25	25	12.5	12.5	25	25
4a	25	25	50	25	50	25
4b	25	25	25	50	25	25
4c	25	25	50	25	100	50
4d	6.25	6.25	12.5	6.25	12.5	12.5
Noroxin	6.25	6.25	6.25	-	-	-
Miconazole	-	-	-	6.25	6.25	6.25

4. Conclusion

Based on the above findings, it was concluded that the synthesized compounds possess antibacterial, antifungal, antimycobacterial and antioxidant properties. The introduction / extension of alkyl groups / resonance conjugation at N of 2-azetidinones enhances the antimicrobial activity of the derivatives.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the article 'A facile and potent synthesis of 2-azetidinones from 2-hydroxy-1-naphthaldehyde and amino acid based Schiff bases'

References

1. G.S. Singh, *Mini-Rev. Med. Chem.*, **4**, 2004, 69.
2. V. Selvamani and S. Mahalakshmi, J. Chem. & Chemical Sci., 7(5), 2017, 376 - 380.
3. U. Sahoo, A.K. Seth, A. Sen, B. Dhanya, J. Patel and R. Chawla, *Res. J. Pharm. Bio. Chem. Sci.*, **1**, 2010, 102.
4. S.V. More, D.V. Dongarkhadekar, R.N. Charan, R.W.N. Jadhav and P. Bhusare, *J. Ind. Chem. Soc.*, **79**, 2002, 768.
5. V.P. Singh, K.K. Saxena, S.K. Bhati and A. Kumar, *J. Global Pharma Tech.*, **2**, 2010, 42.
6. S.K. Srivastava, S. Srivastava and S.D. Srivastava, *Ind. J. Chem.*, **41**, 2002, 2357.
7. B.S. Vashi, D.S. Mehta and V.H. Shah, *Ind. J. Chem.*, **34B**, 1995, 802.
8. B.K. Banik, F.F. Becker and I. Banik, *Bioorg. Med. Chem.*, **12**, 2004, 2523.
9. J.L. Kelley and M.P. Krochmal, *J. Med. Chem.*, **31**, 1998, 1005.
10. E.L. Setti and R.G. Micetich, *Curr. Med. Chem.*, **5**, 1998, 101.
11. B.A. Arthington-Skaggs, M. Motley, D.W. Warnock and C.J. Morrison, *J. Clin. Microbiol.*, **38**, 2000, 2254 - 2260.

12. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd edition, Wayne (Pennsylvania), 2002.