Synthesis and Characterization of Novel N-Substituted-3-chloro-2-Azetidinones as Potential Anticonvulsant Agents.

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ABSTRACT

Various substituted 4(m-hydroxy-p-methoxy phenyl)-1[(6'-fluoro-7'-substituted (1,3)-benzothiazol-2'-yl) amido-2-phenyl] 3-chloro azetidin–2–one containing different functional groups have been synthesized. The lead compounds were characterized by melting point, TLC, calculated elemental analysis, UV, IR, ^{1}HNMR and Mass spectral studies. The compounds were tested for Anticonvulsant studies by PTZ induced method and showed significant activity at low and high concentration compared to standard; still further studies are requested.

Keywords: Anticonvulsant activity, Azetidinone, Benzothiazole, Fluorine.

INTRODUCTION

2-Azetidinones, commonly known as β-lactams, are well-known heterocyclic compounds among the organic and medicinal chemists.[1] The activity of the famous antibiotics such as penicillins, cephalosporins and carbapenems are attributed to the presence of 2-azetidinone ring in them. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing 2-azetidinone ring.[2,3] Such biological activities include antimicrobial,[4] anti-tuburcular,[5] carbonic anhydrase inhibitors,[6] local anaesthatics,[7] anti-inflammatory,[8] anthelmintic,[9] anticonvulsant,[10] hypoglycemic agents activity.[11] The β-lactams also serve as synthons for many biologically important classes of organic compounds.[12] Due to this, the investigation of chemistry and biology of these compounds continue to appeal the synthetic and medicinal organic chemists.[1-4]

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It is well known that the introduction of fluorine atom into an organic molecule causes dramatic changes in its biological profile, mainly due to high electro negativity of fluorine, the strong carbon-fluorine bond and increased solubility in lipids.[13] Therefore it was thought worthwhile to synthesize better kinds of drugs by incorporating azetidinone in benzothiazole moiety.

In search for new biodynamic potent molecule, it was thought worthwhile to incorporate some additional heterocyclic moieties in the β-lactam nucleus and study their biological and pharmacological activity.[14] The review of literature reveal prompted us to synthesize substituted fluorobenzothiazole, azetidinone targeted compounds and those will be screened for anticonvulsant activity.

MATERIALS AND METHODS

Chemicals and Reagents

4-fluoro-3-chloro aniline, Potassium thiocyanate, Glacial acetic acid, Bromine, Anthranillic acid, Pyridine, Vanillin, Ethanol, Conc. Hydrochloric acid, Chloroacetyl chloride, Triethylamine,

N,N^{1}-dimethyl formamide (DMF), various substituted aniline, morpholine, piperazine and diphenylamine.

Experimental Section

Step I: 4-fluoro-3-chloro aniline was treated with potassium thiocyanate (KSCN) in presence of glacial acetic acid and bromine to get 2-amino-6-fluoro-7-chloro-benzothiazole.

Step II: 2-amino-6-fluoro-7-chloro-benzothiazole treated with Anthranillic acid in presence of Pyridine to get 2 (o-amino phenyl amido) 6–fluoro -7-chloro (1,3) benzothiazole.

Step III: 2 (o-amino phenyl amido) 6–fluoro -7-chloro (1,3) benzothiazole reflexed with vanillin and alcohol in presence of Conc.HCl to get 2 (3-hydroxy-4-methoxy benzylidene amino phenyl amido) 6-fluoro-7-chloro-(1,3) benzothiazole or Schiff's base.

Step IV: A Solution of Schiff's base (0.01 mol) in 1,4-dioxane (50ml) was added to well-stirred mixture of Chloroacetyl Chloride (0.95 ml, 0.012 mol) and Triethylamine (1.08 ml, 0.02 mol) at 0^{o} C. The reaction mixture was then stirred for 18 - 20 hrs and kept aside for 3 days at room temperature. The product was recrystallised from N,N' Dimethyl formamide (DMF).

Step V: Azetidine were treated with double the quantities of various substituted aniline, anisidine, PABA and piperazine, refluxed for 2 hours in presence of N,N^{1}-dimethyl formamide (DMF). The mixture was cooled and poured in to crushed ice. The solid separated was filtered off, dried and crystallized from alcohol and benzene.

General Procedures

Melting points were determined in open capillaries and are uncorrected. IR spectra (KBr pellet technique) were recorded using a Perkin-Elmer 237 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AM 400 instrument (at 400 MHz) using tetramethylsilane (TMS) as an internal standard and DMSO-*d6* as a solvent. Chemical shifts are given in parts per million (ppm). Splitting patterns are designated as follows: s- singlet, d- doublet, t- triplet, q- quartet and m-multiplet. Mass spectra (MS) were recorded on Schimadzu GC-MS operating at 70eV. All the synthesized compounds were purified by recrystallization. The reactions were followed up and the purity of compounds was monitored on pre-coated TLC plates and visualizing the spots in ultraviolet light.

In Vitro Anticonvulsant Study

In the present study the mice of either sex, weighing between 18-25 g were selected and divided into control, test and standard.

Before experiment the animal were fasted for 24 hrs with only water *ad-libitum*. Control group received only 0.5 ml DMF as vehicle. Standard group animals were received diazepam (4 mg/kg b.w.) oral test group animals were received the synthesized derivatives at 4 mg/kg b.w. oral in DMF.

Now for the animals of control group pentylene tetrazole (PTZ) 1 ml / 100 g b.w. was administered and actions like straubs tail, jerky movements of whole body and conclusions were observed.

For animals of standard test group PTZ was injected (1 ml/100 g b.w.). After 30 mins animals of standard and test received diazepam and synthesized derivatives respectively.[15] Observations were made and results were tabulated.

RESULTS AND DISCUSSION

Synthesis and pharmacological screening of 4(m-hydroxy-p-methoxy phenyl)-1[(6'-fluoro-7'-substituted (1,3)-benzothiazol-2'-yl) amido-2-phenyl] 3-chloro azetidin–2–one were tested for anticonvulsant activity by PTZ induced method compared to standard Diazepam; showed significant anticonvulsant activity.

Among compounds tested A_3, A_7, A_8 and A_{12} showed significant anticonvulsant activity.

Scheme

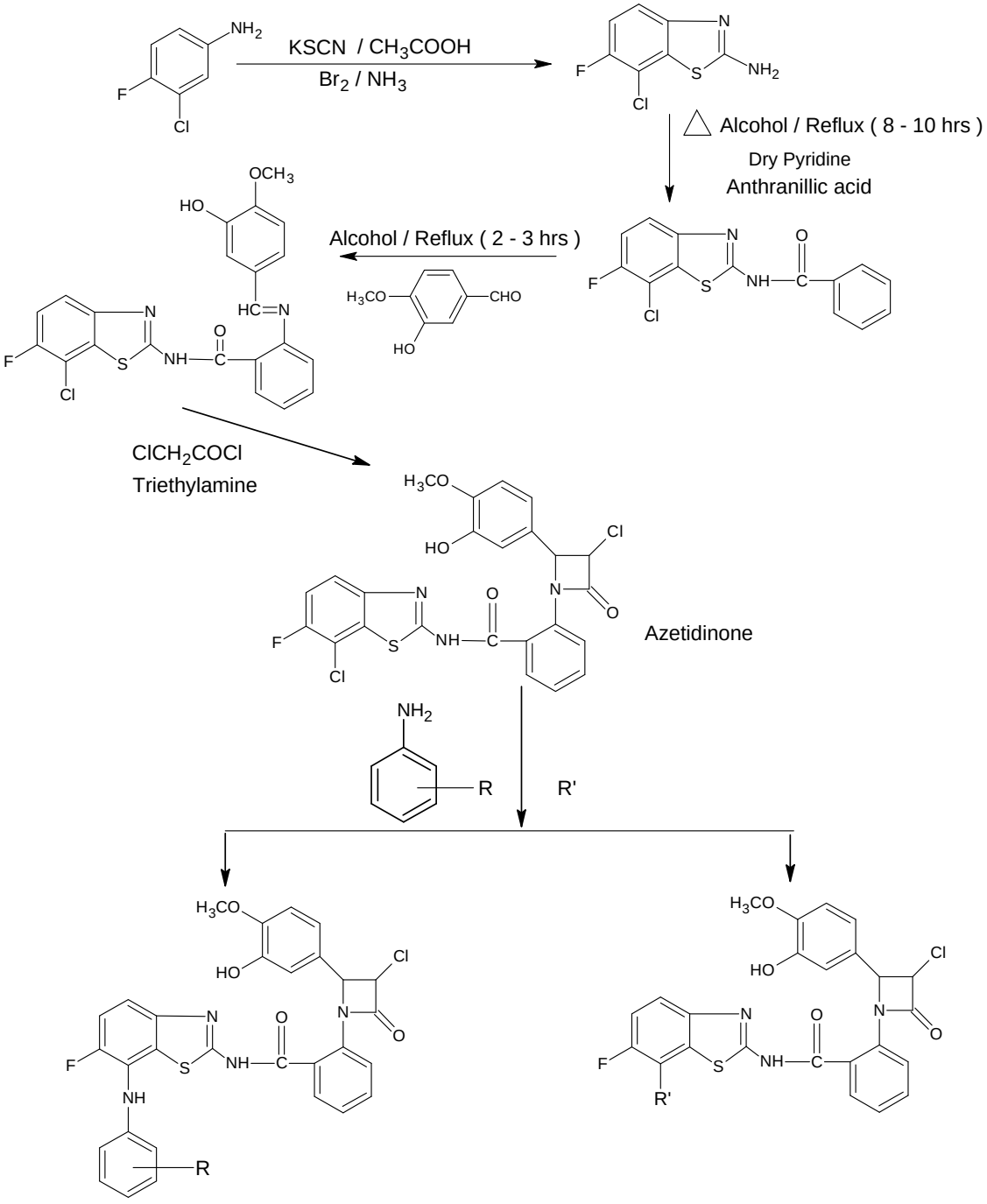
KSCN / CH3COOH
Br2 / NH3
Alcohol / Reflux (8 - 10 hrs)
Dry Pyridine
Anthranillic acid
Alcohol / Reflux (2 - 3 hrs)
ClCH2COCl
Triethylamine
Azetidinone
R
R'
R = o,m,p, nitroaniline
o,m,p, chloroaniline
aniline
o,m,p, anisidine
PABA
R' = piperzine

Table No. 1 Anti Convulsant Activity (PTZ – Induced)

Stock Solution. Test Drugs 5 mg/ml
Mice (Body weight- 18-25gm)
Dose: PTZ – 80 mg/kg I.P.

Animal – Albino
PTZ – 8 mg/ml
Test Drugs – 50 mg / kg orally

SL. No.	Treatment	Dose (mg/kg)	Convulsion			Death / Recovery
			Onset (Sec)	Nature of Severity	Clonic time in Sec	
1	Water + PTZ	5 ml / kg + 80	43	Jerky Movement	55	5/5
2			75		103	
3			63		95	
4			55		75	
5			45		55	
1	A_1 + PTZ	50 + 80	67	Jerky Movement	85	5/5
2			71		93	
3			55		95	
4			56		75	
5			58		95	
1	A_2 + PTZ	50 + 80	71	Jerky Movement	85	5/5
2			62		83	
3			52		95	
4			63		85	
5			45		85	
1	A_3 + PTZ	50 + 80	93	Straub's Tail	450	0/5
2			102		451	
3			103		220	
4			83		395	
5			95		423	
1	A_6 + PTZ	50 + 80	93	Jerky Movement	85	5/5
2			102		73	
3			103		95	
4			83		95	
5			95		75	
1	A_7 + PTZ	50 + 80	63	Jerky Movement	85	3/5
2			52		93	
3			55		95	
4			71		105	
5			63		55	
1	A_8 + PTZ	50 + 80	83	Straub's Tail	450	4/5
2			93		451	
3			105		220	
4			71		390	
5			103		423	
1	A_{10} + PTZ	50 + 80	53	Jerky Movement	85	5/5
2			55		73	
3			55		95	
4			79		75	
5			65		95	

1	A 11 + PTZ	50 + 80	65	Jerky Movement	95	5/5
2			63		83	
3			67		85	
4			73		75	
5			81		95	
1	A 12 + PTZ	50 + 80	52	Jerky Movement	85	4/5
2			53		83	
3			55		95	
4			65		95	
5			75		75	
1	Diazapam + PTZ	50 + 80	97	Straub's Tail	445	0/5
2			105		455	
3			110		210	
4			85		385	
5			93		415	

Table No. 2 Analytical Data

Sl. No	Compound Code	M.P/ B.P°C	% Yield	MOL. FORM	M.Wt.	Calculated %		
						C	H	N
1	A_1	190	78%	$C_{30}H_{21}O_6SN_5FCl$	634	56.83	3.34	11.05
2	A_2	178	82%	$C_{30}H_{21}O_6SN_5FCl$	634	56.83	3.34	11.05
3	A_3	183	75%	$C_{30}H_{21}O_6SN_5FCl$	634	56.83	3.34	11.05
4	A_4	164	72%	$C_{30}H_{21}O_4SN_4FCl_2$	623	57.79	3.39	8.99
5	A_5	132	74%	$C_{30}H_{21}O_4SN_4FCl_2$	623	57.79	3.39	8.99
6	A_6	126	73%	$C_{30}H_{21}O_4SN_4FCl_2$	623	57.79	3.39	8.99
7	A_7	112	76%	$C_{30}H_{22}O_4SN_4FCl$	589	61.17	3.76	9.51
8	A_8	124	65%	$C_{31}H_{24}O_5SN_4FCl$	619	60.14	3.91	9.05
9	A_9	118	69%	$C_{31}H_{24}O_5SN_4FCl$	619	60.14	3.91	9.05
10	A_{10}	158	83%	$C_{31}H_{24}O_5SN_4FCl$	619	60.14	3.91	9.05
11	A_{11}	260	77%	$C_{31}H_{22}O_6SN_4FCl$	633	58.82	3.50	8.85
12	A_{12}	308	85%	$C_{28}H_{25}O_4SN_5FCl$	582	57.78	4.33	12.03

Table No. 3 Characteristics IR absorption bands :

Compound	Ar-NH (in cm^{-1})	C=0 Stretching (in cm^{-1})	C=N Stretching (in cm^{-1})	C=C Stretching (in cm^{-1})	NO_2 (in cm^{-1})	C-F (in cm^{-1})	C – S Stretching (in cm^{-1})	Sec.Ar. Amine (in cm^{-1})	C – Cl Stretching (in cm^{-1})	C-O-C Stretching (in cm^{-1})	Ar-OH Stretching (in cm^{-1})
A_1	3350	1750	1550	1710	1450	1130	720	1300	840	1250	1390
A_2	3370	1710	1525	1680	1450	1160	720	1340	840	1250	1390
A_3	3370	1700	1540	1660	1420	1160	725	1310	850	1255	1380
A_4	3380	1730	1540	1680	-	1155	720	1300	850	1250	1380
A_5	3400	1765	1540	1690	-	1170	725	1310	820	1250	1380
A_6	3290	1720	1530	1680	-	1160	725	1300	840	1250	1380
A_7	3390	1755	1510	1690	-	1150	720	1255	840	1220	1380
A_8	3350	1720	1540	1685	-	1165	725	1310	830	1250	1390
A_9	3310	1730	1550	1650	-	1130	725	1310	840	1245	1380
A_{10}	3400	1750	1560	1660	-	1170	730	1300	850	1230	1385
A_{11}	3320	1700	1530	1640	-	1165	730	1310	840	1270	1380

Table No. 4 ^{1}H NMR Spectral Data

Sl No.	Compound Code	Hydrogen	δ(ppm)	Multiplity	Solvent
1	A_3	-Ar-H- -NH- β lactum 2H – Proton	7.0 – 7.8 5.4 6.6	Multiplet Singlet Doublet	$CDCl_3$
2	A_6	-Ar-H- - NH - β lactum 2H – Proton	7.0 – 7.8 5.3 6.6	Multiplet Singlet Doublet	$CDCl_3$

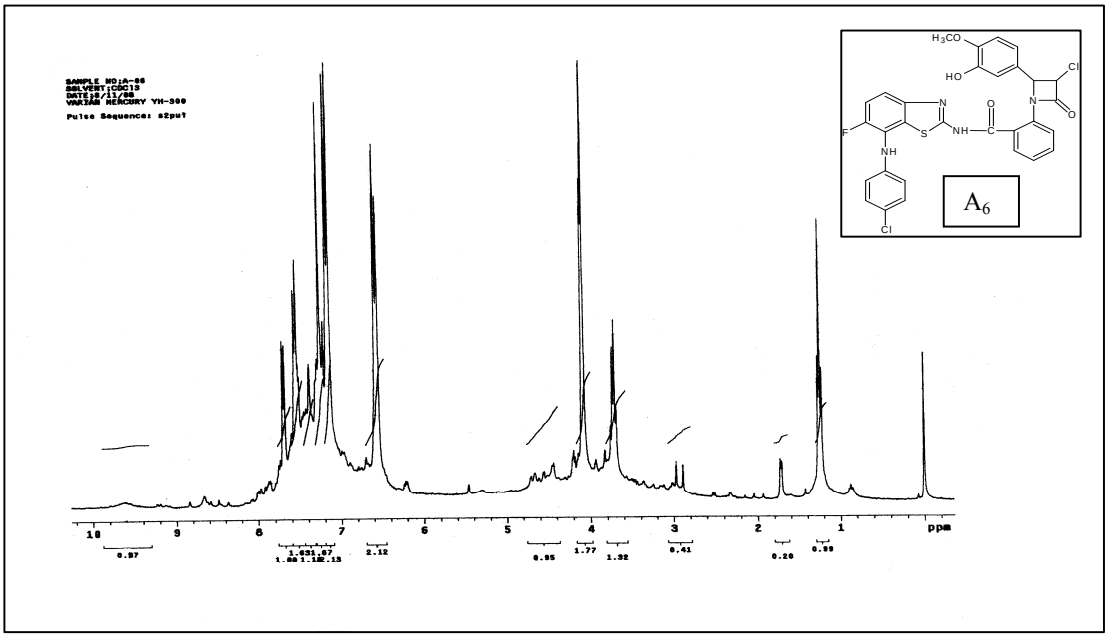

Fig 1. NMR Spectra - A_6

Table No. 5 Mass Spectral Data

Sl no	Compound Code	Calc. Mol. weight	Mol Formula	Fragmentation	m/z
1	A_3	634.03	$C_{30}H_{21}O_6SN_5FCl$	$M^{+1}(CH_3O, Cl, NO_2)$ $M^{+2}(C_6H_3, C_3NO)$ M^{+3} $\{(C_6H_4)_2O\}$	517.5 379.4 201.3
2	A_6	623.48	$C_{30}H_{21}O_4SN_4FCl_2$	$M^{+1}(-CH_3)$ M^{+2} $\{C_6H_3(OH)$-O, Cl, O$\}$ M^{+3} (N-C-C-C_9Cl) M^{+4} $\{(C_6H_4)_2\}$	613.9 454.7 369.8 203.2

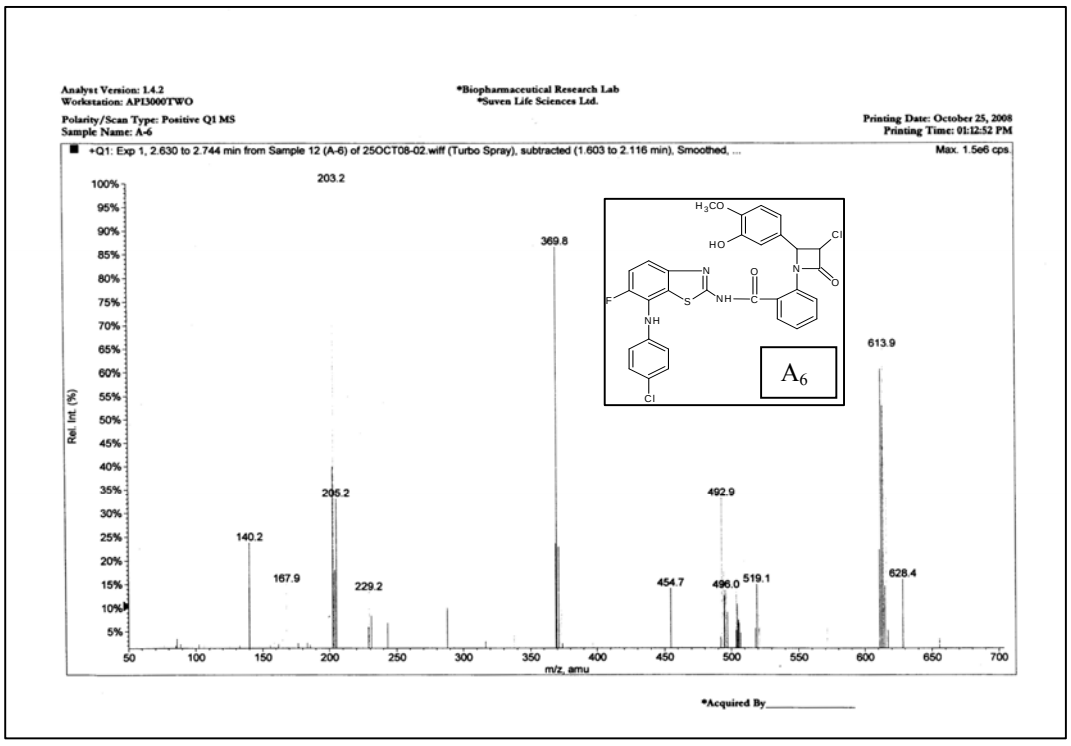

Fig 2. Mass Spectra - A_6

CONCLUSION

Result of present study demonstrate that, a new class of different aromatic aniline, anisidine, PABA, piperzine, encompassing azetidinone derivatives were synthesized and evaluated as antibacterial agents. The newly synthesized heterocyclics exhibited promising anticonvulsant activity using PTZ induced method. The anticonvulsant studies showed significant activity at low and high concentration compared to standard. It can be concluded that this class of compounds certainly holds great promise towards good active leads in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

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