Synthesis of Valproic acid derivatives and their evaluation for anticonvulsant activity

N Upmanyu, S Gupta, J Grover, P Mishra

Citation

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Abstract

Valproic acid is simple branched chain carboxylic acid used in epilepsy. Valproic acid derivatives like acid chloride and amide were prepared by the reaction of valproic acid with thionyl chloride and ammonia. These acid chloride derivatives were reacted with hydrazine hydrate to give the corresponding hydrazides. These hydrazides were reacted with formaldehyde and different secondary amines to give substituted derivatives. The structures of the newly synthesized compounds were elucidated on the basis of analytical and spectral data. The compounds synthesized were then screened for anticonvulsant activity. Various studies were conducted on development of various prodrugs and derivatives of valproic acid. The present study showed that all synthesized amide derivatives (RDG1…RDG8) except RDG2 were more active than sodium valproate in chemical induced model as well as MES model. Amongst all the derivatives RDG3 was found to be more effective than even valpromide.

INTRODUCTION

Valproic acid is simple branched chain carboxylic acid used in epilepsy. Valproic acid increases GABA (-amino butric acid) synthesis and release and potentiates by this mechanism GABAergic transmission in specific brain regions (Kadam, Mahadik and Bothra, 1998). Valproic acid also reduces the release of excitatory amino acid -hydroxy butyric acid and to attenuate neuronal excitation mediated by activation of N-methyl-D- aspartate glutamate receptors. (Mclean and Macdonald, 1986). Valproic acid is a broad-spectrum antiepileptic drug effective against all seizure types.

Various studies were conducted on development of various prodrugs and derivatives of valproic acid (Mergen etal) and other drugs, which shows anticonvulsant activity and reported in literature (Patil, Shyam and Dimmock, 1991) Valproic acid is reacted with thionyl chloride to give acid chloride of it and ammonia reacted with valproic acid to give Valpromide(Furniss etal, 1989) [2].This valpromide, reacted with formaldehyde to give N-(Hydroxymethyl) valpromide (Mishra , Agrawal and Shakya , 1991) [3]. This acid chloride is reacted with hydrazine- hydrate to give hydrazide of valproic acid (4). This hydrazide is reacted with formaldehyde and different secondary amine in ethanol to give N (N'N'-Dialkylamino) methyl valpromide (Singh, Bindal and Dixit, 1975) (5a-f). The scheme of these reactions is shown in scheme 1. Newly synthesized compounds were characterized by IR, NMR and elemental analysis. These compounds were screened for better anticonvulsant property.

MATERIALS AND METHODS

Purity of the compounds was checked by TLC. Melting points were determined in open capillaries and are uncorrected. IR spectra (KBr) are recorded on the JASCO FT/ IR- 470 Spectrophotometer.^{1}H NMR spectra was recorded on Burker AC-300 MHz Spectrophotometer by using CDCl3 as solvent and quantitative elemental analysis of nitrogen by using Perkin Elmer-2400 elemental analyser. For anticonvulsant activity wistar rats of either sex, weighing between 150-250g were used. All the animals were maintained under standard conditions and had access to pelleted animal feed and water.

N-HYDROXY METHYL DERIVATIVES OF VALPROMIDE

Valpromide (2) (0.04M), formalin (0.25M) and aqueous potassium carbonate solution 6-7 ml(20% w/v) were taken in a round bottom flask fitted with reflux condenser and refluxed for 2 hours. After cooling, the flask was placed in refrigerator overnight. The crystals of N-hydroxy methyl compound (3), which separated out were washed with cold distilled water to remove potassium carbonate and excess of formaldehyde. The product was recrystallized from ethanol

(90%w/v) and dried. The I.R. spectrum of this compound exhibited peaks at 3600 cm^{-1}(OH) and 3305.9cm^{-1}(NH) indicating free hydroxyl and amide group. The NMR spectrum of this compound exhibited signals at 6.7 ð (s, 1H, amide NH) and 4.09 ð (t, 1H, of hydroxyl). The physical and elemental analysis data of the compounds given in Table 1.

Figure 1

Table 1: Physical and elemental analysis data of compounds

Constituent (X)	Compound code	% Yield	Melting point (degree c)	Percent Nitrogen	
				Calculated	Found
H	Valpromide (RDG)	70	122	9.78	9.37
CH_2OH	RDG 1	60	106	8.08	7.73
NH_2	RDG 2	75	198	17.70	16.62
$CH_2N(CH_3)_2$	RDG 3	57	114	13.98	13.06
$CH_2N(C_2H_5)_2$	RDG 4	54	110	12.27	13.12
$CH_2N(C_3H_7)_2$	RDG 5	53	104	10.92	9.42
$CH_2N(C_4H_9)_2$	RDG 6	50	118	9.85	9.51
CH_2N C_4H_9 CH_3	RDG 7	50	90	11.56	12.52
$CH_2N(C_3H_7)_2$	RDG 8	51	108	10.92	9.67

PREPARATION OF HYDRAZINE DERIVATIVES OF VALPROIC ACID

Hydrazine derivative was synthesized by taking hydrazine hydrate and acid chloride. Acid chloride was synthesized by taking 8 ml valproic acid (7.2g, 0.05 M) was taken in chloroform 25mL in a round-bottomed flask equipped with reflux condenser and an outlet. Thionyl chloride (7.5 ml, 0.085 M) was added drop wise and the reaction mixture was refluxed for 4 hours. The excess of chloroform and thionyl chloride was removed under reduced pressure to produce acid chloride.

Flat-bottomed flask equipped with two-way assembly fitted with dropping funnel and an outlet. The acid chloride as prepared above was taken in the flask and the hydrazine hydrate 2.4mL (0.05 M) was taken in dropping funnel. This hydrazine hydrate was added drop-wise to the acid chloride with constant stirring. Crystals of hydrazine derivative (4) formed were recrystallized with dehydrated alcohol. The I.R. spectrum of this hydrazide exhibited peaks at 3400 cm^{-1} of N-H(s) and at 1704.$^{7cm-1}$(NH) indicating free hydroxyl and amide group. The NMR spectrum of this compound exhibited signals at 6.7 ð (s, 1H, amide NH) and 4.09 ð (t, 1H, of hydroxyl). The physical and elemental analysis data of the compounds given in Table 1.

SYNTHESIS OF N (N'N'-DIALKYLAMINO) METHYL VALPROMIDE

To a solution of valpromide 4g(0.028 M) in ethanol 19-20 ml, was added formalin 2.24mL(37%w/v, 0.028 M) followed by the addition of dialkyl (0.028 M) under constant stirring. Solvent was distilled off under reduced pressure to obtain product. It was recrystallized from hot water.

ANTICONVULSANT ACTIVITY

Anticonvulsant activity of synthesized compounds by using phenylenetetrazole (PTZ) induced and maximal electroshock seizure (MES) model of epilepsy. (Vogel, 2002)

PENTYLENETETRAZOLE INDUCED CONVULSIONS

Pentylenetetrazole is an convulsive agent used for the induction of epilepsy in test animal.Wistar rats of either sex (150-250 g) were used in studies and divided into control and other groups. Control group received only the vehicle (propylene glycol) and other groups were administered each compounds. A 20 min after injecting the animals were administered pentylenetetraole (60 mg/kg i.p.). Animals were observed for convulsions, sedation and death for 1 hour. The observations are recorded in table 2 and table 3.

Figure 2

Table 2: Pentylenetetrazole model of epilepsy (evaluation of valproic acid derivatives for anticonvulsant activity)

Drug	Dose (mg/kg, i.p.)	Seizure-I		Seizure-II		Jerking	No of animal died/total no of animal treated	Other properties (sedation)
		Onset	Duration	Onset	Duration			
Vehicle administered	0.5 mL	50 sec-3 min	40 sec	9-11 min	20 sec	+	(4/6)	-
Sodium Valproate	200		-	-	-	-	(0/6)	+
	150	2-5 min	30 sec	-	-	+	(0/6)	-
Valpromide	150	-	-	-	-	-	(0/6)	++
	100	-	-	-	-	-	(0/6)	+
	50	-	-	-	-	-	(0/6)	+
	25	-	-	-	-	-	(0/6)	-
RDG 1	150	-	-	-	-	-	(0/6)	+
	100	2-5 min	10 sec	-	-	+	(0/6)	-
	50	2-4 min	15-20 sec	9-11 min	10 sec	+	(0/6)	-
RDG 2	400	6-7 min	15 sec	-	-	-	(0/6)	+++
	200	4-5 min	20 sec	-	-	+	(0/6)	++
	150	2-3 min	20 sec	-	-	+	(0/6)	++
	100	1-2 min	30 sec	10-11 min	10 sec	+	(0/6)	+
RDG 3	100	-	-	-	-	-	(0/6)	++
	150	-	-	-	-	-	(0/6)	++
	25	-	-	-	-	-	(0/6)	++
	15	1-2 min	10 sec	-	-	+	(0/6)	-

Total no of animals used for each group=6, (+) present, (-) absent.

Intensity of sedation: + animals stopped moving but responded to physical stimuli, ++ animals had ataxia on moving, +++ animals did not respond to physical stimuli but responded to painful stimuli, ++++ absence of wrighting reflex as well as no response to painful stimuli.

Figure 3

Table 3: Pentylenetetrazole model of epilepsy (evaluation of valproic acid derivatives for anticonvulsant activity)

Drug	Dose (mg/kg, i.p.)	Seizure-I		Seizure-II		Jerking	No of animal died/total no of animal treated	Other properties (sedation)
		Onset	Duration	Onset	Duration			
RDG 4	100	-	-	-	-	-	(0/6)	++
	50	-	-	-	-	-	(0/6)	++
	25	4-5 min	5 sec	11-12 min	20 sec	+	(0/6)	-
RDG 5	100	--	--	--	--	-	(0/6)	+++
	50	--	--	--	--	--	(0/6)	++
	25	3-4 min	10 sec	9-10 min	25 sec	+	(0/6)	+
RDG 6	100	-	-	-	-	-	(0/6)	+++
	50	5-6 min	20 sec	-	-	+	(0/6)	+
RDG 7	150	-	-	-	-	-	(0/6)	++++
	100	-	-	-	-	-	(0/6)	+
	50	1-3 min	5 sec	-	-	+	(0/6)	-
RDG 8	150	-	-	-	-	-	(0/6)	++++
	100	-	-	-	-	-	(0/6)	++
	50	2-4 min	30 sec	-	-	+	(0/6)	+

Total no of animals used for each group=6, (+) present, (-) absent.

Intensity of sedation: + animals stopped moving but responded to physical stimuli, ++ animals had ataxia on moving, +++ animals did not respond to physical stimuli but responded to painful stimuli, ++++ absence of wrighting reflex as well as no response to painful stimuli.

MAXIMAL ELECTROSHOCK CONVULSIONS

To induce convulsions current of 150 mA was delivered through the electrodes dipped in normal saline at 50 Hz for 0.2 sec (Srivastava and Gupta, 2001). In this method only those rats were selected who responded to electroshock as mentioned above. The selected rats were administered either the vehicle or test compounds (i.p.) and 20 mins after injection, convulsions were induced. The rats were observed for presence or absence of hind limb extension and also for any sedation and mortality for next one hour. All the observations are represented in table 4.

Figure 4

Table 4: Effect against maximal electroshock seizure model (evaluation of valproic acid derivatives for anticonvulsant activity)

Drug	Dose (mg/kg, i.p.)	Seizure (Hind limb extension duration)	Sedation
Vehicle administered	0.5 mL	+ 30 sec	-
Sodium Valproate	200	-	+
	150	+ 20 sec	-
Valpromide	50	-	+
	25	-	+
	15	+ 5 sec	-
RDG 1	150	-	+
	50	-	-
	25	-	-
RDG 2	200	+ 15 sec	++
	150	+ 25 sec	++
RDG 3	50	-	++
	25	-	+
	15	-	-
	5	+ 15 sec	-
RDG 4	50	-	++
	25	-	-
	15	+ 20 sec	-
RDG 5	50	-	++
	25	-	+
	15	+ 20 sec	-
RDG 6	100	-	+++
	50	-	+
	25	+ 25 sec	–
RDG 7	50	-	+
	25	-	-
	15	+ 20 sec	-
RDG 8	50	-	++
	25	-	+
	15	+ 25 sec	-

Total no of animals used for each group=6, (+) present, (-) absent.

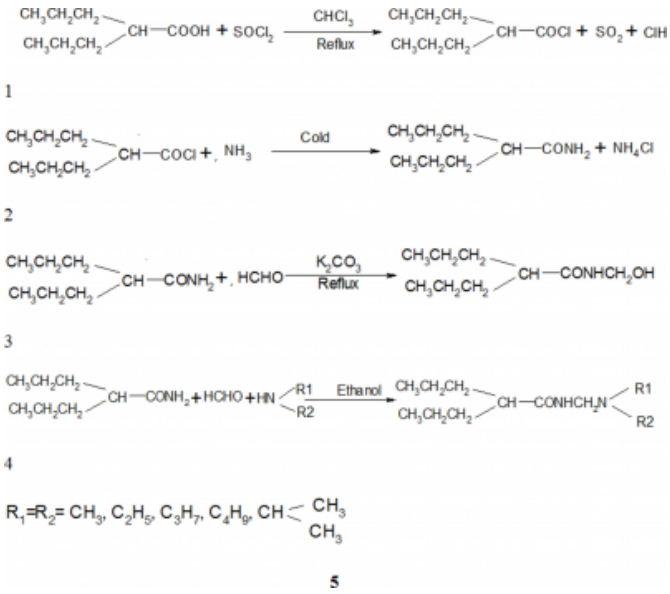
Intensity of sedation: + animals stopped moving but responded to physical stimuli, ++ animals had ataxia on moving, +++ animals did not respond to physical stimuli but responded to painful stimuli, ++++ absence of wrighting reflex as well as no response to painful stimuli.

RESULTS AND DISCUSSION

Among the compounds tested for anticonvulsant activity only the compound 5a was proved to possess more efficacy, being considerably more active than sodium valproate in MES and PT2 induced seizure and was also more active than compound 2 in MES seizure. However the present study also pointed out that compound 5a was also slightly more sedative than compound 2.

Figure 5

Scheme 1 : Synthetic Route



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CORRESPONDENCE TO

E-mail: suryatony@yahoo.co.in Surya Prakash B.N Gupta C/o Amrit Bandhu, Jawahar Chowk, Satna (M.P.)- 485001-INDIA

References

r-0. Furniss B S, Hannaford A J, Smith P W G and Tatchell A R, 1989, Vogel's Textbook of Practical Organic Chemistry, 5th Edn, (ELBS Publishers, Longman Group UK Ltd., London), 692-693, 708.
r-1. Kadam S S, Mahadik K R and Bothara K G, Principles of Medicinal Chemistry, 1998, 6 th Edn, (Nirali Prakashan, Pune), 138.
r-2. Mclean J J and Macdonald R L, "Sodium valproate, but not ethosuximide, produces use- and voltage- dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture" J Pharmacol Exp Ther, 1986, 237(3), 1001-1011.

r-3. Mergen F, Lambert D M, Saraiva J C, Poupaert J H and Dumont P, 1991, “Antiepileptic activity of 1,3-dihexadecanoyl-amino-2-valproyl-propan-2-ol, a prodrug of valproic acid” J Pharm Pharmacol, 43(11) 815-816.
r-4. Mishra P, Agrawal R. K. and Shakya A K, “Indian J Pharm Sci, 1991, “Synthesis and Biological evaluation of some 1,3,4-thiadiazole derivatives”, 53(5), 201-204.
r-5. Patil S A, Shyam K and Dimmock J R, Pharmazie, 1991, “Some Mannich bases of 1-aryl-1-ethanones and related ketones for anticonvulsant activities and they found that some compounds of the series showed good anticonvulsant activity”46 (7), 538-539.
r-6. Singh G B, Bindal M C and Dixit S C, J Indian Chem Soc, 1975 “, Synthesis of N-Mannich bases of Isovaleramide”, 3, 438-439.
r-7. Srivastava A K and Gupta Y K, Indian J Physiol Pharmacol,, 2001, “Synthesis and Evaluation of amino acid analogue of valproic acid” 45(4), 475-480.
r-8. Vogel H G, EDs, Drug Discovery and Evaluation: Pharmacological Assays, 2002 (Springer Verlag Berlin Heidelberg, New York), 2nd Edn, 422-494.

Author Information

N. Upmanyu
Dept. Of Pharm. Sci. Dr H. S. Gour Vishwavidayalaya

S.P. Gupta
Rajiv Gandhi Institute of Pharmacy

J. Grover
Dept. Of Pharmacology All India Institute of Medical Sciences

P. Mishra
Dept. Of Pharm. Sci. Dr H. S. Gour Vishwavidayalaya