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Synthesis and Pharmacological Screening of Bio-active Molecule Fluorobenzothiazole Comprising Sulfonamido Imidazolinone Derivatives

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6-Fluoro-7-substituted-2-[(2′-phenyl-4′-benzylidenyl-5′-oxo-imidazolin-1′-ylamino)benzene-*p*- sulphonamido] (1,3) benzothiazoles have been synthesized and evaluated for anti inflammatory and anthalmintic activity. Structure of these products has been established by IR, ^{1}H NMR data. Significant activities were observed for some members of the series.

Key Words: Fluorine, Benzothiazoles, Sulfonamids, Imidazolinone.

INTRODUCTION

The sulfonamide[1-3] drugs were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infection in human beings. The introduction of trimethoprim and sulphamethoxazole has resulted in increased use of sulfonamide for the treatment of specific microbial infection. Benzothiazoles[4-6] with sulphonyl group, imidazolone[7-10], *etc*. were reported to posses various pharmacological activity of clinical importance.

However, little is known about substituted benzothiazoles having sulphonamido moiety and imidazole with sulphonamido group. Therefore, in present work, the sulphonamido group is linked with benzothiazole ring and imidazolone group to get good biodynamic leads.

Imidazolinones have been reported to possess antifungal[11-14] anti-inflammatory[15-17] antiviral[18] antitubercular[19,20] and antihistamine activity.

Recently, 1,2,4-tri substituted-5-imidazolinones have been reported to possess mono amino oxidase (MAO) inhibitory and anticonvulsant activity. Benzylidine derivatives are also found to possess MAO inhibitory activity.

EXPERIMENTAL

Melting point were determined by open capillary tube method and are uncorrected. Thin layer chromatography (TLC) was run on silica gel G plates using butanol, ethyl acetate, chloroform (1:2:1) as developing

solvent for the purity of the compounds IR spectra were recorded on Shimadzu FT IR-8400S spectrophotometer by using KBr pellet technique.

Synthesis of 6-fluoro-7-substituted-2-(*p*-hydrazino benzene sulphonamido) (1, 3) benzothiazole: 10 mL of conc. HCl was added drop wise with stirring to hydrazine hydrate (12 mL, 0.2 mol) at 5-10ºC followed by ethylene glycol 40 mL. To the above solution 0.1 mol of 6-fluoro-7-substituted-2-(*p*-amino benzene sulphonamido) (1,3) benzothiazoles in portion were added and the resulting mixture was refluxed for 2 h, cooled, poured in crushed ice. The solid separated, was filtered, dried and recrystallized from ethanol.

Preparation of *p*-acetamido benzene sulphonyl chloride: A 500 mL two necked flask was equipped with a dropping funnel and a reflux condenser, attached the top of the later to calcium guard tube for the absorption of hydrogen chloride. 20 g (0.148 mol) of dry acetanilide was placed in the flask and 50 mL (90 g, 0.77 mol) of a AR grade of chlorosulphonic acid in the dropping funnel. Chlorosulphonic acid was added in small portions and the contents of flask were shaken from time to time to ensure thorough mixing. When the addition has been made the reaction mixture was heated on a water bath for 1 h in order to complete the reaction. It was allowed to cool and the oily mixture was poured with stirring into 300 g of crushed ice contained in a 1 L beaker. This operation was carefully carried out in the fume cupboard since the excess of chlorosulphonic acid, reacts vigorously with the water. The flask was rinsed with a little ice water and rinsing was added to the contents of the beaker. The mixture was stirred for several minutes, the solid lump material was broken to obtain even suspension of the granular white solid. Solid *p*-acetamido benzene sulphonyl chloride formed was filtered at the pump and washed with cold water. It was pressed and drained well, kept for drying.

Condensation of 2-amino-6-fluoro-7-chloro-benzothiazole and *p*-acetamido benzene sulphonyl chloride: 2-Amino-6-fluoro-7-chloro (1, 3) benzothiazole (0.013 mol) was taken in pyridine (4 mL) and acetic anhydride (20 mL), to this *p*-acetamido benzene sulphonyl chloride (0.01 mol) were added and the mixture was kept in water bath for 2 h. The reaction mixture then poured in to 20 mL of ice cold water. The solid obtained was filtered and recrystallized from dil. ethanol (80 %) to get pure compound 6-fluoro-7-chloro-2-(*p*-acetamido benzene sulphonamido) (1,3)-benzothiazole.

General procedure for synthesis of 6-fluoro-7-substituted-2-(*p*-acetamido benzene sulphonamido) (1,3) benzothiazoles: The 0.01 mol of 6-fluoro-7-chloro-2-(*p*-acetamido benzene sulphonamido) (1,3) benzothiazole was treated with equimolar quantity of various substituted

anilines, PABA, morpholine, piperazine, dimethylamine, diphenylamine and refluxed for 2 h in presence of DMF (dimethylformamide) then the mixture was cooled and poured in to crushed ice.

The solid separated was filtered off, dried and recrystallized from benzene and super dry alcohol (1:1).

Hydrolysis of the 6-fluoro-7-substituted-2-(*p*-acetamido benzene sulphonamido) (1,3) benzothiazoles: The derivatives obtained were then hydrolyzed by boiling them in 50 mL of 80 % acetic acid for 4 to 5 h and the contents were poured on to crushed ice. The obtained hydrolyzed derivatives were filtered at suction and dried (**Scheme-I**).

Preparation of 4-benzylidene-2-phenyl-oxazol-5-one (oxazolone): A mixture of 27 g (26 mL, 0.25 mL) of redistilled benzaldehyde, 45 g (0.25 mol) of benzoyl glycine 77 g (71.5 mL, 0.75 mol) of acetic anhydride and 20.5 g (0.25 mol) of anhydrous sodium acetate was placed in a 500 mL conical flask and heated on an electric hot plate with constant shaking. As soon as the mixture has liquified completely, the flask was transferred to water bath and refluxed for 2 h. 100 mL of ethanol was added slowly to the contents of the flask. The mixture was allowed to stand overnight. The crystalline product obtained was filtered by suction. It was washed with two 25 mL portions of boiling water and dried at 100°C.

The yield of pure oxazolone is 40 g (64 %) m.p. 165-166°C. Recrystallization from benzene raised the m.p. to 167-168°C.

A mixture of 0.01 mol of hydrolyzed derivatives each taken separately (6-fluoro-7-substituted-2-(*p*-amino benzene sulphonamido) (1,3) benzothiazole and 2-phenyl4-benzylidene-5-oxazolinone (2.49 g. 0.01 mol) was refluxed in pyridine for 6-8 h. Excess of pyridine was distilled off and resulting mass was poured on to crushed ice and neutralized with dil. HCl, filtered and product was recrystallized from ethanol.

Synthesis of 6-fluoro-7-substituted-2-(*p*-hydrazino benzene sulphonamido) (1,3) benzothiazole: 10 mL of conc. HCl was added drop wise with stirring to hydrazine hydrate (12 mL, 0.2 mol) at 5-10ºC followed by ethylene glycol 40 mL. To the above solution 0.1 mol of 6-fluoro-7-substituted-2-(*p*-amino benzene sulphonamido) (1,3) benzothiazoles in portion were added and the resulting mixture was refluxed for 2 h, cooled, poured in crushed ice. The solid separated, was filtered, dried and recrystallized from ethanol.

Synthesis of 6-fluoro-7-substituted-2-[(2' -phenyl-4'-benzylidenyl-5'-oxo-imidazolin-1'-yl amino)benzene-*p*-sulphonamido] (1,3) benzothiazoles: A mixture of 6-fluoro-7-substituted-2-(*p*-hydrazino benzene sulphonamido) (1,3) benzothiazole and 2-phenyl-4-(benzylidene)-5-oxazolinone was refluxed in pyridine for 6-8 h and latter excess of pyridine was distilled off. The resulting mass was poured on to crushed ice and

neutralized with dil. HCl, filtered and product was recrystallized from ethanol (**Scheme-II**). The characterization data of the synthesized compounds is given Tables 1 and 2.

The denaturation of proteins as one of the causes of inflammation is well documented[21-23]. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins. Mizushima[24] and other have employed protein denaturation as an *in vitro* screening model for antiinflammatory compounds. Bovine serum albumin (Loba Chem) diclofenac sodium (standard) and all other chemicals of analytical grade.

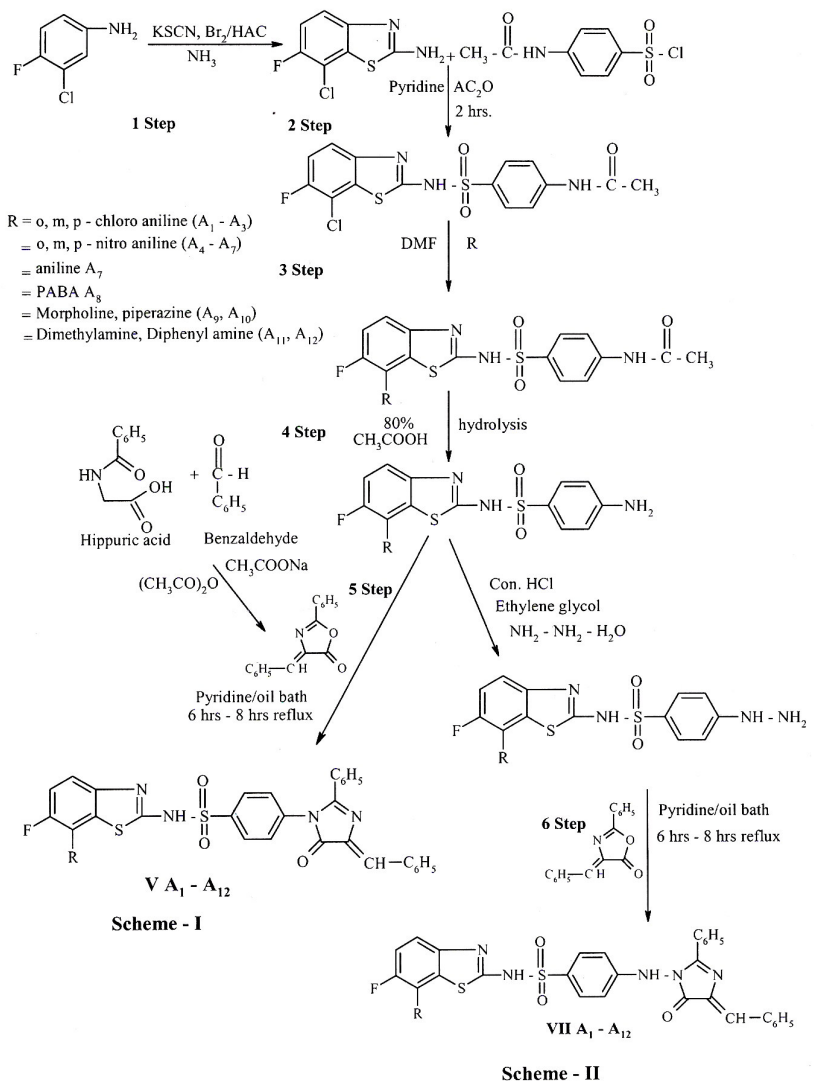

TABLE-1
ANALYTICAL DATA

Compd. code	m.p. (ºC)	Yield (%)	m.f.	m.w.	C (%)	H (%)	N (%)
V A_1	142	108.90	$C_{35}H_{23}N_5O_3S_2FCl$	679.50	61.81	3.38	10.30
V A_2	148	90.00	$C_{35}H_{23}N_5O_3S_2FCl$	679.50	61.81	3.38	10.30
V A_3	160	120.00	$C_{35}H_{23}N_5O_3S_2FCl$	679.50	61.81	3.38	10.30
V A_4	145	119.00	$C_{35}H_{23}N_6O_5S_2F$	690.00	60.86	3.33	12.17
V A_5	142	71.40	$C_{35}H_{23}N_6O_5S_2F$	690.00	60.86	3.33	12.17
V A_6	155	97.00	$C_{35}H_{23}N_6O_5S_2F$	690.00	60.86	3.33	12.17
V A_7	172	93.16	$C_{35}H_{24}N_5O_3S_2F_1$	645.00	65.10	3.72	10.85
V A_8	155	97.08	$C_{36}H_{24}N_5O_5S_2F$	689.00	62.69	3.48	10.15
V A_9	152	94.04	$C_{33}H_{26}N_5O_4S_2F$	639.00	61.97	4.06	10.95
V A_{10}	146	111.40	$C_{33}H_{27}N_6O_3S_2F$	638.00	62.06	4.23	13.16
V A_{11}	187	116.00	$C_{31}H_{24}N_5O_3S_2F$	597.00	62.31	4.02	11.70
V A_{12}	170	99.60	$C_{41}H_{28}N_5O_3S_2F$	721.00	68.23	3.88	9.70
VII A_1	156	48.90	$C_{35}H_{24}N_6O_3S_2FCl_1$	694.50	60.47	3.45	12.09
VII A_2	202	89.60	$C_{35}H_{24}N_6O_3S_2FCl_1$	694.50	60.47	3.45	12.09
VII A_3	105	82.70	$C_{35}H_{24}N_6O_3S_2FCl_1$	694.50	60.47	3.45	12.09
VII A_4	200	47.10	$C_{35}H_{24}N_7O_5S_2F$	705.00	59.57	3.40	13.90
VII A_5	240	111.10	$C_{35}H_{24}N_7O_5S_2F$	705.00	59.57	3.40	13.90
VII A_6	210	112.00	$C_{35}H_{24}N_7O_5S_2F$	705.00	59.57	3.40	13.90
VII A_7	202	115.00	$C_{35}H_{25}N_6O_3S_2F$	660.00	63.63	3.78	12.72
VII A_8	155	60.00	$C_{36}H_{25}N_6O_5S_2F$	701.00	61.36	3.55	11.93
VII A_9	218	105.00	$C_{33}H_{27}N_6O_4S_2F$	654.00	60.55	4.12	12.81
VII A_{10}	210	86.02	$C_{33}H_{28}N_7O_3S_2F$	653.00	60.64	4.28	15.00
VII A_{11}	220	82.30	$C_{31}H_{25}N_6O_3S_2F$	612.00	60.78	4.08	13.72
VII A_{12}	202	71.40	$C_{41}H_{29}N_6O_3S_2F$	736.00	66.84	3.90	11.40

Inhibition of albumin denaturation was studied according sto Muzushima and Kabayashi with significant modification. The test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M pH 4 7.4). Final concentration of DMF in all solutions was less than 2.5 %. The test solution (1 mL) containing different concentrations of drug was mixed with 1 mM albumin solution (1 mL) in phosphate buffer and incubated at 27º ± 1ºC for 15 min Keeping the reaction mixture at 60ºC ± 1º in water bath for 10 min. Induced denaturation. After cooling the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where drug was added. Each experiment was done in triplicate and average is taken[25].

TABLE-2
CHARACTERISTICS IR ABSORPTION BANDS OF SIMILAR COMPOUNDS (V A_1 TO VII A_{11})

Compd. code	Ar-NH_2 str. (cm^{-1})	-C=N- str. (cm^{-1})	Ar-Sec-NH str. (cm^{-1})	C=C str. (cm^{-1})	C-F str. (cm^{-1})	C-Cl str. (cm^{-1})	NO_2 (cm^{-1})	SO_2NH (cm^{-1})	Imidazoline carbonyl group (cm^{-1})	C-O-C (cm^{-1})
AFCB	3474	1645	–	1543	1188	678	–	–	–	–
NPNFCB	3131	1654	–	1542	1272	737	–	1367	–	–
FOB	3452	1649	–	1543	1273	730	–	1368	–	–
V A_1	3140	1610	1330	1490	1160	718	–	1370	1650	–
V A_2	3140	1610	1330	1490	1160	718	–	1370	1650	–
V A_3	3138	1608	1332	1482	1152	710	–	1362	1642	–
V A_4	3140	1610	1330	1490	1160	–	804	1370	1650	–
V A_5	3135	1612	1329	1490	1162	–	805	1369	1650	–
V A_6	3135	1612	1329	1490	1162	–	805	1369	1650	–
V A_7	3140	1615	1330	1490	1165	–	–	1370	1650	–
V A_8	3140	1610	1335	1490	1160	–	–	1375	1655	–
V A_9	3145	1620	1340	1480	1155	–	–	1360	1650	1560
V A_{10}	3140	1615	1330	1490	1165	–	–	1370	1655	–
V A_{11}	3145	1620	1340	1480	1155	–	–	1375	1650	–
V A_{12}	3140	1610	1330	1490	1160	–	–	1370	1650	–
VII A_1	3140	1610	1330	1490	1160	718	–	1370	1650	–
VII A_2	3140	1610	1330	1490	1160	718	–	1370	1650	–
VII A_3	3138	1608	1332	1482	1152	710	–	1362	1642	–
VII A_4	3135	1612	1329	1490	1162	–	805	1369	1650	–
VII A_5	3135	1612	1329	1490	1162	–	805	1369	1650	–
VII A_6	3135	1612	1329	1490	1162	–	805	1369	1650	–
VII A_7	3140	1615	1330	1490	1165	–	–	1370	1650	–
VII A_8	3140	1610	1335	1490	1160	–	–	1375	1655	–
VII A_9	3145	1620	1340	1480	1155	–	–	1360	1650	1555
VII A_{10}	3140	1615	1330	1490	1165	–	–	1370	1655	–
VII A_{11}	3145	1620	1340	1480	1155	–	–	1375	1650	–

All the newly synthesized compounds were tested for anthelmintic activity according to method described in detail by Kalesaraj and Kurup[26]. *Perituma posthuma* (earthworm obtained from Horticultural Department) of nearly equal size (8 ± 1 cm) were selected for present study.

Albendazole was diluted with normal saline to obtain 0.1, 0.2, 0.5 % served as standard and poured into petridishes. The synthesized compounds were prepared in minimum quantity of dimethyl formamide (DMF) and diluted to 15 mL with normal saline to obtain 0.1, 0.2, 0.5 mg/mL concentration were taken in three petridishes. Normal saline serves as control for standard. Six earthworms of nearly equal size were placed in each petridish at room temperature. The time taken to complete paralysis and death were recorded. The mean paralysis and mean lethal time for each sample was recorded (each reading was taken in triplicate). The time taken by worms to become motionless was noted as paralysis time and to ascertain the death, earthworms were frequently applied with external stimuli, which stimulates and induce movement in the earthworms, if alive[27].

RESULTS AND DISCUSSION

Antiinflammatory activity: Synthesized compounds of 6-fluoro-7-substituted-2-(2'-phenyl 4'-benzylidenyl-5'-oxo-imidazolin-l'-yl)-*p*-benzene sulphonamido] (1,3)-benzothiazole and 6-fluoro-7-substituted-2-[(2'-phenyl-4'-benzylidenyl-5'-oxo-imidazolin-1'yl-amino)-*p*-benzene sulphonamido] (l,3)-benzothiazole were tested for antiinflammatory activity by *in vitro* method compared to standard Diclofenac sodium; showed acceptable antiinflammatory activity. Among the compounds tested V A_4, V A_5, V A_{10} and VII A_7, VII A_{10}, VII A_{11} showed promising anti-inflammatory activity (Table-3).

TABLE-3
ANTIINFLAMMATORY ACTIVITY

Name of the compounds	Absorbance value (Mean ± SE)	Inhibition of denaturation (%)
Control	0.087 ± 0.001	–
V A_1	0.098 ± 0.001	12.64
V A_2	0.097 ± 0.002	11.47
V A_3	0.100 ± 0.003	14.94
V A_4	0.111 ± 0.001	27.58
V A_5	0.114 ± 0.002	31.03
V A_6	0.099 ± 0.002	13.79
V A_7	0.098 ± 0.001	12.64
V A_8	0.101 ± 0.001	17.20

Name of the compounds	Absorbance value (Mean ± SE)	Inhibition of denaturation (%)
V A_9	0.102 ± 0.002	17.24
V A_{10}	0.105 ± 0.001	20.68
V A_{11}	0.095 ± 0.001	9.19
V A_{12}	0.097 ± 0.002	11.49
VII A_1	0.098 ± 0.001	12.64
VII A_2	0.097 ± 0.003	11.49
VII A_3	0.100 ± 0.002	14.94
VII A_4	0.099 ± 0.001	13.79
VII A_5	0.101 ± 0.001	16.09
VII A_6	0.102 ± 0.002	17.24
VII A_7	0.102 ± 0.001	18.39
VII A_8	0.100 ± 0.001	17.27
VII A_9	0.103 ± 0.002	14.54
VII A_{10}	0.104 ± 0.002	18.39
VII A_{11}	0.097 ± 0.001	19.54
VII A_{12}	0.111 ± 0.001	11.49
Diclofenac sodium	0.161 ± 0.001	85.05

Anthelmintic activity: Synthesized compounds of 6-fluoro-7-substituted-2-(2'-phenyl 4'-benzylidenyl-5'-oxo-imidazolin-l'-yl)-p-benzene sulphonamido] (1,3)-benzothiazole and 6-fluoro-7-substituted-2-[(2'-phenyl-4'-benzylidenyl-5'-oxo-imidazolin-l'yl-amino)-*p*-benzene sulphonamido] (l,3)-benzothiazole were tested for anthelmintic activity using earthworms, (*Perituma pasthuma*). Among the compounds tested; V A_1, V A_3, V A_4, V A_{11}, V A_{12} and VII A_1, VII A_9, VII A_{11}, VII A_{12} showed significant paralytic time of earthworms, compared to standard drug Albendazole, at all 0.1, 0.2, 0.5 % concentrations of compounds (Table-4).

TABLE-4
ANTHELMINITIC ACTIVITY

Name	Conc. (%)	Time (min)		Name	Conc. (%)	Time (min)	
		Paralysis	Death			Paralysis	Death
Control	0.9	–	–	V A_{12}	0.1	60	170
					0.2	40	143
					0.5	30	120
Albendazole	0.1	50	70	VII A_1	0.1	48	140
	0.2	45	63		0.2	35	110
	0.5	40	55		0.5	30	98

Name	Conc. (%)	Time (min)		Name	Conc. (%)	Time (min)	
		Paralysis	Death			Paralysis	Death
	0.1	35	150		0.1	52	150
V A_1	0.2	21	130	VII A_2	0.2	39	125
	0.5	19	120		0.5	32	110
	0.1	38	160		0.1	59	158
V A_2	0.2	22	152	VII A_3	0.2	45	130
	0.5	20	125		0.5	40	110
	0.1	35	148		0.1	62	145
V A_3	0.2	20	120	VII A_4	0.2	42	119
	0.5	18	110		0.5	35	112
	0.1	40	175		0.1	65	145
V A_4	0.2	29	160	VII A_5	0.2	48	120
	0.5	21	125		0.5	40	109
	0.1	32	170		0.1	70	168
V A_5	0.2	25	145	VII A_6	0.2	56	135
	0.5	23	120		0.5	45	118
	0.1	48	189		0.1	80	170
V A_6	0.2	35	165	VII A_7	0.2	64	149
	0.5	30	130		0.5	50	125
	0.1	49	192		0.1	64	180
V A_7	0.2	38	170	VII A_8	0.2	49	136
	0.5	32	125		0.5	35	101
	0.1	59	194		0.1	52	175
V A_8	0.2	44	170	VII A_9	0.2	39	155
	0.5	32	135		0.5	31	110
	0.1	55	198		0.1	60	185
V A_9	0.2	39	180	VII A_{10}	0.2	42	160
	0.5	29	120		0.5	32	125
	0.1	75	188		0.1	70	162
V A_{10}	0.2	45	145	VII A_{11}	0.2	50	140
	0.5	35	120		0.5	38	105
	0.1	48	150		0.1	52	145
V A_{11}	0.2	38	119	VII A_{12}	0.2	39	120
	0.5	30	110		0.5	30	98

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