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SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-ARYL - 3-CHLORO –1– NICOTINAMIDO–2–AZETIDINONES AS POTENTIAL ANTICONVULSANT AND ANTIMYCOBACTERIAL AGENTS

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ABSTRACT

In the present study novel series of 4 - aryl – 3 - chloro – 1 – nicotinamido – 2 -azetidinones were synthesized and characterized by means of IR, ^{1}H- NMR, Mass spectral analysis. The compounds were screened for anticonvulsant and antimycobacterial activities. Antimycobacterial activity was screened using standard Strain $H_{37}R_V$ and two Human Strains (Human strain-I and Human strain-II) isolated from patients suffering from pulmonary tuberculosis. The minimum inhibitory concentration (MIC) of C_3, C_5, C_6, C_9, against standard Strain $H_{37}R_V$ was found to be 50-100 µg/mL, for Human strain-I and Human strain-II, the MIC was found to be 100-200 µg/mL. Anticonvulsant activity was tested in Wistar rats by maximal electro shock (MES) method. Compounds C_1, C_2, C_3, C_4, C_5, C_6, C_8, C_{13} and C_{14}, showed significant anticonvulsant activity and compounds C_9, C_{10}, C_{11} and C_{12} showed moderate activity.

Key words: Nicotinic acid, Azetidinone, Anticonvulsant, Antimycobacterial.

INTRODUCTION

Nicotinic acid is effective in the treatment of all types of hyperlipoproteinemias[1] and azetidinones is active as anticonvulsant[2,3] and antitubercular[4-6]. Recently, 2-azetidinones have been assessed for antiparkinsonism[7]; anti-inflammatory[2,4]; herbicidal[4].

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They also function as an enzyme inhibitors[4] and effective on the central nervous system[4,8]. The azetidinones were tested as antidepressants[7]; sedatives[7] and are also associated with hypnotic[2, 8], antimicrobial[2, 4], antiviral[2] and anaesthetic[2] activities. In the present study a novel series of azetidinones were synthesized and characterized by means of IR, ^{1}H NMR, Mass spectral analysis. The compounds were screened for anticonvulsant and antimycobacterial activity.

EXPERIMENTAL

In the present study, nicotinic acid (0.03 mol), phosporous pentachloride (0.05 mol) and anhydrous carbon tetra chloride (20 ml) were refluxed for two hour at 100^{o}C and the solvent was distilled off. The remaining solid material of nicotinoyl chloride was added to hydrazine hydrate (0.1 mol) at 5^{o}C in an ice bath. The above mixture, after complete addition, was stirred for 5 hours at room temperature and finally washed with 10% sodium bicarbonate solution. The precipitate formed was filtered and dried, which on addition with different aromatic aldehydes gives the Schiff bases. The Schiff base so formed on treatment with chloroacetyl chloride and triethylamine as basic catalyst in 1,4-dioxan gives products **C_1- C_{14}**.

Biological investigation

The anticonvulsant activity of the compounds were evaluated by maximal electro shock (MES) method using rats where the electroshock is applied through the corneal electrodes. The animals were divided into 16 groups ; each comprising 6 animals. In that, 14 groups were served for testing the synthesized compounds, one as control and one as standard (phenytoin 25 mg/kg of body weight).

In vitro antimycobacterial screening was done for the synthesized compounds **C_3, C_5, C_6, C_9,** screened against standard Strain **$H_{37}R_V$** and two human strains **(Human strain-I and Human strain-II)** isolated from patients suffering from pulmonary tuberculosis in different concentrations from 12.5, 25, 50, 100, 200, 400 µg/mL and the isoniazid was used as standard at a concentration of 50 µg/mL.

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compound were recorded on ABB Bomem FTIR spectrometer MB104 with KBr pellets. ^{1}H NMR was recorded on 300 MHz-Bruker DPX200. The chemical shifts are reported as parts per million downfield from tetramethylsilane. Mass spectra were recorded on Finnigan MAT8230. The purity of the compounds were checked by TLC on precoated SiO_2 gel (HF_{254} 200 mesh) aluminium plates (E Merck).

Synthesis of nicotinic acid hydrazide

A mixture of nicotinic acid (0.03 mol) (4.1 g) and phosphorous pentachloride (0.05 mol) (10.3g) in anhydrous carbon tetra chloride (20 mL) was refluxed for 2 hour at 100°C. Solvent was distilled off and the solid acid chloride thus obtained was used for further reaction. To the nicotinoyl chloride (0.03 mol) hydrazine hydrate was added (0.1 mol) dropwise below 5°C and the resultant mixture was stirred for 5 hour at room temperature. A solid that separated out, which was washed with aqueous sodium bicarbonate ($NaHCO_3$) (10%) and dried in vaccuo. It was recrystallised from methanol[9].

General method of synthesis of Schiff bases (1-14)

A mixture of equimolar quantities of aromatic aldehyde and nicotinic acid hydrazide were refluxed for 1 hour in 50 mL of ethanol with few drops of glacial acetic acid. The reaction mixture was poured in ice-cold water. The separated product was filtered out. The Schiff bases obtained was used for final step to form substituted 2-azetidinones[10].

General method of synthesis of azetidinones C_1- C_{14}

A mixture of Schiff base (0.002 mol) and triethyl amine (0.004 mol) was dissolved in 1,4-dioxan (50 mL). To this, well stirred cooled solution of chloroacetyl chloride (0.004 mol) was added drop wise during 20 mins. The reaction mixture was then stirred for further 3 hours and left at room temperature for 48 hours. The resultant mixture was concentrated, cooled, poured into ice-cold water, dried and recrystallised from n-hexane / spirit, which gave compounds[11] **C_1** to **C_{14}.**

3-Chloro 4-(2-hydroxy phenyl) N-nicotinamido 2-azetidinone- (C_1)

Yield = 52.66%, mp 205°C, IR(KBr)cm^{-1}; 1717 (β-lactam, C=O) 3019(Ar-H) 752 (C-Cl) 1627(C=N) 3196(Ar-OH);^{1}H NMR($CDCl_3$) δ; 11.3(s,1H Ar-OH), 8.7(s, 1H, CONH) 6.9-7.4(m, 7H,Ar-H) 3.7(d, 1H, N- CH) 4.7(d,1H, CH-Cl); EI-MS m/z (M^+): 317 (B^+): 288

3-Chloro 4-(3-hydroxy phenyl) N-nicotinamido 2-azetidinone - (C_2)

Yield = 62.80%, mp 209°C, IR(KBr)cm^{-1}; 1674 (β-lactam, C=O) 3026 (Ar-H) 748(C-Cl) 1625(C=N) 3200(Ar-OH);^{1}H NMR($CDCl_3$) δ; -11.2(s,1H Ar-OH), 8.7(s, 1H, CONH) 6.9-7.4(m, 7H,Ar-H) 4.6(d,1H, CH-Cl) 3.2(d, 1H, N- CH); EI-MS m/z (M^+): 317 (B^+): 288

3-Chloro 4-(4-hydroxy phenyl) N-nicotinamido 2-azetidinone –(C_3)

Yield = 80.01% mp 195^{0}C, IR(KBr)cm^{-1};1770 (β-lactam, C=O) 3055(Ar-H) 769(C-Cl) 1625(C=N) 3200(Ar-OH); ^{1}H NMR($CDCl_3$) δ; 11.2(s,1H Ar-OH), 8.6(s, 1H, CONH) 6.9-7.9(m, 7H,Ar-H) 4.5(d,1H, CH-Cl) 3.2(d, 1H, N- CH); EI-MS m/z (M^+):317 (B^+):288

3-Chloro 4-(3,4-dihydroxy phenyl) N-nicotinamido - 2-azetidinone –(C_4)

Yield = 70.20% mp 215^{0}C, IR(KBr)cm^{-1}; 1772(β-lactam, C=O) 3062(Ar-H) 765(C-Cl) 1667(C=N) 3199(Ar-OH);^{1}H NMR($CDCl_3$) δ; 10.8(s,1H Ar-OH), 8.6(s, 1H, CONH) 6.9-7.7(m, 7H,Ar-H) 3.9(d,1H, CH-Cl) 2.4(d, 1H, N- CH); EI-MS m/z (M^+): 317(B^+): 288

3-Chloro 4-(4-methyl phenyl) N-nicotinamido - 2-azetidinone –(C_5)

Yield = 67.97% mp 208^{0}C, IR(KBr)cm^{-1}; 1681 (β-lactam, C=O) 3056(Ar-H) 712(C-Cl) 1621(C=N) 2851,2923(Ar-CH_3);^{1}H NMR($CDCl_3$) δ; 8.6(s, 1H, CONH) 7.2-7.7(m, H,Ar-H) 4.6(d,1H, CH-Cl) 3.7(d, 1H, N- CH); 2.4(Ar-CH_3). EI-MS m/z (M^+):315(B^+):290

3-Chloro 4-(4-methoxy phenyl) N-nicotinamido -2-azetidinone – (C_6)

Yield = 58.33% mp 183^{0}C, IR(KBr)cm^{-1}; 1734 (β-lactam, C=O) 3048(Ar-H) 780 (C-Cl) 1619(C=N) 1251(Ar-OCH_3);^{1}H NMR($CDCl_3$) δ; 8.6(s, 1H, CONH) 6.9-7.7(m, 7H,Ar-H) 4.6(d,1H, CH-Cl) 3.8(Ar-OCH_3) 3.6(d, 1H, N- CH); EI-MS m/z (M^+): 331 (B^+): 65

3-Chloro 4-(3,4,5-trimethoxy phenyl) N-nicotinamido 2-azetidinone –(C_7)

Yield = 61.48% mp 194^{0}C, IR(KBr)cm^{-1}; 1749(β-lactam, C=O)3061 (Ar-H) 764 (C-Cl) 1622(C=N) 1232(Ar-OCH_3) 1237 (Ar-OCH_3);^{1}H NMR($CDCl_3$) δ; 8.5(s, 1H, CONH) 7.0-7.2(m, 7H,Ar-H) 4.6(d,1H, CH-Cl) 3.7(d, 1H, N- CH); EI-MS m/z (M^+): 391 (B^+):65

3-Chloro 4-(4-hydroxy 3-methoxy phenyl) N-nicotinamido 2-azetidinone- (C_8)

Yield = 45.03% mp 196^{0}C, IR(KBr)cm^{-1}; 1778(β-lactam, C=O) 3042(Ar-H) 753(C-Cl) 1627(C=N) 3185(Ar-OH) 1237(Ar-OCH_3);^{1}H NMR($CDCl_3$) δ; -9.8(s,1H Ar-OH), 8.6(s, 1H, CONH) 7.1-7.6(m, 7H,Ar-H) 4.6(d,1H, CH-Cl) 3.9(Ar-OCH_3) 3.7(d, 1H, N- CH); EI-MS m/z (M^+): 347 (B^+): 288

3-Chloro 4 - (p-dimethyl amino phenyl) N-nicotinamido 2-azetidinone – (C_9)

Yield = 61.75% mp 204^{0}C, IR(KBr)cm^{-1}; 1772 (β-lactam, C=O) 3048(Ar-H) 743 (C-Cl) 1606(C=N) 2851, 2920(N-CH_3);^{1}H NMR($CDCl_3$) δ; 8.5(s, 1H, CONH) 6.7-7.7(m, 7H,Ar-H) 4.7(d,1H, CH-Cl) 3.7(d, 1H, N- CH); EI-MS m/z (M^+): 344 (B^+): 290

3-Chloro 4-(p-nitro phenyl) N-nicotinamido 2-azetidinone – (C_{10})

Yield = 47.68% mp 205^{0}C, IR(KBr)cm^{-1}; 1783(β-lactam, C=O)3062 (Ar-H) 745 (C-Cl) 1628(C=N) 1346(Ar-NO_2);^{1}H NMR($CDCl_3$) δ; 8.5(s, 1H, CONH) 6.4-7.6(m, 7H,Ar-H) 4.6(d,1H, CH-Cl) 4.2(d, 1H, N- CH); EI-MS m/z (M^+): 346 (B^+): 330

3-Chloro 4-(3-nitro phenyl) N-nicotinamido 2-azetidinone – (C_{11})

Yield = 55.18% mp 216^{0}C, IR(KBr)cm^{-1}; 1741 (β-lactam, C=O)3039 (Ar-H) 734(C-Cl) 1628(C=N) 1355(Ar-NO_2);^{1}H NMR($CDCl_3$) δ; 8.7(s, 1H, CONH) 7.2-8.3(m, 7H,Ar-H) 4.6(d,1H, CH-Cl) 3.7(d, 1H, N- CH); EI-MS m/z (M^+): 346 (B^+): 330

3-Chloro 4-(p-chloro phenyl) N-nicotinamido 2-azetidinone – (C_{12})

Yield = 45.76% mp 181^{0}C, IR(KBr)cm^{-1}; 1785 (β-lactam, C=O) 3048(Ar-H) 756(C-Cl) 1624(C=N);^{1}H NMR($CDCl_3$) δ; 8.6(s, 1H, CONH) 7.2-7.7(m, 7H,Ar-H) 4.6(d,1H, CH-Cl) 4.2(d, 1H, N- CH); EI-MS m/z (M^+): 336 (B^+): 290

3-Chloro 4-(2-chloro phenyl) N-nicotinamido -2-azetidinone – (C_{13})

Yield = 62.94% mp 188^{0}C, IR(KBr)cm^{-1}; 1771 (β-lactam, C=O) 3028(Ar-H) 747 (C-Cl) 1621(C=N) ;^{1}H NMR($CDCl_3$) δ; 9.0(s, 1H, CONH) 7.0-8.2(m, 7H,Ar-H) 4.0(d,1H, CH-Cl) 3.7(d, 1H, N- CH); EI-MS m/z (M^+): 336 (B^+): 290

3-Chloro 4-(cinnamyl) N-nicotinamido -2-azetidinone – (C_{14})

Yield = 68.66% mp 206^{0}C, IR(KBr)cm^{-1}; 1763 (β-lactam, C=O) 3048(Ar-H) 748(C-Cl) 1621(C=N);^{1}H NMR($CDCl_3$) δ; 8.3(s, 1H, CONH) 6.6-7.5(m, 7H,Ar-H) 4.2(d,1H, CH-Cl) 3.9(d, 1H, N- CH); EI-MS m/z (M^+): 327 (B^+): 106

Anticonvulsant evaluation by Maximal Electro Shock (MES) method[12]

In MES method, convulsions electric shock is applied through the corneal electrodes, producing optic stimulation cortical excitation. The MES convulsions are divided into five phases such as (a) Tonic flexion, (b) Tonic extension, (c) Clonic convulsion, (d) Stupor and (e) Recovery or death. A drug is known to possess anti-

convulsant property if it reduces or abolishes the extensor phase of MES convulsions. For the evaluation of anticonvulsant activity, the total 16 groups of animals were kept fasting for 10- 14 hours. After that the synthesized compounds was administered to each group at a dose of 50mg/kg of bodyweight. 1% C.M.C. was used as vehicle control and phenytoin was used as a standard drug. The activities of each group were measured after the interval of half-an hour are compounds administering including control and standard. Results and data are given in Table 4.

Table 1. Characterization data of compounds

Compound	Ar	Melting point (ºC)	Yield (%)
C_1	2-Hydroxy phenyl	205	53
C_2	3-Hydroxy phenyl	209	63
C_3	4-Hydroxy phenyl	195	80
C_4	3,4-Dihydroxy phenyl	215	70
C_5	4-Methyl phenyl	208	68
C_6	4-Methoxy phenyl	183	58
C_7	3,4,5-Trimethoxy phenyl	194	61
C_8	4-Hydroxy 3-methoxy phenyl	196	45
C_9	4-Dimethylaminophenyl	204	62
C_{10}	4-Nitro phenyl	205	48
C_{11}	3-Nitro phenyl	216	55
C_{12}	4-Chloro phenyl	181	46
C_{13}	2-Chloro phenyl	188	63
C_{14}	Cinnamyl	206	68

Antimycobacterial evaluation

The synthesized compounds **C_3, C_5, C_6** and **C_9** were evaluated for antitubercular activity against standard strain $H_{37}R_V$ and with 2 human strains of *Mycobacterium tuberculae*. The results and data are given in Tables 2 and 3.

Table 2 Antimycobacterial activity

Compounds	Strain	Concentration µg/mL					
		12.5	**25**	**50**	**100**	**200**	**400**
C_3	$H_{37}R_V$	+++	++	-	-	-	-
C_5		+++	+++	++	-	-	-
C_6		+++	+++	++	-	-	-
C_9		+++	+++	+	-	-	-
C_3	Human Strain I	+++	++	+	-	-	-
C_5		+++	+++	++	-	-	-
C_6		+++	+++	++	+	-	-
C_9		+++	+++	+	+	-	-
C_3	Human Strain II	+++	++	+	+	-	-
C_5		+++	++	++	+	-	-
C_6		+++	+++	++	+	-	-
C_9		+++	+++	++	+	-	-

-	:	No growth of mycobacterium tuberculosis
+	:	Growth of mycobacterium tuberculosis below 100 colonies
++	:	Growth of mycobacterium tuberculosis between 100- 200 colonies
+++	:	Growth of mycobacterium tuberculosis above 200 colonies

Table 3. Minimum inhibitory concentration

Compound	Minimum inhibitory concentration		
	$H_{37}R_V$	**Human Strain I**	**Human Strain II**
C_3	50	100	200
C_5	100	100	200
C_6	100	200	200
C_9	100	200	200

Materials required

(i) **LOWENSTEIN-JENSEN (L.J)** medium slopes containing various concentrations of the compounds

(ii) Control strain $H_{37}R_{V.}$

(iii) Two strains of *Mycobacterium tuberculosis* isolated from patients suffering from pulmonary tuberculosis.

Preparation of drug containing slopes

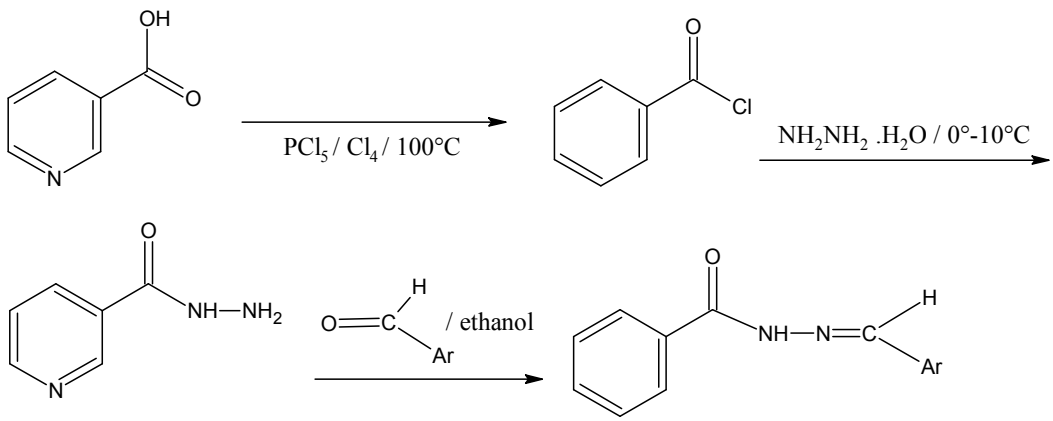
The synthesised compounds were dissolved in DMSO and added to the egg fluid salt solution in such a way that to give final concentration of 12.5, 25, 50, 100, 200, 400 µg of the compound/ mL of the medium. The above was inspissated at 90^0C for 50 mins only once. (Note: No reinspissation, as the drug may be destroyed).

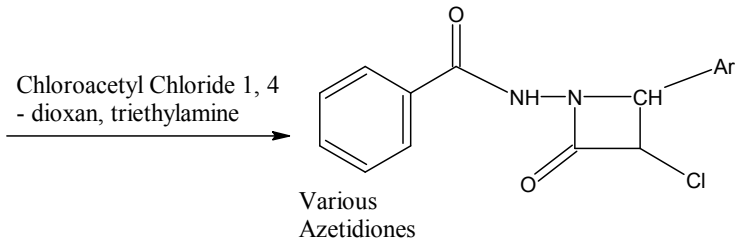
Preparation of the bacterial suspension

The bacterial culture of a control strain and 2 test strain about 2/3 loop full (3 mm internal diameter) is mixed with 1 mL sterile distilled water in BIJOU bottle containing 3-5 glass beads, shaken in a vertexed bottle for about 1 mm to get a uniform suspension.

Susceptibility test procedure

1 loop full of bacterial suspension was inoculated on L.J. medium slopes containing the test compounds. A drug free slope is also included as a control. All the slopes were incubated at 37^0C for 15 days.





Scheme 1

Table 4. Anti-convulsant activity of some synthesised compounds by maximal electro shock method

Compound	Mean time in various phases of convulsions (seconds) ± standard error mean.		Recovery	M.E.S. Test	Percentage protection (% abolition of tonic extensor phase)
	Flexion	Extensor			
Control	6.833 ± 0.40	10.00 ± 0.73	✓	0/6	-
C_1	3.667 ± 0.21	1.83 ± 0.83	✓	3/6	50 %
C_2	3.33 ± 0.21	2.16 ± 0.74	✓	2/6	33.33%
C_3	4.66 ± 0.33	2.00 ± 0.69	✓	2/6	33.33%
C_4	**4.33 ± 0.49**	**4.66 ± 0.49**	✓	**0/6**	-
C_5	5.33 ± 0.61	5.00 ± 0.57	✓	0/6	-
C_6	4.33 ± 0.42	1.00 ± 0.63	✓	4/6	66.66%
C_7	6.66 ± 0.66	7.30 ± 0.61	✓	0/6	-
C_8	4.66 ± 0.49	1.00 ± 0.63	✓	5/6	83.33%
C_9	5.66 ± 0.33	9.16 ± 0.74	✓	0/6	-
C_{10}	6.66 ± 0.33	9.83 ± 0.60	✓	0/6	-
C_{11}	6.16 ± 0.47	8.66 ± 0.49	✓	0/6	-
C_{12}	6.50 ± 0.42	9.66 ± 0.61	✓	0/6	-
C_{13}	4.66 ± 0.22	3.66 ± 0.33	✓	0/6	-
C_{14}	4.83 ± 0.47	5.33 ± 0.88	✓	0/6	-
Standard	4.00 ± 0.36	0.00 ± 0.00	✓	6/6	100%

RESULTS AND DISCUSSION

All the synthesized compounds exhibit significant to moderate anticonvulsant activity. Compounds **C_1, C_2, C_3, C_4, C_5, C_6, C_8, C_{13}, C_{14},** showed significant anticonvulsant activity in the flexion phase. Other compounds showed moderate activity. In the extensor phase **C_7** showed most significant activity when compared with **C_1, C_2, C_3, C_4, C_5, C_6, C_8, C_{13},** and **C_{14}.** Compounds **C_9, C_{10}, C_{11}, C_{12}** showed reduction in the duration of the flexion and extensor phase. **C_5** and **C_7** least active in flexion and showed significant activity in extensor phase. Compounds **C_3, C_5, C_6, C_9** showed significant anti-tubercular activity.

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