



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 -azetidiones were synthesized and characterized by means of IR, ¹H- NMR, Mass spectral analysis. The compounds were screened for anticonvulsant and antimycobacterial activities. Antimycobacterial activity was screened using standard Strain H₃₇R_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 C₃, C₅, C₆, C₉ against standard Strain H₃₇R_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 C₁, C₂, C₃, C₄, C₅, C₆, C₈, C₁₃ and C₁₄, showed significant anticonvulsant activity and compounds C₉, C₁₀, C₁₁ and C₁₂ showed moderate activity.

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

INTRODUCTION

Nicotinic acid is effective in the treatment of all types of hyperlipoproteinemias¹ and azetidiones is active as anticonvulsant^{2,3} and antitubercular^{4,6}. Recently, 2-azetidiones have been assessed for antiparkinsonism⁷; anti-inflammatory^{2,4}; herbicidal⁴.

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They also function as an enzyme inhibitors⁴ and effective on the central nervous system^{4,8}. The azetidiones were tested as antidepressants⁷; sedatives⁷ and are also associated with hypnotic^{2, 8}, antimicrobial^{2, 4}, antiviral² and anaesthetic² activities. In the present study a novel series of azetidiones were synthesized and characterized by means of IR, ¹H NMR, Mass spectral analysis. The compounds were screened for anticonvulsant and antimycobacterial activity.

EXPERIMENTAL

In the present study, nicotinic acid (0.03 mol), phosphorous pentachloride (0.05 mol) and anhydrous carbon tetra chloride (20 ml) were refluxed for two hour at 100°C and the solvent was distilled off. The remaining solid material of nicotinoyl chloride was added to hydrazine hydrate (0.1 mol) at 5°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 C₁- C₁₄.

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 C₃, C₅, C₆, C₉, screened against standard Strain H₃₇R_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. ¹H 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 SiO₂ gel (HF₂₅₄ 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 (NaHCO₃) (10%) and dried in vacuo. It was recrystallised from methanol⁹.

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-azetidinones¹⁰.

General method of synthesis of azetidinones C₁- C₁₄

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 compounds¹¹ C₁ to C₁₄.

3-Chloro 4-(2-hydroxy phenyl) N-nicotinamido 2-azetidinone- (C₁)

Yield = 52.66%, mp 205⁰C, IR(KBr)cm⁻¹; 1717 (β-lactam, C=O) 3019(Ar-H) 752 (C-Cl) 1627(C=N) 3196(Ar-OH);¹H NMR(CDCl₃) 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 - (C₂)

Yield = 62.80%, mp 209⁰C, IR(KBr)cm⁻¹; 1674 (β-lactam, C=O) 3026 (Ar-H) 748(C-Cl) 1625(C=N) 3200(Ar-OH);¹H NMR(CDCl₃) 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 –(C₃)

Yield = 80.01% mp 195⁰C, IR(KBr)cm⁻¹; 1770 (β-lactam, C=O) 3055(Ar-H) 769(C-Cl) 1625(C=N) 3200(Ar-OH); ¹H NMR(CDCl₃) 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 –(C₄)

Yield = 70.20% mp 215⁰C, IR(KBr)cm⁻¹; 1772(β-lactam, C=O) 3062(Ar-H) 765(C-Cl) 1667(C=N) 3199(Ar-OH); ¹H NMR(CDCl₃) 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 –(C₅)

Yield = 67.97% mp 208⁰C, IR(KBr)cm⁻¹; 1681 (β-lactam, C=O) 3056(Ar-H) 712(C-Cl) 1621(C=N) 2851, 2923(Ar-CH₃); ¹H NMR(CDCl₃) 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-CH₃). EI-MS m/z (M⁺): 315(B⁺): 290

3-Chloro 4-(4-methoxy phenyl) N-nicotinamido -2-azetidinone – (C₆)

Yield = 58.33% mp 183⁰C, IR(KBr)cm⁻¹; 1734 (β-lactam, C=O) 3048(Ar-H) 780 (C-Cl) 1619(C=N) 1251(Ar-OCH₃); ¹H NMR(CDCl₃) δ; 8.6(s, 1H, CONH) 6.9-7.7(m, 7H, Ar-H) 4.6(d, 1H, CH-Cl) 3.8(Ar-OCH₃) 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 –(C₇)

Yield = 61.48% mp 194⁰C, IR(KBr)cm⁻¹; 1749(β-lactam, C=O) 3061 (Ar-H) 764 (C-Cl) 1622(C=N) 1232(Ar-OCH₃) 1237 (Ar-OCH₃); ¹H NMR(CDCl₃) 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- (C₈)

Yield = 45.03% mp 196⁰C, IR(KBr)cm⁻¹; 1778(β-lactam, C=O) 3042(Ar-H) 753(C-Cl) 1627(C=N) 3185(Ar-OH) 1237(Ar-OCH₃); ¹H NMR(CDCl₃) 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-OCH₃) 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 – (C₉)

Yield = 61.75% mp 204⁰C, IR(KBr)cm⁻¹; 1772 (β-lactam, C=O) 3048(Ar-H) 743 (C-Cl) 1606(C=N) 2851, 2920(N-CH₃); ¹H NMR(CDCl₃) 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 – (C₁₀)

Yield = 47.68% mp 205⁰C, IR(KBr)cm⁻¹; 1783(β-lactam, C=O)3062 (Ar-H) 745 (C-Cl) 1628(C=N) 1346(Ar-NO₂); ¹H NMR(CDCl₃) 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 – (C₁₁)

Yield = 55.18% mp 216⁰C, IR(KBr)cm⁻¹; 1741 (β-lactam, C=O)3039 (Ar-H) 734(C-Cl) 1628(C=N) 1355(Ar-NO₂); ¹H NMR(CDCl₃) 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 – (C₁₂)

Yield = 45.76% mp 181⁰C, IR(KBr)cm⁻¹; 1785 (β-lactam, C=O) 3048(Ar-H) 756(C-Cl) 1624(C=N); ¹H NMR(CDCl₃) 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 – (C₁₃)

Yield = 62.94% mp 188⁰C, IR(KBr)cm⁻¹; 1771 (β-lactam, C=O) 3028(Ar-H) 747 (C-Cl) 1621(C=N); ¹H NMR(CDCl₃) 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 – (C₁₄)

Yield = 68.66% mp 206⁰C, IR(KBr)cm⁻¹; 1763 (β-lactam, C=O) 3048(Ar-H) 748(C-Cl) 1621(C=N); ¹H NMR(CDCl₃) 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) method¹²

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 (°C)	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 C₃, C₅, C₆ and C₉ were evaluated for antitubercular activity against standard strain H₃₇R_V and with 2 human strains of *Mycobacterium tuberculosis*. The results and data are given in Tables 2 and 3.

Table 2 Antimycobacterial activity

Compounds	Strain	Concentration $\mu\text{g/mL}$					
		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 *H₃₇R_V*.
- (iii) Two strains of *Mycobacterium tuberculosis* isolated from patients suffering from pulmonary tuberculosis.

Preparation of drug containing slopes

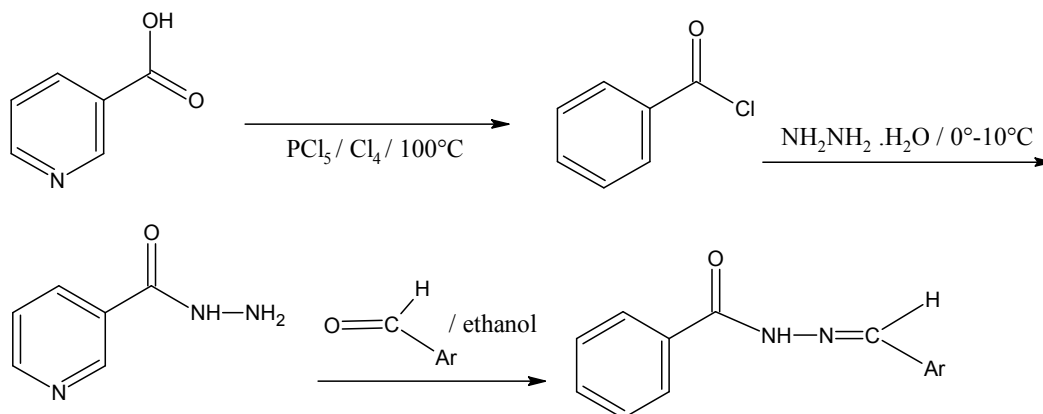
The synthesised compounds were dissolved in DMSO and added to the egg fluid salt solution in such a way that to give final concentration of 12.5, 25, 50, 100, 200, 400 μg of the compound/ mL of the medium. The above was inspissated at 90⁰C for 50 mins only once. (Note: No reinspissation, as the drug may be destroyed).

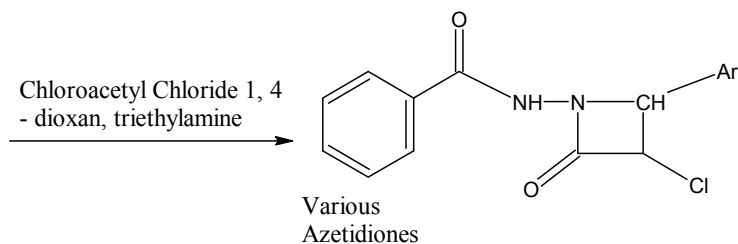
Preparation of the bacterial suspension

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 37⁰C 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) \pm standard error mean.		Recovery	M.E.S. Test	Percentage protection (% abolition of tonic extensor phase)
	Flexion	Extensor			
Control	6.833 \pm 0.40	10.00 \pm 0.73	✓	0/6	-
C₁	3.667 \pm 0.21	1.83 \pm 0.83	✓	3/6	50 %
C₂	3.33 \pm 0.21	2.16 \pm 0.74	✓	2/6	33.33%
C₃	4.66 \pm 0.33	2.00 \pm 0.69	✓	2/6	33.33%
C₄	4.33 \pm 0.49	4.66 \pm 0.49	✓	0/6	-
C₅	5.33 \pm 0.61	5.00 \pm 0.57	✓	0/6	-
C₆	4.33 \pm 0.42	1.00 \pm 0.63	✓	4/6	66.66%
C₇	6.66 \pm 0.66	7.30 \pm 0.61	✓	0/6	-
C₈	4.66 \pm 0.49	1.00 \pm 0.63	✓	5/6	83.33%
C₉	5.66 \pm 0.33	9.16 \pm 0.74	✓	0/6	-
C₁₀	6.66 \pm 0.33	9.83 \pm 0.60	✓	0/6	-
C₁₁	6.16 \pm 0.47	8.66 \pm 0.49	✓	0/6	-
C₁₂	6.50 \pm 0.42	9.66 \pm 0.61	✓	0/6	-
C₁₃	4.66 \pm 0.22	3.66 \pm 0.33	✓	0/6	-
C₁₄	4.83 \pm 0.47	5.33 \pm 0.88	✓	0/6	-
Standard	4.00 \pm 0.36	0.00 \pm 0.00	✓	6/6	100%

RESULTS AND DISCUSSION

All the synthesized compounds exhibit significant to moderate anticonvulsant activity. Compounds C₁, C₂, C₃, C₄, C₅, C₆, C₈, C₁₃, C₁₄, showed significant anticonvulsant activity in the flexion phase. Other compounds showed moderate activity. In the extensor phase C₇ showed most significant activity when compared with C₁, C₂, C₃, C₄, C₅, C₆, C₈, C₁₃, and C₁₄. Compounds C₉, C₁₀, C₁₁, C₁₂ showed reduction in the duration of the flexion and extensor phase. C₅ and C₇ least active in flexion and showed significant activity in extensor phase. Compounds C₃, C₅, C₆, C₉ showed significant anti-tubercular activity.

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