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ORIGINAL RESEARCH ARTICLE

Synthesis of 10-[7,11-(2,4 di substituted phenyl)-3-oxo-9-aminoimino-2,4-diazaspiro [5,5] phenothiazine derivatives as anticonvulsant Activity

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ABSTRACT

10-chloroacetyl-2-trifluoro methyl phenothiazine **(1)**. 10-[7,11-(2,4 di substituted phenyl)-3-oxo-9-aminoimino-2,4-diazarpiro [5,5] undecane 1,5-dione] acetyl-2-tri fluoro methyl phenothiazines **(2-27)** were prepared in present study. The newly synthesized compounds were screened for their anticonvulsant activity against electrically (MES) and chemically (PTZ, picrotoxin and bicuculline) induced seizures and compared with the standard drugs phenytoin sodium, Lamotrigine and Sodium valproate. The compound **13** was found to be most potent compound of this series.

Keywards: phenothiazine derivatives, anticonvulsant activity & acute toxicity.

INTODUCTION:

phenothiazine derivatives have been found to possess potent wide spectrum CNS activities viz. anticonvulsant [1], antipsychotic [2], antidepressant [3] and neuroleptic [4]. Furthermore, new pharmacophores like triazole, oxadiazole and thiadiazole having different heterocyclic ring have been reported to exhibit anticonvulsant activity [5-14]. Based on these findings, we have synthesized the newer phenothiazine derivatives by incorporating the above mentioned new pharmacophores with the hope to possess better anticonvulsant activity. The introduction of various heterocyclic/aliphatic moieties at 10- phenothiazine led to the discovery of promethazine [10-(2-methyl aminopropyl) phenothiazine hydrochloride], chloropromazine [2-chloro-10-3-(3-dimethylaminoproypl) phenothiazine hydro-chloride], which possess potent anti-histaminic and CNS depressant activities respectively. All the compounds were tested pharmacologically for their anti-convalsant activities. The structure of all these newly synthesized compounds was confirmed on the basis of spectral (IR, ^{1}HNMR and mass) and analytical data.

RESULT & DISCUSSION:

Anticonvulsant activity of 10-chloro acetyl-4-fluoral phenothiazine; 10-[7,11-diaryl-3-oxo/thiaoxo-9-aminoimino-2,4-diazospiro [5,5] undecane 1,5-dione; 10-[7,11-(2,4-di substituted)diphenyl-3-oxo-9-amino imino-2,4-diazospiro [5,5] undecane 1,5-dione]acetyl phenothiazines.

In table II revealed the anticonvulsant activity of phenothiazines derivatives all the compounds 2-21 exhibited variable degree of (30-90%) anticonvulsant. Compound (13) was showed maximum anticonvulsant activity (90%) against MES protection in compounds (2-11) oxo derivatives of phenothiazines compounds 3, 7, 10 & 11 showed same degree of protection (70%) against MES. Compound (6) showed 30% protection. In Compounds (12-21), 10-[7,11-di aryl-3-thioxo-9-aminoimino-2,4-diazaspiro [5,5] undecane 1,5-dione] acetyl phenothiazines exhibited significance protection in the range of (30-90%) against MES protection. In these compounds, compound 16 showed 40% protection. Compound (13), 10-[7,11-{di (4-methoxy phenyl)}-3-thioxo-9-amino imino-2, 4-diazospiro [5,5] undecane 1,5-dione]acetyl phenothiazine exhibited maximum 90% protection against MES it was further tested in three graded doses (7.5, 15 and 30 mg/kg i.p.) it may be concluded that compound having 4-methoxy group has showed good protection MES moreover spiro thiobarbiturate compounds (12-21) were found to

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be more active than spiro barbiturate compounds (2-11) hence it can be concluded.

1. Presences of thia substituted phenothiazines moiety have shown better anticonvulsant activity than the compounds having oxo substituted phenothiazines moiety.
2. 2-fluorophenyl substituted phenothiazines ring showed more potent activity than other substituted phenothiazines.
3. The presence of electronegative group (fluorine) plays a pivitol role to increase the anticonvulsant activity.

All the compounds showed ALD_{50} values > 1600 mg/kg i.p. suggesting a good safety margin. However the most potent compounds 13 & 14 exhibited an ALD_{50} > 3200 mg/kg i.p. maximum dose tested.

Pharmacology

All the newly synthesized compounds were tested for anticonvulsant activity. Anticonvulsant activity was determined by supra maximal electro shock seizure pattern test (SMES) and pentylene tetrazole method. The effect of unknown compounds was compared with the standard drug phenytoin sodium, lamotrigine and sodium valproate. Propylene glycol treated group served as control. All the newly synthesized compounds were also screened for their approximate lethal dose (LD_{50}).

Electrically induced seizures

Supramaximal electroshock seizure pattern test (SMES): This activity was performed by following the method of Qifeng et al. [15]. Mice were divided into the groups of 10 animals each and pregnancy was excluded. The mice were treated with the test compounds and phenytoin sodium at a dose of 30 mg/kg p.o. After 1h they were subjected to the shock of 25 mA by convulsiometer through ear electrodes for 0.25 s. Abolition of the hind limb extensor component of the seizure is de.ned as protection, and results are expressed as number of animals protected/number of animals tested.

Chemically induced seizures

Pentylenetetrazole (PTZ), picrotoxin and bicuculline induced seizures

For the chemically induced convulsion test according to the method of Fischer [16, 17], pentylenetetrazole/picrotoxin/bicuculline was dissolved in suf.cient 0.9% saline to allow subcutaneous injections to mice. The animals given subcutaneous pentylenetetrazole (scPTZ)/picrotoxin/bicuculline in a dose of 80 mg/kg in the scruff of neck were observed for at least 30 min for the presence or absence of seizure. Standard drug in this model was sodium valproate (80 mg/kg p.o.) and was injected 60 min prior to PTZ/ picrotoxin/bicuculline challenge. The failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5sce duration) is de.ned as protection.

Acute toxicity study

The compounds were investigated for this acute toxicity (LD_{50}) in albino mice by following the method of smith [18]. Test compounds were administered orally as gum acacia suspension in one group and the same volume of normal saline in another group of animals consisting six mice each in graded doses. During the study, the animals were allowed to take water and food adlibidum. After 24 h of drug administration percent mortality in each group was observed. From the data obtained LD_{50} was calculated.

Experimental

General

All reagents and solvents were of analytical grade and used directly. The chemicals and solvents used for the experimental work here commercially procured from E. Merck, HIMEDIA, CDH, s. d. and Qualigens, India. All reagents and solvents were generally used as received from the commercial supplier. Reactions were routinely performed in oven-dried borosil glassware. The melting points of compounds were determined in open capillaries with the help of thermonic melting point apparatus and were uncorrected. Homogeneity of all the newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G coated plate of 0.5 mm thickness. The eluent was a mixture of different polar and nonpolar solvents in different proportions, and spots were visualized under iodine chamber. Elemental analysis (C, H, N) of all the compounds were performed on CHN analyzer, Carlo Erba 1108 analyzer at the Central Drug Research Institute (Lucknow, India). The IR spectra were recorded on Perkin Elmer 881 FTIR spectrophotometer (nmax in cm_1). The 1H NMR spectra were recorded in $CDCl_3$ and DMSO-d6 on Brucker DRX-300 FTNMR instrument.

10-chloroacetyl-2-tri floro methyl phenothiazine (1):

It was prepared according to the reported method (Jaiswal et al. 1981). To a solution of phenothiazine (0.01 mole) in benzene (dry, 50 ml), chloroacetyl chloride (0.02 mole) was added drop by drop at 0-5ºC. Reaction mixture was stirred for 2 hr. at room temperature the solid thus separated out was filtered and washed with petroleum ether and recrystallised from chloroform.

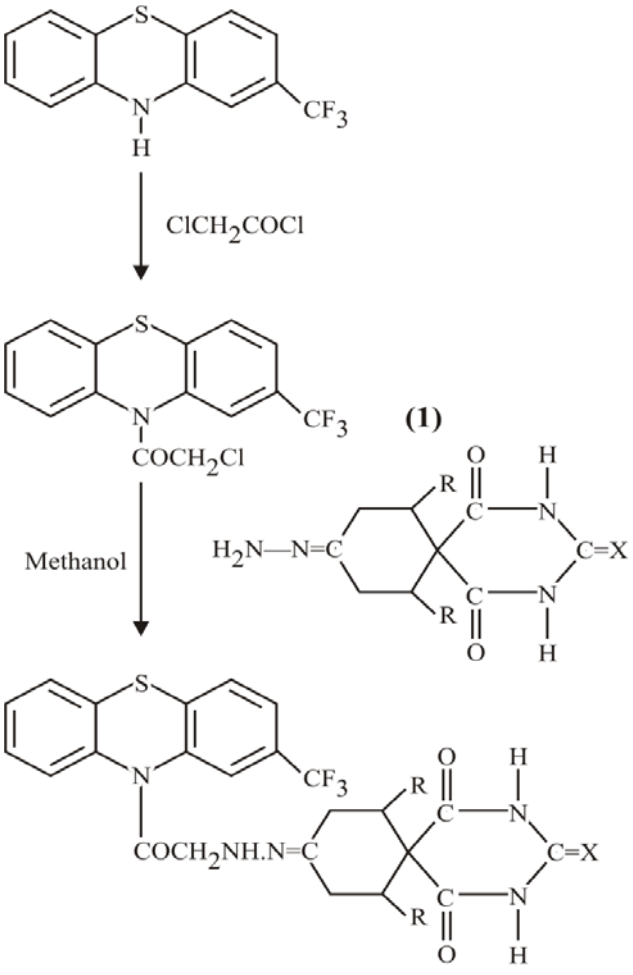
Yield 72% (reported 76%) m.p. 182ºC (reported 183ºC), Compound **1a** (Found: C, 52.16; H, 2.74; N, 4.28; Calc. for $C_{15}H_9NF_3ClOS$: C, 52.41; H, 2.64; N, 4.07 %). IR (KBr) ν_{max} in cm^{-1} : 768 (C-S-C), 3048 (N-H), 1310 (C-F), 3010 (aromatic C-H), 1540 (C⎓C of aromatic ring) ^{1}H NMR ($CDCl_3$) δ 8.21-6.85 (m, 7H, Ar-H), 3.21 (s, 2H, -$COCH_2Cl$), ppm MS : $[M]^+$ at m/z (343.75).

10-[7,11-(2,4 di substituted phenyl)-3-oxo-9-aminnoimino-2,4-diazarpiro [5,5] undecane 1,5-dione] acetyl-2-tri floro methyl phenothiazines (2).

To a solution of 10-chloroacetyl 2-tri floro methyl phenothiazine (0.01 mole) in methanol (dry, 50ml), 7,11-(2,4-disubstituteddiphenyl)-3-oxo-9-aminoimino-2,4-diazarpiro [5,5] undecane 1,5-dione] (0.01 mole) was added with stirring the reaction mixture then refluxed for 6-8 hours. Excess of solvent was distilled of and residue thus obtained washed with petroleum either (40-60ºC) and recrystallised from ethanol/water.

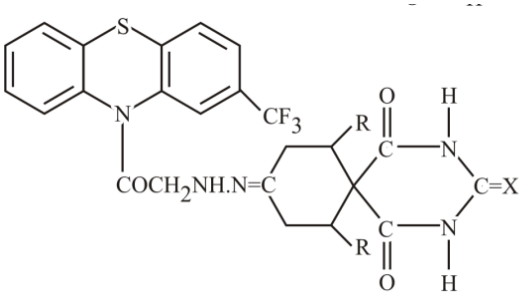
Yield 65%, m.p. 174ºC, Compound **2** (Found: C, 61.27; H, 4.18; N, 9.66; Calc. for $C_{38}H_{32}N_5F_3O_6S$: C, 61.37; H, 4.34; N, 9.42 %). IR (KBr) ν_{max} in cm^{-1} : 775 (C-S-C), 3065 (N-H), 1340 (C-F), 1676 (C=O), 845 (C-Cl), 1300 (C-N), 3025 (aromatic C-H), 1545 (C⎓C of aromatic ring).^{1}H NMR ($CDCl_3$) δ 9.22 (bs, 2H, 2X,NHCO), 7.56-6.89 (m, 15H, Ar-H), 4.83 (hump, 1H, -CH_2N<u>H</u>), 4.46 (dd, 2H, 7H and 11H of undecane ring), 3.59 (d, 2H, C<u>H</u>$_2$NH), 3.57 (dd, 4H, 8H and 10 H of undecane ring), 3.42 (m, 6<u>H</u>, 2-OCH_3 attached with phenyl ring) ppm. MS: $[M]^+$ at m/z (743.75).

Other 10-[7, 11-(3,5-di substituted) di phenyl-3-oxo/thia-9-aminoimino-2,4-diazaspiro [5,5] undecane 1,5-dione] acetyl phenothiazines (3-21) were prepared by the above similar method and their physical and analytical data are given in (**Table I)**.



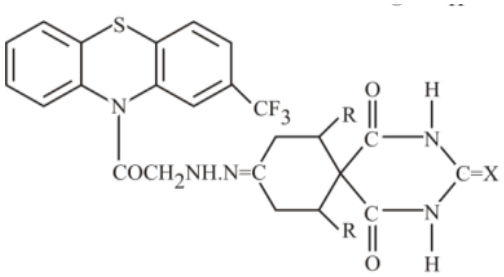
(2-21)

Table-I: Physical and analytical data of 10-[7, 11-(2,4-di substituted) diphenyl-3-oxo-9-amino imino-2,4-diazospiro [5,5] undecane 1,5-dione] acetyl-2-tri floro methyl phenothiazines. (2-21)



Com.	R	X	Mol. Wt	M.P°C	Yield %	Recrystalisation solvent	Mol.formula	Element Analysis					
								% C		% H		% N	
								Calcd	Found	Calcd	Found	Calcd	Found
2	2-OCH_3,C_6H_5	O	743.7	174	65	Ethanol/Water	$C_{38}H_{32}N_5F_3O_6S$	61.37	61.32	4.34	4.12	9.42	9.32
3	4-OCH_3,C_6H_5	O	743.7	172	62	DMF/Water	$C_{38}H_{32}N_5F_3O_6S$	61.37	61.30	4.34	4.15	9.42	9.50
4	2-OH,C_6H_5	O	715.7	168	60	Benzene	$C_{36}H_{28}N_5F_3O_6S$	60.41	60.18	3.94	3.88	9.79	9.92
5	4-OH,C_6H_5	O	715.7	169	61	Benzene	$C_{36}H_{28}N_5F_3O_6S$	60.41	60.20	3.94	3.72	9.79	9.86
6	–C_6H_5	O	683.7	162	58	DMF/Water	$C_{36}H_{28}N_5F_3O_4S$	63.24	63.19	4.13	4.22	10.24	10.40
7	2-F,C_6H_5	O	719.6	170	55	Ethanol/Water	$C_{36}H_{26}N_5F_5O_4S$	60.08	60.34	3.64	3.54	9.73	9.62
8	4-F,C_6H_5	O	719.6	160	52	Ethanol/Water	$C_{36}H_{26}N_5Br_2F_3O_4S$	60.08	60.35	3.64	3.42	9.73	9.65
9	2-Br,C_6H_5	O	841.4	164	50	DMF/Water	$C_{36}H_{26}N_5Br_2F_3O_4S$	51.38	51.22	3.11	3.18	8.32	8.64
10	4-Br,C_6H_5	O	841.4	158	54	DMF/Water	$C_{36}H_{26}N_5Br_2F_3O_4S$	51.38	51.25	3.11	3.19	8.32	8.60
11	4-Cl,C_6H_5	O	752.5	155	52	Ethanol/Water	$C_{36}H_{26}N_5F_3Cl_2O_4S$	57.45	57.63	3.48	3.68	9.31	9.52
12	2-OCH_3,C_6H_5	S	759.8	180	50	DMF/Water	$C_{38}H_{32}N_5F_3O_5S_2$	60.07	60.24	4.24	4.44	9.22	9.50
13	4-OCH_3,C_6H_5	S	759.8	178	52	DMF/Water	$C_{38}H_{32}N_5F_3O_5S_2$	60.07	60.26	4.24	4.44	9.22	9.52
14	2-OH,C_6H_5	S	731.7	165	58	Ethanol/Water	$C_{36}H_{28}N_5F_3O_5S_2$	59.09	59.39	3.86	3.62	9.57	9.78
15	4-OH,C_6H_5	S	731.7	163	55	DMF/Water	$C_{36}H_{28}N_5F_3O_5S_2$	59.09	59.36	3.86	3.72	9.57	9.82
16	–C_6H_5	S	699.7	155	52	DMF/Water	$C_{36}H_{28}N_5F_3O_3S_2$	61.79	61.92	4.03	4.06	10.01	10.12
17	2-F,C_6H_5	S	735.7	171	58	Benzene	$C_{36}H_{26}N_5F_5O_3S_2$	58.77	58.65	3.56	3.46	9.52	9.62
18	4-F,C_6H_5	S	735.7	168	52	Benzene	$C_{36}H_{26}N_5F_5O_3S_2$	58.77	58.62	3.56	3.46	9.52	9.70
19	2-Br,C_6H_5	S	857.5	166	56	Methanol/Water	$C_{36}H_{26}N_5F_3Br_2O_3S_2$	50.42	50.32	3.06	3.12	8.17	8.32
20	4-Br,C_6H_5	S	857.5	163	54	Ethanol/Water	$C_{36}H_{26}N_5F_3Br_2O_3S_2$	50.42	50.31	3.06	3.10	8.17	8.40
21	4-Cl,C_6H_5	S	768.6	167	50	DMF/Water	$C_{36}H_{26}N_5Cl_2F_3O_3S_2$	56.25	56.08	3.41	3.56	9.11	9.38

Table-II: Anticonvulsant activity of 10-[7, 11-(2,4-di substituted) diphenyl-3-oxo-9-amino imino-2,4-diazospiro [5,5] undecane 1,5-dione] acetyl-2-tri floro methyl phenothiazines. (2-21



Comp.	R	X	Dose (mg/kg i.p.)	Anticonvulsant activity (SMESc)		ALD$_{50}$(mg/kg i.p.)
				No. of animals exhibiting convulsion	% seizure protection	
	P.G.[a]		2 ml.	10	0	
	P.S.[b]		7.5	7	30	
			15	5	50*	
			30	2	80***	
2.	$-C_6H_5$	O	30	6	40*	> 1600
3.	2-F,C_6H_5	O	7.5	3	70**	> 1600
			15	4	60**	
			30	3	70**	
4.	4-F,C_6H_5	O	30	3	70**	> 1600
5.	2-Br,C_6H_5	O	30	4	60**	> 1600
6.	4-Br,C_6H_5	O	30	7	30	> 1600
7.	4-Cl,C_6H_5	O	30	3	70**	> 1600
8.	2-OCH_3,C_6H_5	O	30	7	30	> 1600
9.	4-OCH_3,C_6H_5	O	30	6	40*	> 1600
10.	2-OH,C_6H_5	O	30	3	70**	> 1600
11.	4-OH,C_6H_5	O	30	3	70**	> 1600
12.	$-C_6H_5$	S	30	6	40*	> 1600
13.	2-F,C_6H_5	S	7.5	7	30	> 3200
			15	5	50*	
			30	1	90***	
14.	4-F,C_6H_5	S	7.5	7	30	> 3200
			15	5	50*	
			30	2	80***	
15.	2-Br,C_6H_5	S	30	3	70**	> 1600
16.	4-Br,C_6H_5	S	30	6	40*	> 1600
17.	4-Cl,C_6H_5	S	30	4	60**	> 1600
18.	2-OCH_3,C_6H_5	S	30	6	40*	> 1600
19.	4-OCH_3,C_6H_5	S	30	5	50*	> 1600
20.	2-OH,C_6H_5	S	30	4	60**	> 1600
21.	4-OH,C_6H_5	S	30	30	70***	> 1600

*** P<0.05; ** P<0.01; *** P <0.001**

a. P.G. = Propylene glycol standard for control group.

b. Phenytoin Sodium = reference standard for anticonvulsant activity.

c. Subramaximal electroshock seizure pattern test.

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