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Application of Green Chemistry Principle in Synthesis of Phenytoin and Its Biogical Evaluation as Anticonvulsant Agents

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Abstract: Phenytoin (5,5'-dipenylimidazolidine-2,4-dione**)** is the prime example of anticonvulsant agent. According to reported procedure, it is synthesized by condensation of benzil and urea in presence of base (30% w/v NaOH) using ethanol as solvent which itself acts as CNS stimulant. Removal of solvent after synthesis is most difficult and non-assured process. In case of phenytoin transformation in polymorphism plays an important role when solvent other than water is used. About 30% extra cost is calculated if solvent other than water is used. Therefore by application of green chemistry principle phenytoin was synthesized by condensation of benzil and urea in presence of base (30 % NaOH) and water as green solvent. This compound was characterized on the basis of its spectral (IR, ^{1}H NMR) data and evaluated for anticonvulsant activity using MES induced and PTZ induced seizure models in Swiss albino mice. Significant anticonvulsant activity was found by using 25 mg/kg and 50 mg/kg of phenytoin compared with standard phenytoin at 25 mg/kg dose.

Keywords: Phenytoin, Green chemistry, Green solvent, Anticonvulsant activity

Introduction

Green chemistry is the design of chemical products and process that eliminates the use and generation of hazardous substances[1]. Using green solvent, like water, synthesis of biologically active moiety with high percentage yield as well as purity is the one of objective of green chemistry. Purity of few drugs like CNS acting required high profile of purity and safety for pertaining biological activity. Synthesis of CNS acting moiety with at par purity could be achieved by omitting interfering solvents. Epilepsy is a common

neurological condition, affecting 0.5 to 1% of the population worldwide (45-10 million people)[2]. Epilepsy may be defined as paroxysmal, self-sustaining and self-limiting cerebral dysarrhythmia characterized by an abnormal and excessive electroence-phalogram (EEG) discharge and a loss of consciousness. Major types of epilepsies are Generalized seizure and Partial seizure[3]. Phenytoin (5, 5-diphenylimidazolidine-2,4-dione) is the first anticonvulsant agent often cited as a prime example of anticonvulsant acting as a sodium channel blocker[4,5]. Generally, according to reported procedure, it is synthesized by condensation of benzil and urea in presence of base (30% w/v NaOH) using ethanol as solvent which itself acts as CNS stimulant[6]. Removal of solvents after synthesis is most difficult and non-assured process. In case of Phenytoin transformation in polymorphism plays an important role when solvent other than water is used. About 30% extra cost is calculated if solvent other than water is used. Therefore in present work, Phenytoin was synthesized by condensation of benzil and urea in presence of base (30% NaOH) and water, a green solvent and anticonvulsant activity was performed by MES and PTZ induced seizure models.

Experimental

All the chemicals used for synthesis were of LR (Laboratory Reagent) grade. TLC (Thin Layer Chromatography) was performed on microscopic glass slides (2 x 7.5 cm) coated with silica gel-G, using chloroform: ethyl acetate (7:3) as a solvent systems and the spots were visualized by exposure to iodine vapours. The IR spectrum of synthesized compound was recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide. The ^{1}H NMR was recorded in DMSO-D6 using NMR Varian-Mercury 300MHz spectrometer and chemical shifts are given in parts per million, downfield from tetramethylsilane (TMS) as an internal standard from University of Pune.

Synthesis of 5, 5'-dipenylimidazolidine-2,4-dione

General procedure

5.3 g (0.025 mol) of benzil, 3 g (0.05 mol) of urea, 15 mL of 30% w/v sodium hydroxide solution and 40 mL of water was placed in a 250 mL round bottom flask and refluxed for 2 hours. After cooling to room temperature, the reaction mixture was poured into 100 mL of water with stirring. It was allowed to stand for 15 minutes and filtered under suction to remove the insoluble by-product. The filtrate thus obtained was cooled and acidified by using concentrate hydrochloric acid. The precipitates obtained were separated by filtration. The crude product obtained was washed with cold water. IR and ^{1}H NMR spectra of synthetised phenytoin are represented by Figure 1 and Figure 2 respectively. The physical and spectral data matched with that of standard given in Table 1, 2 and 3 respectively.

Table 1. Physical data of phenytoin

S. No.	Parameters	Results
1	Color, state	Colorless, crystalline
2	Percentage Yield, %	44
3	TLC	(Chloroform: Ethyl acetate), (7:3)
4	R_f Value	0.64
5	Melting point, ^{0}C	296-297 (297-298)

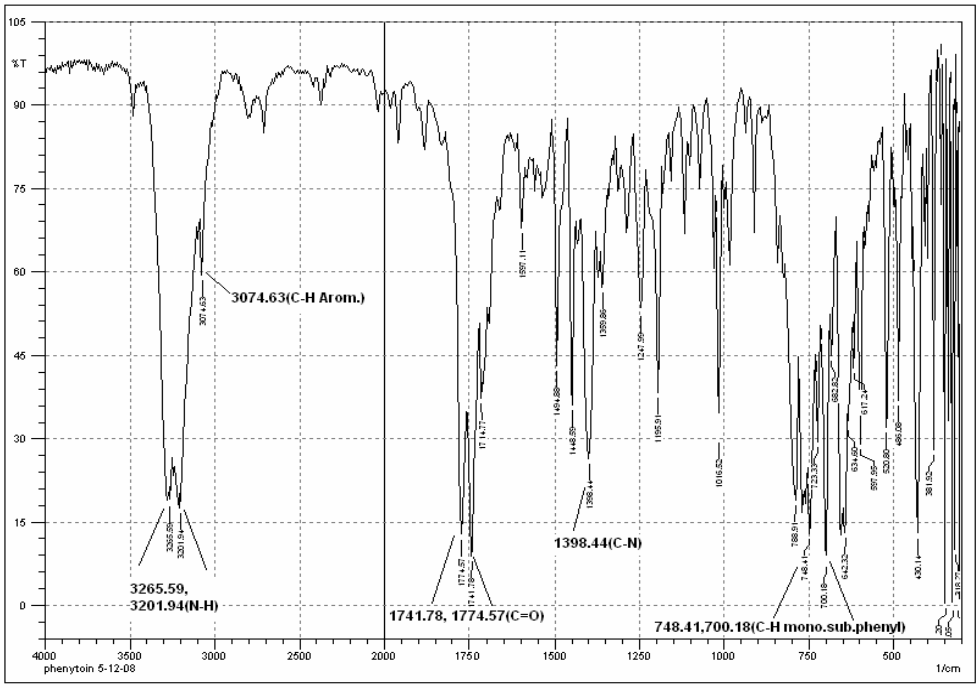


Figure 1. IR (KBr) cm^{-1} spectrum of synthesized phenytoin

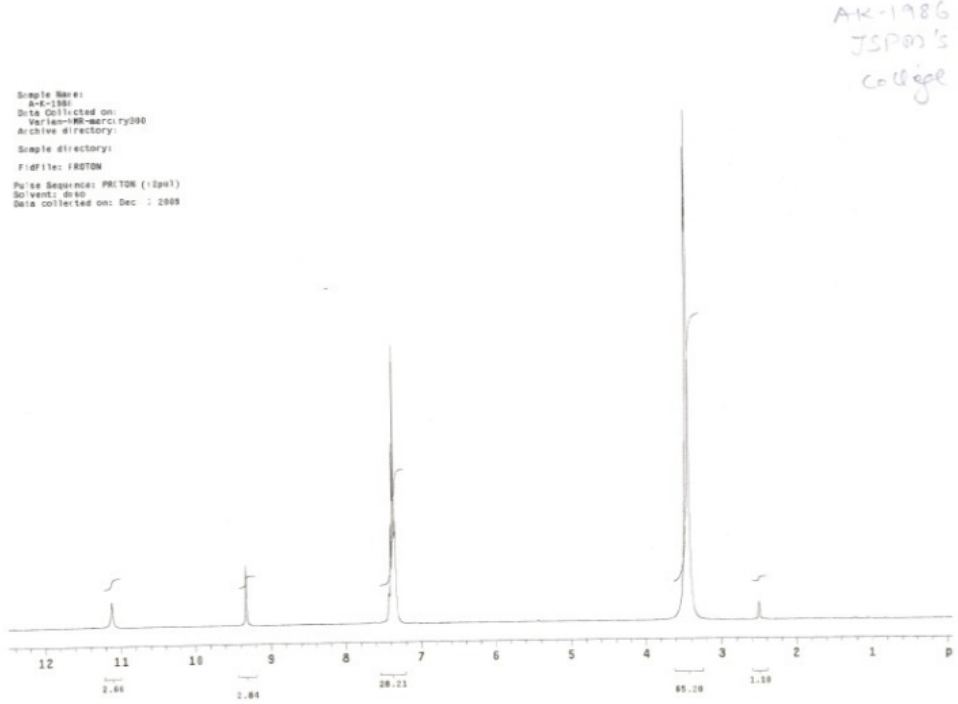


Figure 2 ^{1}H NMR spectrum of synthesized phenytoin in DMSO D6

Table 2. IR Spectral data of phenytoin

S. No.	Functional groups	IR (KBr) cm^{-1}
1	C-H Out of plane vibrations of mono-substituted phenyl ring	748.41, 700.18
2	C-N Stretching	1398.44
3	C=O stretching	1741.78, 1774.57
4	C-H Stretching of aromatic ring	3074.63
5	N-H Stretching	3265.59, 3201.94

Table 3. ^{1}H NMR spectral data of phenytoin

S. No.	No. of protons	Chemical shift (δ)
1	(1H),*s*	11.12
2	(1H),*s*	9.33
3	(10H), *m*	7.34-7.42

Anticonvulsant activity

The Anticonvulsant activity of the compound was measured by MES (Maximal electroshock) induced seizure test and PTZ (Pentylenetetrazole) induced seizure test.

MES induced seizure test[7,8]

The *Swiss albino* mice of either sex weighing 25-30 g were used for the test. They were divided into four groups each containing 6 mice. 0.1% gum acacia solution was used as a vehicle for preparation of suspension of Phenytoin. Control group was injected with normal saline solution or gum acacia solution 10 mL/kg (p.o.). Phenytoin 20 mg/kg (i.p.) was used as a standard. Each group test 1 and test 2 was treated orally with 20 mg/kg and 40 mg/kg (p.o.) of Phenytoin respectively. They all received current of 48mA for 2s duration through electroconvulsiometer using ear electrodes after 60 min of oral administration of test formulation. The animals were observed closely for 2 minutes. The duration of tonic flexion and latency of onset of clonus was recorded. A complete abolition of hind limb tonic extension was considered as 100% protection. The ability to prevent this feature was considered as an indication of anticonvulsant activity. Data of MES induced seizure test of synthesized phenytoin is given in Table 4 and represented with the Figure 3.

Table 4. Data of Anticonvulsant activity of Synthetic Compound Phenytoin on MES-induced seizures in mice.

S. No.	Groups	Dose mg/kg	Latency of Onset of phases Time, s		
			Tonic flexion	Tonic extension	Clonus
1	Control	Saline water	0.00	11.92±673	15.49± 432
2	(Std. Drug) Phenytoin	25	4.83±0.412***	2.10±514***	9.00± 656**
3	Test Compound	25	2.83 ±0.329***	5.00 ± 0.231***	13.21± 0.129*
		50	3.15 ±0.231***	2.83± 231***	10.26 ± 984**

(Values are expressed as mean ±S.E.M. n=6 $^{***}p < 0.001$ $^{**}p < 0.01$, $^{*}p < 0.05$ compared with Normal control ANOVA followed by Dunnet's t- test)

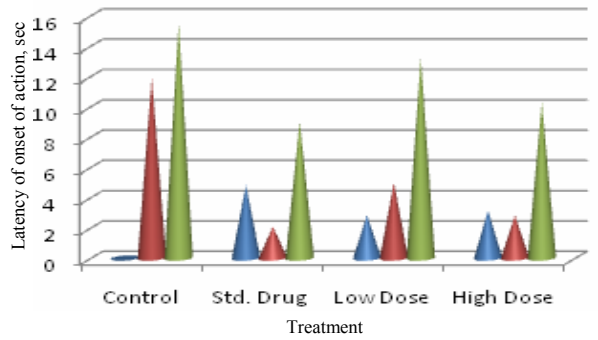


Figure 3. MES induced test on mice of synthetic compound Phenytoin after oral administration

(*Where As Control group - Saline water, Std. Drug - Phenytoin 25 mg/kg, Test compound phenytoin,. Low dose- 25 mg/kg, High dose-50 mg/kg body wt. of Swiss albino mice; Series 1 - tonic flexion, series 2- tonic extension, series 3- clonic phase PTZ induced seizure test*[9])

Swiss albino mice of either sex with a body weigh between 18 & 12 g, were used the test compound or the reference drug was injected subcutaneous or Intraperitoneal or given orally two group of 10 mice. Another group of 10 mice serve as control 15 min after oral administration subcutaneously injection 30 min after intraperitoneal injection or 60 min, after oral administration, 60 mg per kg PTZ was injected subcutaneously. Each animal was placed in to an individual plastic cage, for observation lasting in seizures and tonic clonic convulsion was recovered. At least 80% of the animals in the control group were show to convulsion. Data of PTZ induced seizure test is given in Table 4 and represented with the Figure 4.

Table 4. Data of anticonvulsant activity by PTZ-induced seizures in mice

Groups	Dose mg/kg	Onset of seizures, min	Onset of death, min	% inhibition of onset of death, %
Control	Saline water	2.89±0.239	5.21±0.321	0
Phenytoin Std.	25	6.36±0.872**	13.22±0.641**	73.69
Phenytoin Test	Low Dose 25	6.33±0.231*	9.36±0.683**	61.23
	High Dose 50	7.58±0.1302**	10.91±0.456*	69.52

*(Values are expressed as mean ± S.E.M. n=6 ** p < 0.01 * p < 0.05 compared with vehicle control ANOVA followed by Dunnet's t- test)*

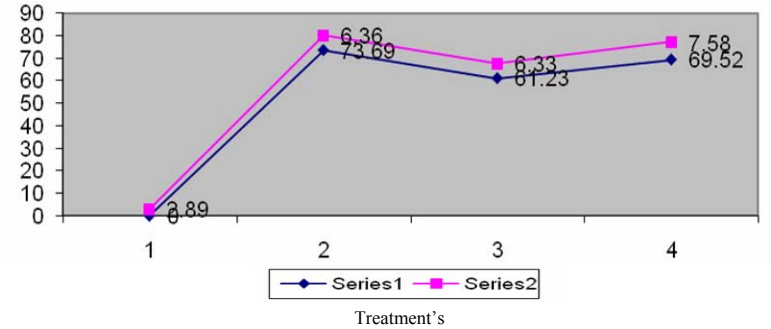
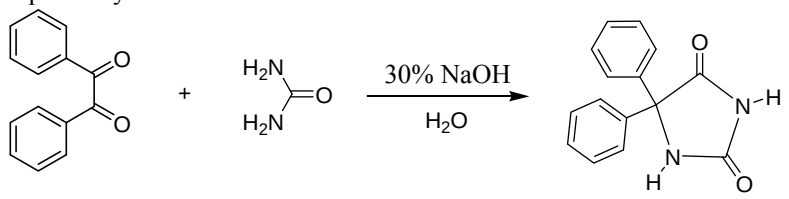


Figure 4. PTZ induced test on mice of Synthetic compound Phenytoin after oral administration

Results and Discussion

Phenytoin (5,5'-dphenylimidazoldine-2,4-dione) was synthesized by condensation of benzil and urea in presence of 30% NaOH solution and water as a green solvent. It was obtained as a solid melting in the range 296-297 ^{0}C. The solid state IR (KBr, cm^{-1}) spectrum of the compound reveals a characteristic aromatic stretch at 3074.63 cm^{-1}. Sharp N-H stretching vibrations are seen at 3265.59 and 3201.94 cm^{-1}. The stretching vibrations of C=O group are seen around at 1741.78 and 1774.57 cm^{-1}. The ^{1}H NMR (DMSO, ppm) data of the compound reveal a signal between 7.34-7.42 for the aromatic protons. Presence of characteristic singlet at 9.33 and 11.12 assigned to protons attached to imide and amide nitrogen respectively.



Benzil (0.025 mol) | Urea (0.05 mol) | Phenytoin 5,5'-dphenylimidazoldine-2,4-dione

The compound has shown significant anticonvulsant activity with increase latency of onset of phases in Time (s) as compared to standard in MES induced seizure test. PTZ induced seizure test have shown 61.23% and 69.52% inhibition of onset of death.

Conclusion

Phenytoin is synthesized by application of principle of green chemistry with high purity profile as well as having safety by omitting the use of ethanol. Water a green solvent is used instead of ethanol in synthesis of phenytoin .There is reduction in time and ultimately cost as compare to conventional procedure of synthesis of phenytoin. Thus we conclude that the synthesized compound have potential in the green chemistry.

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References

1. Kidwai M, *Green Chemistry in India; Pure Appl Chem.,* 2001, **73**, 1261-1263.
2. Bell G S and Sander J, *Seizure*, **2002**, 11 (Suppl. A), 306-314.
3. Tripathi K D, Essentials of Medical Pharmacology; Jaypee Publications: New Delhi, 2003, 495-498**.**
4. Bigge C and Boxer P, *Annu Rep Med Chem.,* 1994, **29**, 13-22.
5. Wong M G, Defina J A and Andrews P R, *J Med Chem.,* 1986, **29,** 562-572
6. Furniss B, Hannaford J, Smith P W G and Tatchell A R**,** Vogel's Textbook of Practical Organic Chemistry; 5th Edition Dorling Kindersley: New Delhi, 2008, 1153.
7. Vogel H, Drug discovery and Evaluation Pharmacological Assays: Springer Publication: Berlin, 2002, 422.
8. Achliya G S, Wadodkar G S and Dorle A K, *J Ethnopharmacolo.*, 2004, **94(1)**, 77-83
9. Nguelefack T B and Astamo A D, *J Ethnopharmacolo.*, 2006, **106**, 70-75.