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Synthesis, Characterization and Biological Evaluation of Some Substituted Thiohydantoins

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ABSTRACT Thiohydantoins have been proven to have anticonvulsant and antidepressant activity. Some derivatives of thiohydantoin containing both aryl group and benzylidene nucleus were synthesised and their anticonvulsant activity were investigated by pentylenetetrazole induced convulsions. It was found that the activity of Compound C was found to be significant when compared to that of the other three synthesized thiohydantoin compounds. Clonazepam is a marketed potent anticonvulsant drug. Compound C was compared with the clonazepam drug (0.25µg/10g).

(Thiohydantoins, synthesis, anticonvulsant activity)

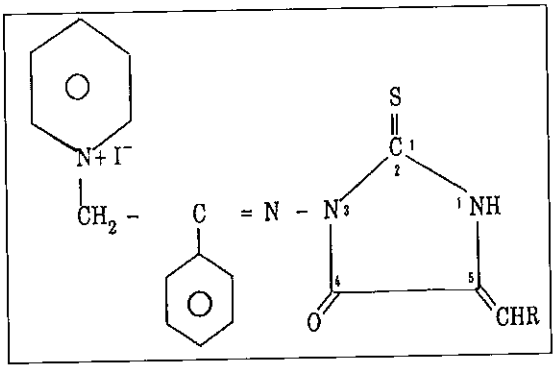
INTRODUCTION

Thiohydantoins are analogous to hydantoins. They are also referred to as thioimidazolidine or glycolylthiourea. Thiohydantoins contain a five membered ring in which 3 carbon atom and 2 nitrogen atoms are present in a fused ring system. A thiocarbonyl group and carbonyl group is also present. A methyl group is present in its ring structure. Thiohydantoins are considered to possess considerable anticonvulsant and antidepressant activity [1]. The C_5 atom of hydantoin can be considered to possess an active methylene group and is therefore a suitable position for base catalyzed condensation with aldehydes [2]. From the literature survey it shows that increasing the carbon chain and including the aromatic group in the nitrogen atom and presence of benzylidene nucleus causes enhancement in anticonvulsant activity [3, 4].

The anticonvulsant activity was performed in mice treated with pentylenetetrazole by pentylenetetrazole induced convulsions [5, 6, and 7]. The above facts motivated us to synthesize some derivatives of thiohydantoin containing both aryl group and benzylidene nucleus and perform their anticonvulsant activity.

MATERIALS AND METHODS

Reaction of 4-oxo-2-thioimidazolidinyl-3-(methyl-phenyl-imino)-pyridinium iodide with different aromatic aldehydes yielded four compounds (A - D) respectively.



Where R =
1) Para-dimethylaminobenzaldehyde-[4-N $(CH_3)_2$ C_6 H_4]
2) 3,4,5-trimethoxybenzaldehyde-[3, 4, 5 -OCH_3 C_6H_4]
3) Anisaldehyde (4-methoxybenzaldehyde)-[4-OCH_3 C_6H_4]
4) 4-nitrobenzaldehyde-[4-NO_2 C_6 H_4]

The main synthetic pathway that was used to perform the synthesis of substituted thiohydantoins is as follows:

N-acetophenone pyridinium iodide (1) on treatment with thiosemicarbazide gives the N-acetophenone pyridinium iodide thiosemicarbazone (II). Cyclisation of (II) with monochloroacetic acid in the presence of anhydrous pyridine affords a product 4-oxo-2-thioimidazolidinyl-3-(methyl-phenyl-imino)-pyridinium iodide (III). Reaction of (III) with aromatic aldehyde in the presence of acetic acid, sodium acetate results in 3-(5-arylidine-4-oxo-2-thio-imidazolidinyl)-N-(methyl-phenyl-imino)-pyridinium iodide (IV). In the present study the various aromatic aldehydes like, para-dimethylaminobenzaldehyde, 3, 4, 5-trimethoxybenzaldehyde, 4-nitrobenzaldehyde, anisaldehyde are used to produce different substituted thiohydantoins. All the four synthesized substituted thiohydantoin compounds were characterized by the following methods. Melting points of the synthesized compounds were taken in open capillary tube and are uncorrected (Toshniwal melting point apparatus). Infrared Spectra were recorded on Win-Bommen B-104 IR spectrophotometer using KBr pellets. ^{1}HNMR spectra were taken on a Jeol GSX-400 NMR spectrophotometer using TMS as internal standard. Solvents and reagents used for the study were purified by standard methods. Thin layer chromatography was performed using plates coated with silica gel GF of 0.25 mm thickness and eluted by suitable solvent system. Spots were visualised in the UV-light chamber and iodine chamber.

MATERIALS AND METHODS

General Details of Synthesis of Compounds

N-acetophenone pyridinium iodide (I) [8]
A mixture of anhydrous pyridine (0.2 mol), redistilled acetophenone (0.2 mol) and iodine (0.2 mol) was heated at 100°C in an oil bath for 2 hours (anhydrous condition). The reaction mixture was cooled, and the crystals formed was filtered and recrystallized from ethanol [9, 10] to afford compound I as a brownish crystalline compound. Melting Point: 213°C.

N-acetophenone pyridinium iodide thiosemicarbazone (II)
A mixture of N-acetophenone pyridinium iodide (I) (0.05 mol) and thiosemicarbazide (0.05 mol) in glacial acetic acid was refluxed for 6 hours. Acetic acid was removed by distillation and the crude sample is recrystallized using dilute ethanol [9]. Melting Point: 172°C

4-oxo-2-thioimidazolidinyl-3-(methyl-phenyl-imino)-pyridinium iodide (III)
A mixture of N-acetophenone pyridinium iodide thiosemicarbazone (II) and monochloroacetic acid (equimolar quantities) were dissolved in anhydrous pyridine and refluxed for 40 hours and cooled. It was added to cold water and the product formed is filtered and recrystallized using dilute ethanol [10, 12]. Melting Point: 122°C.

3-(5-arylidine-4 oxo-2-thio-imidazolidinyl)- N-(methyl-phenyl-imino)-pyridinium iodide (IV)
A mixture of 4-oxo-2-thio imidazolidinyl-3-(methyl-phenyl-imino)-pyrdinium iodide (III) (0.01 mol), an appropriate aromatic aldehyde (0.01 mol) and fused sodium acetate (1.0g) in glacial acetic acid was heated under reflux for 8 hours on a sand bath under anhydrous condition. Solvent was distilled off under reduced pressure and the residual pasty mass was washed thoroughly with water. The solid thus obtained, was dried in a vacuum desiccator and recrystallized from ethanol [2]. The reactions are shown over the next two pages in the Figure 1.

The elemental analysis [10, 11] data is shown in Table 1.

The products are also characterized by spectral analysis; the IR and ^{1}H NMR data are shown in Table 2.

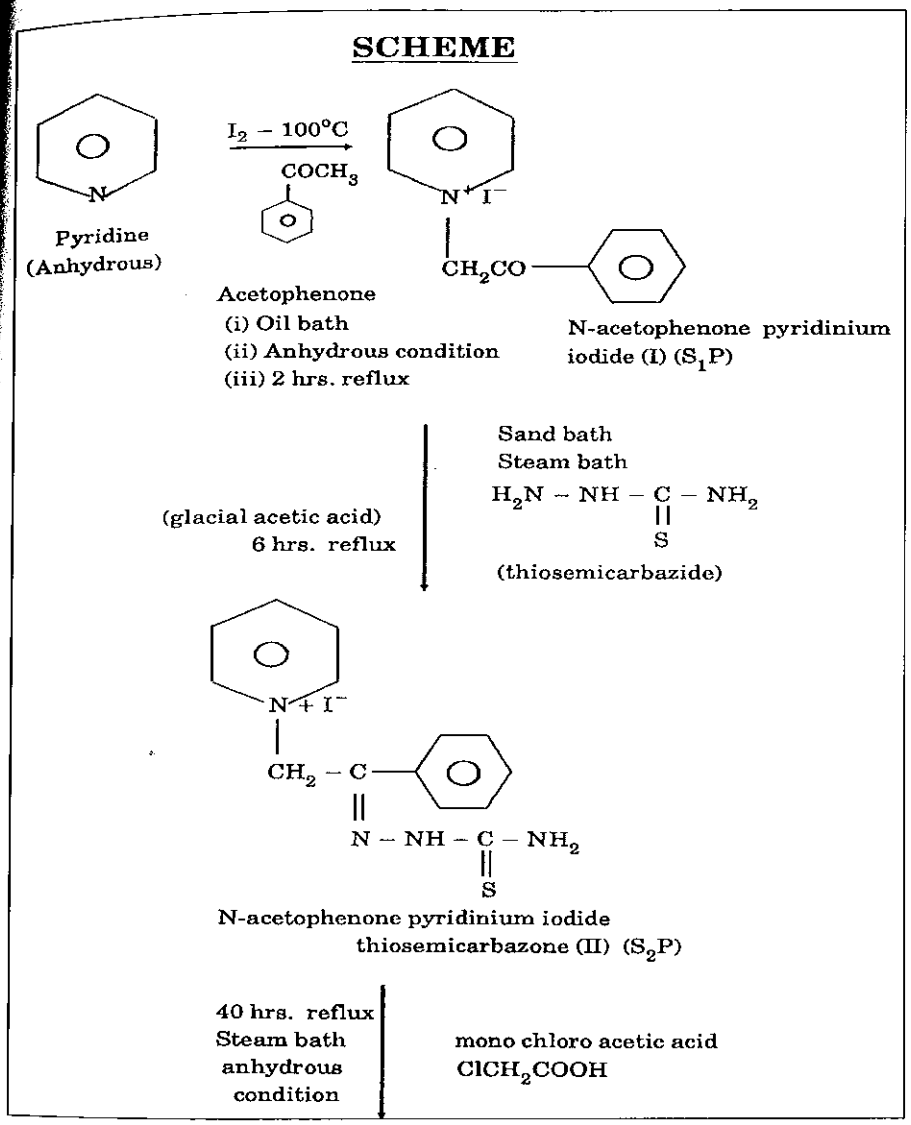


Figure 1. Reaction Pathway showing the formation of substituted thiohydantoins

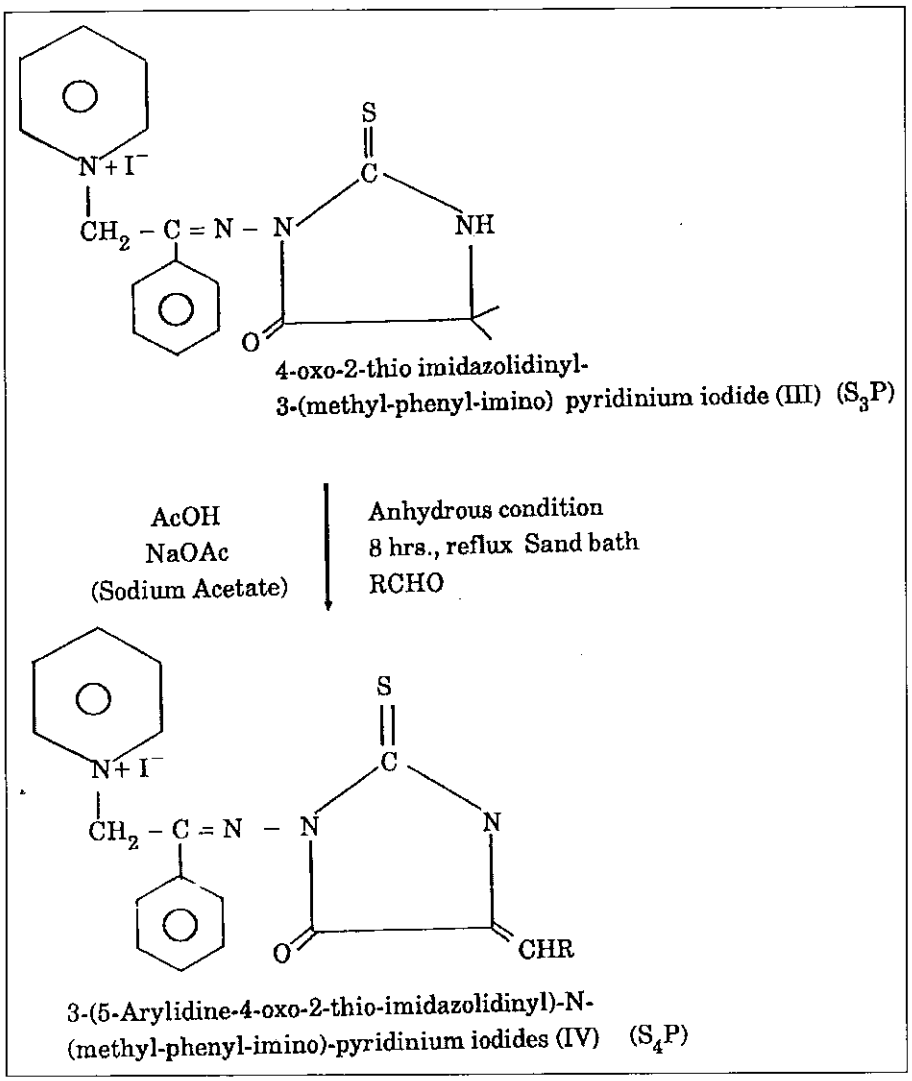
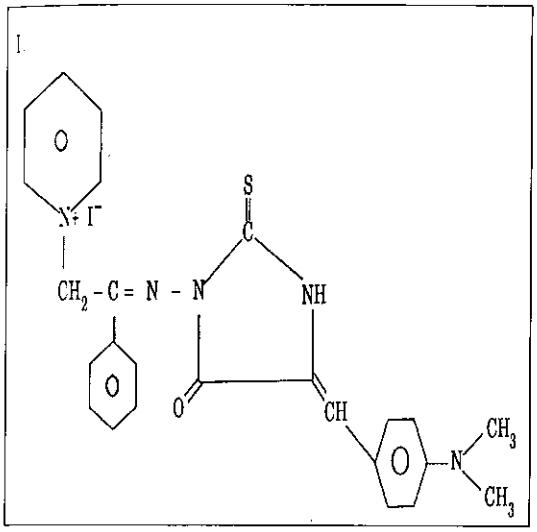


Figure 1. Reaction Pathway showing the formation of substituted thiohydantoins (cont.)

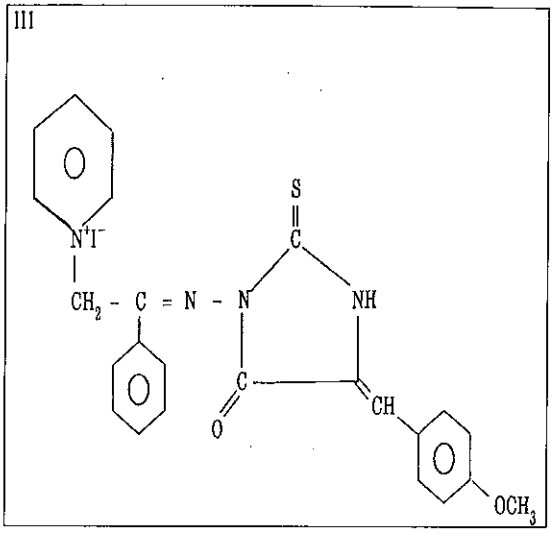
The various aldehydes that have been used are as follows:

1 = Para-dimethylaminobenzaldehyde
2 = 3, 4, 5-trimethoxybenzaldehyde
3 = Anisaldehyde (4-methoxybenzaldehyde)
4 = 4-nitrobenzaldehyde

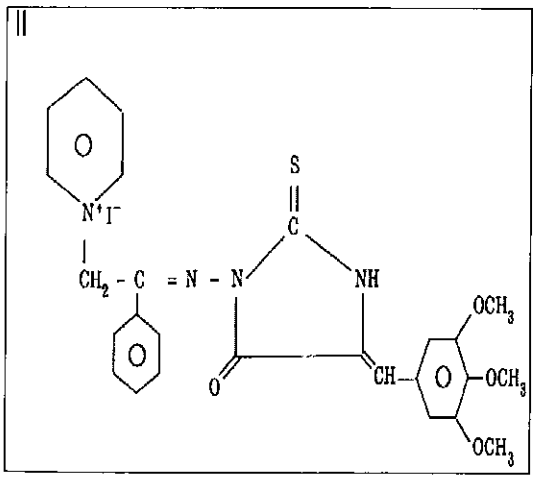
The products are as follows:



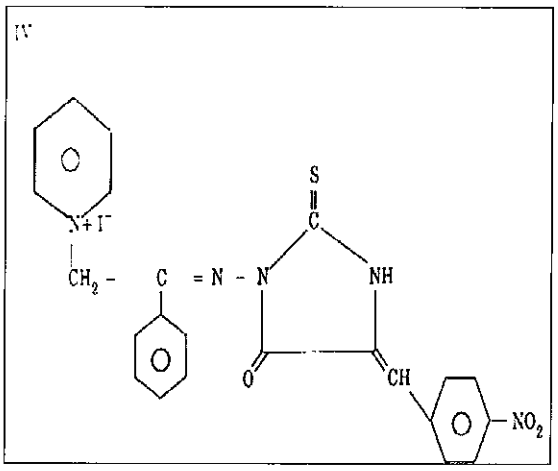
3- [5-(paradimethyl amino arylidine) -4 – oxo- 2 – thio-imidazolidinyl]- N-(methyl-phenyl-imino) pyridinium iodide.
[Compound A = TH-PDAB]



3- [5-(4-trimethoxy arylidine) -4 – oxo- 2 – thio-imidazolidinyl]- N-(methyl-phenyl-imino) pyridinium iodide.
[Compound C = TH-ANIS]



3- [5-(3,4,5-trimethoxy arylidine) -4 – oxo- 2 – thio-imidazolidinyl]- N-(methyl-phenyl-imino) pyridinium iodide.
[Compound B = TH-TMB]



3- [5-(4-nitro arylidine) -4 – oxo- 2 – thio-imidazolidinyl]- N-(methyl-phenyl-imino) pyridinium iodide.
[Compound D = TH-4NB]

Table 1. Elemental Analysis Data

COMP. NO.	R	M. P. (°C)	YIELD (%)	MOLECULAR FORMULA	ANALYSIS OF NITROGEN	
					Calculated	Found
A	4-$N(CH_3)_2C_6H_4$	152	64	$C_{24}H_{24}N_5OSI$	12.30	12.01
B	3,4,5-$OCH_3\,C_6H_2$	160	90	$C_{26}H_{25}N_4O_4SI$	9.10	8.69
C	4-$OCH_3\,C_6H_4$	192	84	$C_{24}H_{21}N_4O_2SI$	10.00	9.80
D	4-$NO_2\,C_6H_4$	181	92	$C_{23}H_{18}N_5O_3SI$	12.28	11.47

Table 2. IR and ^{1}HNMR spectral data

COMP. NO.	IR (KBr) cm^{-1}	^{1}H NMR δ VALUES IN PPM (SOLVENT)
A	1653 (amide carbonyl), 3419 (amide NH)	7.2-7.7 (aromatic protons)(m, J=315.7), 9.7 (NH)(s, J=1)
	1595 (C=N), 1165 (C=S	2.6 (CH_2)(s,J=8), 3.03 (methyl protons)(d,J=) (acetn)(296 K)
B	1683 (amide carbonyl), 3417 (amide NH)	7.2-7.9 (aromatic protons) (m,J=315.7), 9.8 (NH) (s,J=1)
	1590 (C=N), 1126 (C=S)	2.1 (CH_2) (s,J=62.3), 3.92-3.94 (methoxy protons) (t,J=23.2) (Deuteriated Chloroform)(296 K)
C	1678 (amide carbonyl), 3414 (amide NH)	7.2-7.9 (aromatic protons) (m,J=57.5), 9.8(NH)(s,J=2.8)
	1598 (C=N), 1159 (C=S)	2.6 (CH_2) (s,J=3.3), 3.8 (methoxy protons) (s,J=16.4) (Deuteriated Chkoroform))(296 K)
D	1705 (amide carbonyl), 3412 (amide NH)	7.2-7.9 (aromatic protons) (m,J=57.5), 10.1 (NH)(s,J=1)
	1600 (C=N), 1198 (C=S)	2.6 (CH_2) (s,J=2.7) (Deuteriated Chloroform)) (296K)

DETERMINATION OF BIOLOGICAL ACTIVITY

The newly synthesized substituted thiohydantoins were subjected to anticonvulsant activity since thiohydantoins known to possess potent anticonvulsant and antidepressant activity [13]. Albino mice were housed in groups of six for a minimum of three days prior to behavioral study. The mice were free and allowed food and water "*ad libitum*". All animals were starved overnight before dosing into available tap water "*ad libitum*". Gross behavioral changes in mice were studied as suggested by Robert A. Torner [13]. The gross behavioral observations were observed for all the groups at ½, 1, 2, 4, 6, 24, 48, 72 hours after the oral administration of the compound. Arachis oil was used as a solvent to dissolve the compound and to administer the drug in to the animal. The synthesized compounds were administered (per oral) to study the various gross behavioral characters for primary screening that were awareness, mood, motor activity, CNS excitation , posture, motor incoordination, muscle tone, reflexes, autonomic and other such miscellaneous characters like death etc.

It was found that the animals showed behavioural changes only when the dose of the synthesized thiohydantoins was above 200 mg/kg body weight and the various behavioural parameters like the grip strength, rigidity and pain response were found to be the key factors to prove that the compound possess anticonvulsant activity.

LD_{50} [15] primary screening [14] and anticonvulsant activity as determined by protection against leptazol-pentylenetetrazole induced convulsions were performed.

The effect of the synthesized product on pentylenetetrazole induced convulsions was

evaluated as Goodman *et al.* [15]. Mice weighing 18 - 25 g of either sex were grouped in to X groups. Pentylenetetrazole was injected (intraperitoneally) 1 ½ hours after the synthesized compounds were administered (per oral), differences with reference to control were evaluated using Student T Test, has been listed in Table 3, 4 [7, 19]. Any increase in the onset of clonic, tonic, death when compared to control (Grp-1) (pentylenetetrazole) is considered to have anticonvulsant activity [16, 17, and 18].

Clonazepam was used as a standard drug at a dose of 0.25micro gram /10g body wt [19]. Discrepancies $p < 0.05$ was considered statistically significant (Table 5).

Table 3. Effect of synthesized Thiohydantoins upon Pentylenetetrazole induced clonic convulsions, tonic convulsions and death

Animal: Mice			**Solvent used: Arachis oil**		**Route of administration: Oral**	
GROUP	**COMPOUND**	**NO. OF ANIMALS**	**ONSET OF CLONIC CONVULSIONS (sec)**	**ONSET OF TONIC CONVULSIONS (sec)**	**ONSET OF DEATH (sec)**	**PERCENTAGE MORTALITY**
1	PET control 100mg/kg	5	52.6 ± 3.41	254.4 ± 29.96	280.2 ± 35.55	100
2	Compound A (TH-PDAB) 400mg/Kg	5	72.4 ± 0.9***	275 ± 8.6	336.6 ± 35.39*	100
3	Compound A (TH-PDAB) 800mg/Kg	5	94 ± 6.317***	405.6 ± 39.6	396.4 ± 26.09*	100
4	Compound B (TH-TMB) 400mg/Kg	4	41.5 ± 0.96	265.75 ± 2.17	280 ± 2.38	100
5	Compound B (TH-TMB) 800mg/Kg	5	65.4 ± 2.77*	368.2 ± 0.96	388.8 ± 2.61*	100
6	Compound C (TH-ANIS) 400mg/Kg	5	84.8 ± 8.45**	610 ± 75.27**	630 ± 73.75**	100
7	Compound C (TH-ANIS) 800mg/Kg	4	101.3 ± 4.75***	570 ± 38.6**	590.3 ± 39.55**	100
8	Compound D (TH-4NB) 400mg/Kg	5	62.25 ± 5.135*	247.5 ± 44.39	268.25 ± 11.59	100
9	Compound D (TH-4NB) 800mg/Kg	5	85.75 ± 0.912**	199 ± 19.415	338.24 ± 19.76	100
10	Standard Drug Clonazepam (0.25µg/10g)	5	114 ± 20.75**	568 ± 16**	588 ± 18.86**	100

Values are MEAN ± SEM *** $p<0.001$, **$p<0.01$, *$p<0.05$
When compared to that of control (PET) Group I (pentylenetetrazole induced convulsions)

Table 4. Results showing the p values comparing compounds (A, B, C, D) with pentylenetetrazole control

S. NO	COMPOUNDS (mg/kg)	ONSET OF CLONIC CONVULSIONS	ONSET OF TONIC CONVULSIONS	ONSET OF DEATH
1	Compound A (TH-PDAB)			
	400 mg	$p < 0.001$*	$p > 0.5$	$p > 0.1$
	800mg	$p < 0.001$*	$p > 0.5$	$p < 0.05$*
2	Compound B (TH-TMB)			
	400 mg	$p > 0.5$	$p > 0.5$	$p > 0.5$
	800mg	$p < 0.01$*	$p > 0.1$	$p < 0.05$*
3	Compound C (TH-ANIS)			
	400 mg	$p < 0.01$*	$p < 0.01$*	$p < 0.01$*
	800mg	$p < 0.001$*	$p < 0.01$*	$p < 0.01$*
4	Compound D (TH-4NB)			
	400 mg	$p < 0.05$*	$p > 0.5$	$p > 0.1$
	800mg	$p < 0.01$*	$p > 0.5$	$p > 0.1$
5	Standard Drug Clonazepam (0.25µg/10g)	$p < 0.05$*	$p < 0.001$*	$p < 0.001$*

Table 5. Comparing Clonazepam with compound C (TH-ANIS)

COMPOUNDS (mg/kg)	ONSET OF CLONIC CONVULSIONS	ONSET OF TONIC CONVULSIONS	ONSET OF DEATH
Compound C (TH-ANIS)			
400 mg	$p > 0.1$	$p > 0.5$	$p > 0.5$
800mg	$p > 0.5$	$p > 0.5$	$p > 0.5$

RESULTS AND DISCUSSION

From the literature survey, it was understood that 5-benzylidene derivatives of 3-o-chloro and 3-m-benzoyl-2-thiohydantoins and benzylidene derivatives of 3-o, 3-m, 3-p-chlorophenyl-2-thiohydantoins, when screened for pharmacological activities, showed anticonvulsive activity in the electroconvulsive and pentylenetetrazole test. It is now evident that 5-benzylidene groups when introduced into the thiohydantoins produced significant anticonvulsant activity. This motivated us to synthesize some substituted thiohydantoins and screen for its pharmacological activity.

Anticonvulsant activity of the four derivatives of thiohydantoins was performed. From the pharmacological studies carried out on mice for pentylenetetrazole induced convulsions it can be concluded that compound C at both 400mg and 800 mg/kg body weight prolonged all the three phases of pentylenetetrazole induced convulsions i.e clonic, tonic, death. It was also be observed that almost all the synthesized compounds prolonged the onset of clonic convulsions at dose levels of 400 and 800 mg/kg. The onset of death induced by pentylene tetrazole was prolonged significantly by Compound A and Compound B at 800mg/kg. Compound C at both levels namely 400 and 800 mg/kg weight prolonged the onset of death which is statistically significant (Table 4). It was found that the activity of Compound C was found to be significant when compared to that of the other three synthesized thiohydantoin compound. Clonazepam is a marketed potent anticonvulsant drug. Compound C was compared with the clonazepam drug (0.25µg/10g)

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SERIES B

PHYSICAL SCIENCES