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Research Article

SYNTHESIS AND PHARMACOLOGICAL SCREENING OF SOME NEW AZETIDINONE DERIVATIVES

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ABSTRACT

Para-amino benzoic acid on addition with different aromatic aldehyde gives schiff's bases. The schiff base so formed on treatment with chloroacetyl chloride and triethyl amine as a base catalyst in 1-4 dioxan gives various substituted 4-[3-chloro-4-substituted phenyl-2-oxo-azetidin-1-yl] benzoic acid containing different functional groups(2a-2j).The lead compounds were characterized by melting point, TLC, IR, and ^{1}HNMR studies. All the newly synthesized azetidinone derivatives were evaluated for their anticonvulsant activity by maximal electric shock method. Diazepam was used as standard drug. The compound 2b showed more potent anticonvulsant activity than the standard drug diazepam.

Keywords: p- amino benzoic acid, Aromatic aldehyde, Schiff Base, Azetidinones, Anticonvulsant activities.

INTRODUCTION

Azetidinones which are part of antibiotics structure are known to exhibit interesting biological activities. A large number of 3-chloro-2-azetidinones possesses powerful antimicrobial(Rajiv Dua, 2010;Ubhash Pande, 2009; Navin Patel, 2011), anti-inflammatory (Trilok Chandra, 2009;Vijay Kumar, 2009), analgesic(Vijay Kumar,2009), anticonvulsant (Sayyed Hussain, 2011; N Ramlakshmi, 2008), antitubercular (KIlango, 2011; R.B.Patel, 2006), antioxidant(Vaidya,2010), antihyperglycemic(Rajesh Goel,2004), They also function as enzyme inhibitors & are effective on the central nervous system. A series of β-lactam are competitive inhibitors of GABA aminotransferase inhibitors. Inhibition of GABA aminotransferase, the enzyme that catalyzes the degradation of GABA, can produce an anticonvulsant effect. There are structural similarities between monocyclic and bicyclic β-lactam to γ-amino butyric acid and to the known substrates and inhibitors of GABA aminotransferase[13]. They are the carbonyl derivatives of azetidines containing carbonyl group at the position-2. These are also known as 2-azetidinones. Azetidin-2-one, a four-membered cyclic lactam (β-lactam) skeleton has been recognized as a useful building block for synthesis of a large number of organic molecules by exploiting the strain energy associated with it. Azetidin-2-ones (5) is a hydrolytically sensitive colorless solid, melting point 73-74^{0}C, other simple azetidin-2-ones are usually low melting solids and oils.

The Staudinger reaction (ketene- imines cycloaddition reaction) is regarded as one of the most fundamental and versatile methods for the synthesis of structurally diverse 2-azetidinone derivatives. Azetidin-2-ones can also be synthesized by enolate -imines condensations and cyclization reactions. Also it is used in the synthesis of a variety of β-lactam antibiotics.

MATERIAL AND METHODS

Melting points were determined in an open capillary tube using Veego VMP-1 apparatus and are uncorrected. IR spectra were recorded (in KBr) on Shimadzu FT-IR spectrometer. 1H-NMR spectra was recorded on Bruker DRX-300 (300 MHz FT-NMR) using DMSO as solvent and TMS as Internal standard. TLC using silica gel-G checked the purity of the compounds. The spots were developed in iodine chamber and visualized under ultraviolet lamp.

General Procedure

1) Synthesis of N-substituted–benzylidine –p -amino benzoic acid (Schiff Bases) (1a-1j):

P-amino benzoic acid (0.014mol, 2gm) was dissolved in 10 ml ethanol. The appropriate aromatic aldehyde (0.014mol, 1.48gm) was added to the reaction mixture. It was refluxed for 6-7 hours,cooled to R.T. and then poured into crushed ice .The solid obtained was filtered, washed with water and recrystallized with ethanol.

Mobile phase for TLC Benzene: Acetone (4.5:0.5).

2) Synthesis of substituted 3-Chloro-2-Azetidinones (2a-j):

To a stirred solution of substituted schiff bases of PABA (1a-j) (0.01mol), in 1,4 dioxan (25ml), triethylamine (0.01mol,) and chloroacetylchloride (0.01mol) were added drop wise with constant stirring at 0-20^{0}C. The reaction mixture was kept for 30 min and then refluxed for 7-8 hours. Excess of solvent distilled off and the residue was poured into ice-cold water. A solid obtained was filtered and which was recrystallized from ethanol.

Mobile phase for TLC Benzene: Ethanol (7:3).

4 -[3-chloro-4-phenyl-2-oxo-azetidin-1-yl] benzoic acid. (C_1)

Yield 37.81%, mp 210-212^{0}C, IR (KBr) cm-1; 3246.77cm^{-1} (-OH), 3123.18cm^{-1} (Ar C-H). 1719.95cm^{-1} (C=O β-lactum), 1680.16cm^{-1} (C=O), 1547.67 cm^{-1} (Ar C=C), 1346.46cm^{-1} (C-N), 778.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10 (S,1H –COOH),7.6 -8 (m,9H, phenyl), 6.4(d,1H,3-CH of β-lactum), 6.2(d,1H,4-CH of β-lactum),

4 -[3-chloro-4-(4-chlorophenyl)-2-oxo-azetidin-1-yl] benzoic acid. (C_2)

Yield 62.01%, mp 128-130^{0}C, IR(KBr) cm-1; 3196.77cm^{-1}(-OH), 3143.18cm^{-1} (Ar C-H). 1730.95cm^{-1} (C=O β-lactum), 1695.16 cm^{-1}(C=O), 1556.67 cm^{-1} (Ar C=C), 1346.46cm^{-1} (C-N), 775.52cm^{-1} (C-Cl). NMR(DMSO) δ; 10.2 (S,1H –COOH), 8.2-8.4(m,4H,chlorophenyl), 7.7-8 (m,4H, phenyl), 6.6(d,1H,3-CH of β-lactum), 6.4(d,1H,4-CH of β-lactum).

4 -[3-chloro-4-(4-methoxyphenyl)-2-oxo-azetidin-1-yl] benzoic acid. (C_3)

Yield 51.04%, mp 180-182^{0}C,IR (KBr) cm-1: 3150cm^{-1} (-OH), 3080cm^{-1} (Ar C-H), 1710.12cm^{-1}(C=O β-lactum), 1687.77cm^{-1} (C=O), 1574.93 cm^{-1} (Ar C=C), 1453.41cm^{-1} (C-N), 1261.49cm^{-1} (-C-O), 772.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10.2 (S,1H –COOH), 8.2-8.4(m,4H,methoxyphenyl), 7.6-7.9 (m,4H, phenyl), 6.4(d,1H,3-CH of β-lactum), 6.2(d,1H,4-CH of β-lactum), 1.3(s,3H,CH_3).

4-[3-chloro-4-(4-hydroxyphenyl)-2-oxo-azetidin-1-yl]benzoic acid. (C_4)

Yield 43.72%, mp 160-162^{0}C,IR (KBr) cm-1: 3200cm^{-1} (-OH), 3135cm^{-1} (Ar C-H), 1715.12cm^{-1}(C=O β-lactum), 1681.02cm^{-1} (C=O),1570.11 cm^{-1} (Ar C=C),1420.62cm^{-1} (C-N), 1286.56cm^{-1} (-C-O), 772.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10.3 (S,1H –COOH), 7.45-7.86(m,4H,hydroxyphenyl), 7.2-7.5 (m,4H, phenyl), 6.4(d,1H,3-CH of β-lactum), 5.9(d,1H,4-CH of β-lactum), 3.15(s,1H,Ar-OH).

4-[3-chloro-4-(2-hydroxyphenyl)-2-oxo-azetidin-1-yl]benzoic acid. (C_5)

Yield 53.23%, mp 210-212^{0}C,IR (KBr) cm-1: 3200cm^{-1} (-OH), 3135cm^{-1} (Ar C-H), 1715.12cm^{-1}(C=O β-lactum), 1681.02cm^{-1} (C=O),1570.11 cm^{-1} (Ar C=C),1420.62cm^{-1} (C-N), 1286.56cm^{-1} (-C-O), 772.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10.3 (S,1H –COOH), 7.45-7.86(m,4H,hydroxyphenyl), 7.2-7.5 (m,4H, phenyl), 6.4(d,1H,3-CH of β-lactum), 5.9(d,1H,4-CH of β-lactum), 3.15(s,1H,Ar-OH).

4 -[3-chloro-4-(3-nitrophenyl)-2-oxo-azetidin-1-yl] benzoic acid. (C_6)

Yield 67.96%, mp 160-162^{0}C,IR (KBr) cm-1: 3260cm^{-1} (-OH), 3114.18cm^{-1} (Ar C-H), 1710.12cm^{-1}(C=O β-lactum), 1677.16cm^{-1} (C=O), 1527.67 cm^{-1} (Ar C=C), 1503.67 cm^{-1} (NO_2),1343.46cm^{-1} (C-N), 772.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10.3 (S,1H –COOH), 8.6-8.9(m,4H, nitrophenyl), 7.6-8 (m,4H, phenyl), 6.45(d,1H,3-CH of β-lactum), 6.2(d,1H,4-CH of β-lactum).

4 -[3-chloro-4-(4-dimethylaminophenyl)-2-oxo-azetidin-1-yl] benzoic acid. (C_7)

Yield 60.54%, mp 182-184^{0}C,IR (KBr) cm-1: 3245cm^{-1} (-OH), 3123.28cm^{-1} (Ar C-H), 1714.12cm^{-1}(C=O β-lactum), 1684.36cm^{-1} (C=O), 1532.60 cm^{-1} (Ar C=C), 1343.46cm^{-1} (C-N), 772.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10.3(S,1H –COOH), 8.5-8.9 (m,4H, dimethylamino phenyl), 7.6-8 (m,4H, phenyl), 6.6(d,1H,3-CH of β-lactum), 6.3(d,1H,4-CH of β-lactum),1.45(s,6H,CH_3).

4-[3-chloro-4-(4(Z)-2-phenylvinyl)-2-oxo-azetidin-1-yl] benzoic acid. (C_8)

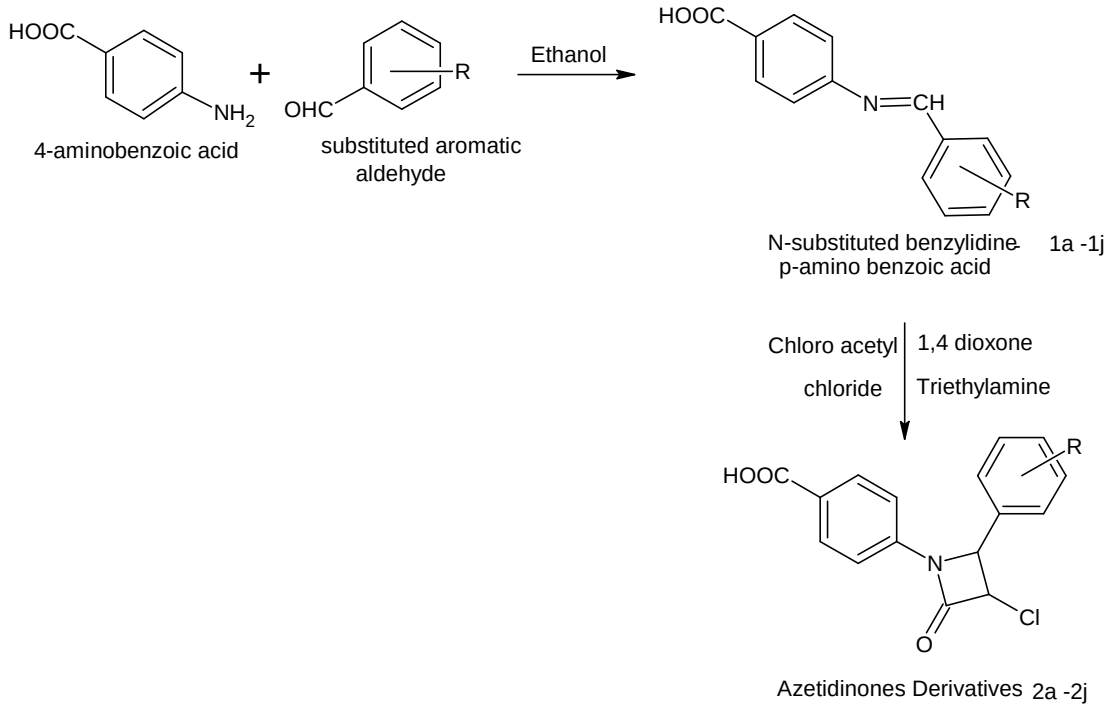
Yield 67.87%, mp 120-122^{0}C,IR (KBr) cm-1: 3245.77cm^{-1} (-OH), 3145cm^{-1} (Ar C-H), 1709.95cm^{-1}(C=O β-lactum), 1677.16cm^{-1} (C=O), 1527.67 cm^{-1} (Ar C=C), 1343.46cm^{-1} (C-N), 772.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10.3 (S,1H –COOH), 7.6-8 (m,9H, phenyl), 6.45(d,1H,3-CH of β-lactum), 6.2(d,1H,4-CH of β-lactum), 6(t,1H,-CH),

4 -[3-chloro-4-(4-nitrophenyl)-2-oxo-azetidin-1-yl] benzoic acid. (C_9)

Yield 69.53%, mp 178-180^{0}C,IR (KBr) cm-1: 3260cm^{-1} (-OH), 3114.18cm^{-1} (Ar C-H), 1709.95cm^{-1}(C=O β-lactum), 1677.16cm^{-1} (C=O), 1527.67 cm^{-1} (Ar C=C), 1503.56 cm^{-1} (NO_2),1343.46cm^{-1} (C-N), 772.52cm^{-1} (C-Cl), NMR(DMSO) δ; 10.3 (S,1H –COOH), 8.6-8.9(m,4H, nitrophenyl), 7.6-8 (m,4H, phenyl), 6.45(d,1H,3-CH of β-lactum), 6.2(d,1H,4-CH of β-lactum).

4 -[3-chloro-4-(3,4,5-trimethoxyphenyl)-2-oxo-azetidin-1-yl] benzoic acid. (C_{10})

Yield 71.77%, mp 180-182^{0}C,IR (KBr) cm-1: 3246.77cm^{-1} (-OH), 3124.18cm^{-1} (Ar C-H), 1719.95cm^{-1}(C=O β-lactum), 1687.16cm^{-1} (C=O), 1530.67 cm^{-1} (Ar C=C),1343.46cm^{-1} (C-N), 1261.49cm^{-1} (-C-O), 772.52cm^{-1} (C-Cl). NMR(DMSO) δ; 10.4 (S,1H –COOH), 8.6-8.9(m,4H, nitrophenyl), 7.6-7.9 (m,4H, phenyl), 6.5(d,1H,3-CH of β-lactum), 6.25(d,1H,4-CH of β-lactum), 1.5(s,9H,CH_3).



Scheme

Table 1: Physical constants of different azetidinone derivatives (2a-2j)

Compound No.	R	Mol. Formula	Mol. Wt	Melting Point (°C)	Rf value	% yield
2a	-C_6H_5	$C_{16}H_{12}O_3NCl$	301.5	210-212	0.79	37.81
2b	4-Cl-C_6H_5	$C_{16}H_{11}O_3NCl_2$	335	90-92	0.83	62.01
2c	4-OCH_3-C_6H_5	$C_{17}H_{14}O_4NCl$	331.5	180-182	0.85	51
2d	4-OH-C_6H_5	$C_{16}H_{12}O_4NCl$	317.5	160-162	0.9	43.72
2e	2-OH-C_6H_5	$C_{16}H_{12}O_4NCl$	317.5	210-212	0.84	53.23
2f	3-NO_2 -C_6H_5	$C_{16}H_{11}O_5N_2Cl$	346.5	160-162	0.88	67.96
2g	4-$N(CH_3)$ -C_6H_5	$C_{18}H_{18}O_3N_2Cl$	345.5	182-184	0.6	60.54
2h	-CH=CH-C_6H_5	$C_{18}H_{14}O_3NCl$	313.5	120-122	0.76	69.87
2i	4-NO_2-C_6H_5	$C_{16}H_{11}O_5N_2Cl$	346.5	178-180	0.86	69.53
2j	3,4,5$(OCH_3)_3$-C_6H_5	$C_{19}H_{18}O_6NCl$	391	180-182	0.82	71.77

Pharmacological Evaluation **Anticonvulsant activity**

The anticonvulsant activity of the compounds was evaluated by maximal electro shock (MES) method using mice where the electroshock is applied through the corneal electrodes. Producing optic stimulation cortical excitation. The MES convulsions are divided into five phases such as (a) Toxic flexion (b) Tonic extension (c) clonic convulsion (d) stupor and (e) Recovery or death. A drug is known to posses anticonvulsant properties. If it reduces or abolishes the extensor phase of MES convulsions, for the evaluation anticonvulsant activity the total 12 groups of animals each containing 6-animals were kept fasting for 10-14 hrs. In that 10 groups were served for testing the synthesized compounds, one as control and one as standard. After that the synthesized compounds were administered to each group at a dose of 25mg/kg of bodyweight, 1% C.M.C. was used as vehicle control and diazepam (5mg/kg of body weight) was used as a standard drug respectively. The activities of each group were measured after the intervals of 60 mins and 120 mins of administration including control and standard.

Results and data are given in table 2.

Neurotoxicity study

Neurotoxicity occurs when the exposure to natural or artificial toxic substances, which are called neurotoxins, alters the normal activity of the nervous system in such a way as to cause damage to nervous tissue. Rota rod test was used for evaluation of minimal neurotoxicity of the synthesized compounds, for the evaluation anticonvulsant activity the total 12 groups of animals each containing 6-animals were kept fasting for 10-14 hrs. In that 10 groups were served for testing the synthesized compounds, one as control and one as standard. After that the synthesized compounds were administered to each group at a dose of 25mg/kg of bodyweight, 1% C.M.C. was used as vehicle control and diazepam (5mg/kg of body weight) was used as a standard drug respectively. After 60 minutes and 120 minutes of administration, animals were placed on the 1 inch diameter rod, rotating at 6 rpm for 1 min. Neurological deficit was indicated by the inability of the animal to maintain the equilibrium for 1 min on the rotating rod in each three trials.

Results and data are given in table 3

Table 2: Anticonvulsant activity data of synthesized compounds (2a-2j)

Group	Treatment	Dose (mg/kg)	Duration of hind limb extensor in seconds (mean±S.E.M)	
			60 minutes	120 min
I	Control	-	52±0.9661	67±0.609
II	Standard	5 mg	11.33±0.4944	23±0.5774
III	2a	25mg	27±0.7303**	44.16±0.6009**
IV	2b	25mg	15±0.5774**	27±0.7303**
V	2c	25mg	26±0.5774**	39.33±0.7601**
VI	2d	25mg	17±0.6831**	26.16±0.7923**
VII	2e	25mg	32±0.8563**	52±0.9661**
VIII	2f	25mg	34.16±0.6009**	48.33±0.8028**
IX	2g	25mg	28.5±0.7638**	65.83±0.6009 ns
X	2h	25mg	50±0.9309 ns	66.16±0.6009 ns
XI	2i	25mg	21±0.5774**	30.66±0.6667**
XII	2j	25mg	32±0.7638**	36.16±0.6009**

Data were analyzed by one-way ANOVA followed by Dunnett's test.

Values are expressed as mean ± S.E.M.

** P<0.01 when compared to control, ns-non significant.

Table 3: Neurotoxicity study data of synthesized compounds

Group	Treatment	Dose (mg/kg)	Fall of latency in sec	
			60 minutes	120 minutes
I	Control	-	-	-
II	Standard	5 mg	25	38
III	2a	25mg	26	-
IV	2b	25mg	-	-
V	2c	25mg	-	-
VI	2d	25mg	-	-
VII	2e	25mg	-	-
VIII	2f	25mg	-	-
IX	2g	25mg	43	-
X	2h	25mg	25	30
XI	2i	25mg	-	-
XII	2j	25mg	-	-

The figure in the table indicate fall of latency in seconds

The dash (-) indicates an absence of neurotoxicity at 60 and 120 minutes time interval.

RESULTS AND DISCUSSION

The synthesis of target compound was accomplished as shown in the scheme. Para amino benzoic acid on condensation gave the known N-substituted –benzylidine- p-amino benzoic Acid **(I)**. Subsequent cycloaddition reaction with chloroacetyl chloride resulted in the synthesis of 3-chloro-2-azetidinones **(II)**.

The title compounds **(2a-2j)** were evaluated for *in vivo* anticonvulsant activity against by MES method. And also evaluated for neurotoxicity study. Most of the compounds exhibited mild to moderate anticonvulsant activity. The results re-veal's that among the tested compounds **2b, 2d, 2i** were found to have better activity, whereas the **2a** and **2c** have moderate activity. Among the tested compounds **2a, 2g** and **2h** exhibited neurotoxicity.

CONCLUSION

We have synthesized successfully a series of azetidinones. The desired product formed was fast, eco-friendly, cheaper, high yield

and easy to handle. The constitution of all the title compounds assigned on the basis of IR, ^{1}HNMR data were found to be in correlation with the desired structure. The present results are worth noticing in the case of anticonvulsants activity of the tested compounds.

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