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Synthesis and Evaluation of New Azetidinone Derivatives for Antibacterial and Antioxidant Activity

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ABSTRACT
Some new derivatives of the 2-azetidinone have been synthesized. All the new compounds were evaluated for their antioxidant and anti bacterial activity.The synthesized derivatives V_a-V_k has been evaluated for their antimicrobial activity. The results of the derivatives were compared with standard drug Moxifloxacin. All the compounds were less active than standard. The compound Vb showed maximum activity for antibacterial activity. All the new Azetidinone derivatives employed in the investigation have been found to have antioxidant activity.
Keywords: 2-azetidinone, antioxidant, anti bacterial activity, Moxifloxacin, synthesis

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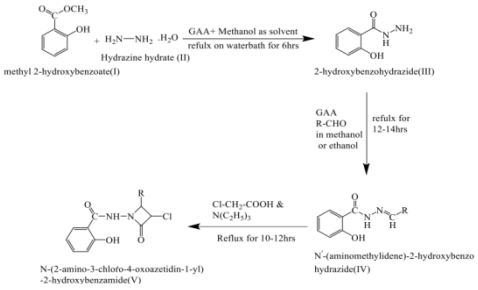
INTRODUCTION

The β-lactams are 4-membered cyclic amides derived from 3-aminopropanoic acids. Though the first member synthesized by Staudinger in 1907, the β-lactams as a class acquired importance since the discovery of penicillin which contains β-lactam unit as an essential structural feature of its molecule, this interest continued because of the therapeutic importance of β-lactam antibiotics and recent finding of new naturally occurring β-lactams. As a result of vigorous research has been accumulated over the years, and the chemistry of azetidinones continues to be blossoming field. Azetidin (a), azetin (b), 2-azetin (c) and azete (d) are the nitrogen analogues of cyclobutane, cyclobutene and cyclobutadiene respectively. Azetidins are well studied, in particular their derivatives the azetidin -2-ones (β-lactams) have received considerable attention mainly because of the antibacterial properties of penicillin and cephalosporins. The chemistry of both 1-and 2- azetidins has been developed only since the mid-1960 and these systems have not yet been comprehensively reviewed [1-5].Azetidin-2-ones are the most extensively studied derivatives of azetidin-2-one, largely as a result of the discovery of the antibacterial properties of penicillin, cephalosporin and cephamycin. Recently, there has been considerable interest in other fused β-lactams, such as clavulanic acid, thienamycin and the related clavulanic acid derivative and the penems. Nonfused β-lactam containing natural products include the nocardicins and the monobactams [5-8].Incorporation of an amide linkage into a four membered ring results in angle strain and some degree of inhibition of amide resonance, rendering β-lactams more susceptible than normal amides to nucleophilic attack at the carbonyl group. β-lactams undergo N(1)-C(2) cleavage on treatment with a variety of nucleophiles and this ability of a β-lactam to act as an acylating agent is generally considered to be responsible for the antibacterial properties of penicillins and cephalosporins. These strained bicyclic β-lactams inhibit bacterial cell wall biosynthesis, apparently by acylating transpeptidases. Polymerization of β-lactams, involving cleavage of the amide bond can be induced by treatment with strongly basic catalysts or by acylating agents. Introduction of a heteroatom substituent at C-4 tends to destabilize the β-lactams, promoting N (1)-C (4) bond cleavage [9-13].According to literature survey, azetidinones were proven to have antimicrobial activities. We have planned to synthesize various Azetidinone derivatives. The plan to synthesize newer heterocyclic molecules is that, many microbial organisms are developing resistance to various antibiotics. So our motto is to design and synthesize such molecues which would have better antibiotic activity against different bacterial and fungal infections.

MATERIAL AND METHODS

Synthesis of N-(2-amino-3-chloro-4-oxoazetidin-1-yl)-2-hydroxybenzamides (Va-Vk)



Scheme

COMPOUND	R(DIFFERENT ALDEHYDE)	MOLECULAR FORMULA(ALDEHYDE)
Va	Benzaldehyde	C_7H_6O
Vb	Veratraldehyde	$C_9H_{10}O_3$
Vc	Salicylaldehyde	$C_7H_6O_2$
Vd	Para Dimethyl Amino benzaldehyde	$C_9H_{11}NO$
Ve	Glutaraldehyde	$C_5H_8O_2$
Vf	Cinnamaldehyde	C_9H_8O
Vg	Furfuraldehyde	$C_5H_4O_2$
Vh	Vanillin	$C_8H_8O_3$
Vi	2-Chloro Benzaldehyde	C_7H_5ClO
Vj	4-Chloro Benzaldehyde	C_7H_5ClO
Vk	Propionaldehyde	C_3H_6O

Table 1. List of substituted Aldehydes

STEP-1: Preparation of 2-hydroxybenzohydrazide(III):-

In a 500 ml RB flask, 1 mole of methylsalicylate and 5 moles of Hydrazine hydrate and 5ml of methanol were placed and the reaction mixture was shaken for 5 minutes and refluxed for 6 hours, the completion of the reaction was monitored by TLC. The mixture was transferred into a petridish and the methanol was allowed to evaporate and the resultant white crystalline solid was collected. The solid was purified by recrystallization in ethanol (yield: 90%, M.Pt: 251-254□, RF:0.57)

STEP-2: Preparation of N'-(aminomethylidene)-2-hydroxybenzohydrazide (IVa-IVk):

In a 500ml RB flask, 1 mole of 2-hydroxybenzohydrazide and 1 mole of any aldehyde derivative and 2-3ml of GAA. To this add methanol or ethanol as solvent and shake it for 5minutes and refluxed it for 12-14hours, the completion of reaction was monitored by TLC. The mixture was transferred into a petridish and the methanol was allowed to evaporate and the resultant crystalline solid was collected. The solid was purified by recrystallization in ethanol.

STEP-3: Preparation of N-(2-amino-3-chloro-4-oxoazetidin-1-yl)-2-hydroxybenzamides (Va-Vk):-

In a 250ml RB flask, mixture of N'-(aminomethylidene)-2-hydroxybenzohydrazide (0.015 M) and required amount of triethylamine (0.02 M) and chloroaceticacid (0.02 M) in ethanol (50 ml) was refluxed for 10-12hrs on water bath to yield 2-azetidinone derivatives (Va-Vk). The completion of reaction was monitored by TLC. After cooling, the solution was poured on crushed ice to precipitate the product. The product was recrystallized from methanol.

EVALUATION ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS

The antibacterial activity of the azetidinone derivatives (Va -Vf) had been assayed against four different strains of bacteria by cup-plate agar diffusion method by measuring the zone of inhibition in mm. The test organisms were sub cultured using nutrient broth medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at37±1^{0}C for24hrs, they were stored in refrigerator. The stock culture were maintained. Bacterial inoculum was prepared by transferring a loopful of culture to nutrient broth in conical flask. The flask were incubated at 37± 1^{0}Cfor 48hrs before the experimentation.

ANTIOXIDANT ACTIVITY

Preparation of standard solution of Ascorbic acid:

Required amount of ascorbic acid was accurately weighed and dissolved in distilled water to prepare1mM stock solution. Solutions of different concentrations of ascorbic acid. ,1µM, 3µM, 1µM,

3µM, 10µM, 30µM, 100µM, 300µM, were prepared from 1mM stock solution. Preparation of DPPH solution;0.5Mm of DPPH was prepared by dissolving 19.71 mg of DPPH in 100ml of methanol. The solution was protected from sunlight to prevent the oxidation of DPPH.

Preparation of Test compounds;

Required amount of test compounds (Va,-Vk) were dissolved in methanol and 1 mM stock solution was prepared. Solutions of different concentrations of test compounds 1 nM to 1 mM were prepared. Estimation of antioxidant activity of Ascorbic acid;0.2 ml of DPPH solution was added to 2.8 ml of ascorbic acid solution in a test tube wrapped with aluminium foil, incubated for 30 min at room temperature and its absorbance was read out at517 nm using UV-Visible double beam spectrophotometer. The results were plotted on a graph and the IC50 values were determined [14,15].

Estimation of antioxidant activity of Test compounds:

The IC50 values of the test compounds were determined by a procedure similar to that described under ascorbic acid.

RESULTS AND DISCUSSION

Table 2. Physical data of synthesized compounds

S.no	Compound	Molecular Weight	Yield	R_F Value	Melting Point
1	Va	316.74	69%	0.43	266-268
2	Vb	376.79	72%	0.59	220-225
3	Vc	332.74	68%	0.72	272-274
4	Vd	359.81	78%	0.53	282-284
5	Ve	310.73	84%	0.64	216-218
6	Vf	344.79	76%	0.74	253-255
7	Vg	306.04	85%	0.77	265-257
8	Vh	362.77	78%	0.80	238-240
9	Vi	351.18	78%	0.58	243-245
10	Vj	351.18	76%	0.67	234-235
11	Vk	268.70	85%	0.63	212-214

Spectrum of N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-hydroxybenzamide (Va) IR KBrcm;3188.60(CONH,str),761.20(CCl),1467.67(C2H2,Aromatic),2942.51(CH,aliphaticalkane),1254.62(CN,aliphaticamine) H NMR Spectrum(CDCL3,400MHZ;δ(ppm)7.8(s,CONH1H),7.4(m,Ar-5H)7.7(s,Ar-1H),7.3(s,Ar-1H),6.9(d,Ar- 2H),5.5(s,Al-1H),5.3(s,Al-H)9.5(s,ph-OH)

Spectrum of N-(3-chloro-2-(2,3-dimethoxyphenyl)-4-oxoazetidin-1-yl)-2-hydroxybenzamide (Vb)IR(KBr cm);3383.15(CONH,str),3061.15(CH, aromatic str), 772.71(CCl), 1546.50(C2H2,aromatic), 2908.02(CH, alkane,str),1285.07(CN,amine),1209.98 (ROR, ether)H NMR Spectrum(CDCL3,400MHZ);δ(ppm)7. 7(s,1H-CONH),7.4(sAr-H),7.1(d Ar-H),6.8(s Ar-H),6.7(s Ar-H),5.4(s Al-1H)5.2(s Al-1H),4.7(m Al-CH3)9.5(s,ph-OH).

Antimicrobial activity of N-(2-amino-3-chloro-4-oxoazetidin-1-yl)-2-hydroxybenzamides (Va-Vk)

Table 3 Antibacterial activity against Escherichia coli-(zone of inhibition in mm)

Compound	50µg/ml	100µg/ml	300µg/ml
Va	9mm	14mm	18mm
Vb	13mm	18mm	23mm
Vc	12mm	16mm	23mm
Vd	11mm	15mm	22mm
Ve	6mm	10mm	17mm
Vf	7mm	12mm	21mm
Vg	9mm	15mm	21mm
Vh	7mm	13mm	20mm
Vi	8mm	13mm	18mm
Vj	6mm	11mm	14mm
Vk	5mm	9mm	11mm
Moxifloxacin	14mm	22mm	24mm
control	2mm	2mm	2mm

Table 4. Anti-oxidant activity of Azetidinone derivatives(V).

S. No	Compound	IC_{50} (µM)
1	Va	82.51
2	Vb	91.3
3	Vc	58.5
4	Vd	62.56
5	Ve	46.28
6	Vf	41.30
7	Vg	73.28
8	Vh	55.37
9	Vi	31.4
10	Vj	37.58
11	Vk	28.1
12	Standard	8.65

DISCUSSION

In this study, we have synthesized a new series of Azetidinone derivatives (Va-Vk). Yields of all synthesized compounds were good. Azetidinones had a subject of numerous investigations. The compounds were confirmed by TLC, melting point and spectral studies such as IR, MASS, and 1HNMR.All the derivatives were subjected to antioxidant activity (DPPH method) and antibacterial activity. The synthesized derivatives Va-Vk has been evaluated for their antimicrobial activity. The results of the derivatives were compared with standard drug Moxifloxacin. All the compounds were less active than standard. The compound Vb showed maximum activity for antibacterial activity. All the new Azetidinone derivatives employed in the investigation have been found to have antioxidant activity. All the compounds were tested at 1nM to 1mM concentrations and results were compared with standard drug (Ascorbic acid) at the same concentrations. Among all the compounds, Va and Vb had effective antioxidant activity 3 1 . 4 µ ml and28.1 µ m l respectively. The compound Vk was found to be possess least antioxidant activity than all the tested compounds with 58.7 µml.

CONCLUSION

Syntheticworkofthesestudieshavepositivelyundergoneasperthepla nandassuch in all the reactions carried, the compounds alone could be obtained. Compounds were synthesized and were analysed by physical and spectral data(IR, NMR,Mass). Synthesized Azetidinone derivatives gave satisfactory results for various evaluations like TLC, melting point, spectral data, antibacterial and antioxidant activities. Characterization of the synthesized as done by IR,[1]HNMR and Mass spectral data.The structures, functional groups and molecular weights of the compounds were confirmed. All the compounds were screened for antibacterial studies. The results of derivatives were compared with standard drug Moxifloxacin. All the compounds were less active than standard. All the series of the compounds showed antioxidant activity.Compound Vb with IC_{50} value of 28.1and Va with IC_{50} value of 31.4mlwas found to be more effective antioxidant.

CONFLICT OF INTEREST

Authors declared there is no conflict of interest

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