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Synthesis and evaluation of phenytoin derivatives as anticonvulsant agents

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2,5-Dioxo-4,4-diphenylimidazolidine-1-carboxylic acid (**2**) was reacted with methyl ester of different amino acids **(1a-c)** and substituted benzhydrols **(3a-b)** in pyridine and in the presence of N,N dicyclohexyl carbodiimide (DCC) to yield a series of the title compounds, methyl 2-(2,5-dioxo-4,4-diphenylimidazolidine-1-carboxamido) substituted propanoate (**4a-c**) and benzhydryl 2,5-dioxo-4,4-diphenyl imidazolidine-1-carboxylate (**5a-b**).

The structures of these compounds were established on the basis of their spectral (IR, ^{1}H-NMR) data. These newly synthesized derivatives of phenytoin were evaluated in terms of anticonvulsant activity.

Key Words: Phenytoin, hydantoin, anticonvulsant activity.

Introduction

Epilepsy is one of the most common neurological disorders, affecting about 1% of the world's population and characterized by recurrent seizure attacks.[1] It is estimated that in one-third of these patients seizures are not adequately controlled by existing drugs. Furthermore, the drugs available have shown significant side-effects, and many have narrow therapeutic indices and are difficult to formulate. For example, to exert its anticonvulsant therapeutic effect, the drug must reach its receptors in the central nervous system (CNS). Yet, many of the drugs exhibit physicochemical and protein-binding properties that would not permit crossing of the blood-brain barrier (BBB).[2]

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Phenytoin is one of the most widely used drug in the therapy of epilepsy. However, its low solubility in water, both as free acid and sodium salt, makes its administration to patients difficult and seldom satisfactory. Phenytoin is given orally as sodium salt in a strong alkaline solution, since it requires a pH between 10 and 12 to be maintained in solution. The alkalinity of this dosage form often causes gastric irritation, which is a serious drawback. Phenytoin can also be given by the intramuscular route, but the product commonly precipitates at the injection site, leading to unreliable blood levels of the drug. Moreover, absorption of intramuscular phenytoin is very slow, and so it is not appropriate for treating epileptic seizures, in which a loading dose of the product is required. For parenteral use, sodium phenytoin is formulated in aqueous alkaline solution (pH 12) containing 40% of propylene glycol and 10% of ethanol. The risks associated with the use of this formulation are obvious, taking into account its high pH as well as the precipitation of the free acid.[3]

The classic prodrug approach to improve membrane permeability of drug molecules employs lipophilic derivatives to increase passive membrane penetration. In recent years, different nutrient transporters (i.e. oligopeptide, amino acid, and glucose transporters) have been identified and cloned. The active nutrient transport systems have become a target for prodrug design.[4]

In this study, we have attempted to demonstrate the feasibility of utilizing natural amino acids and benzhydrols as a promoiety that can transport a model compound across the BBB.

Experimental

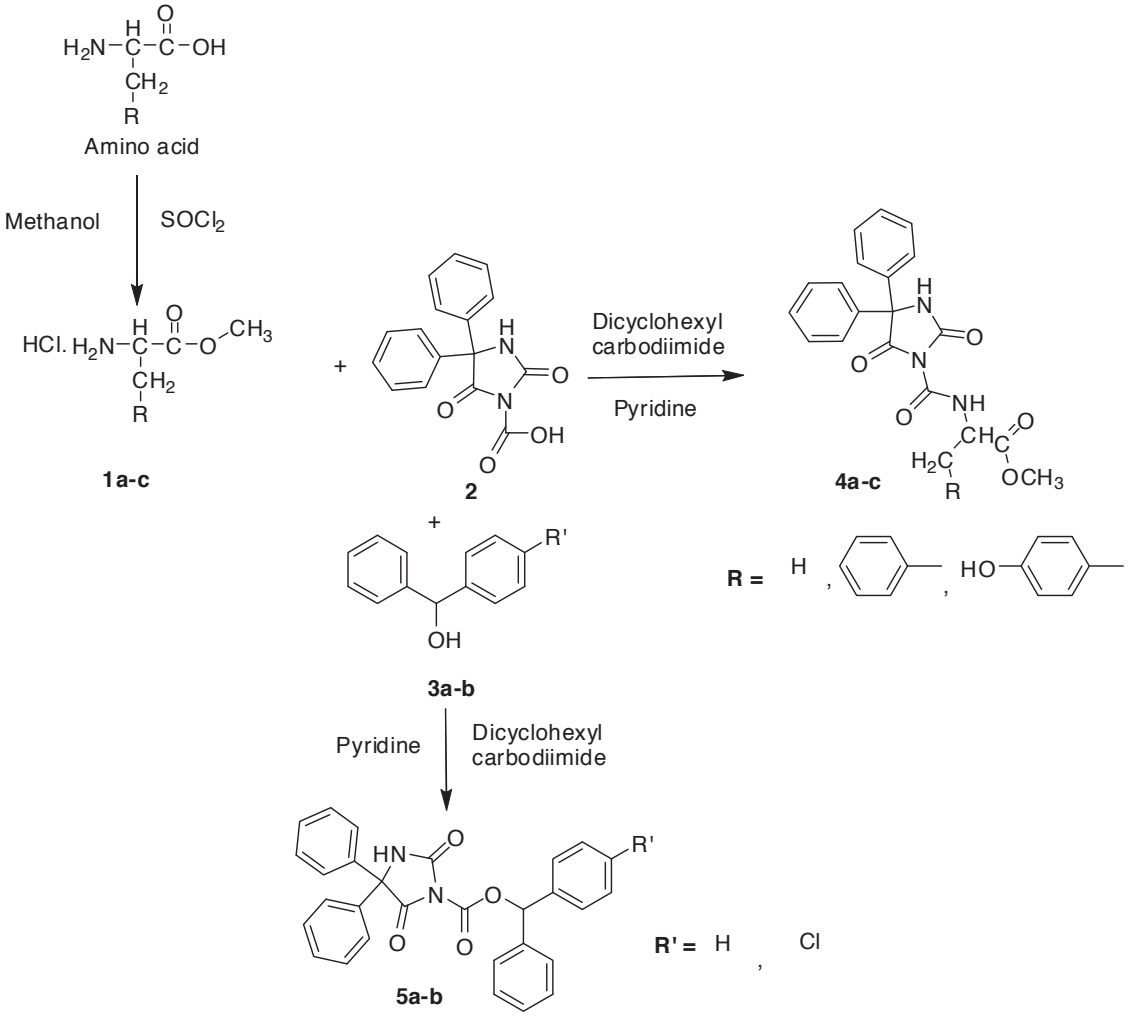
Chemistry

All chemicals used were of synthetic grade. The purity of compounds was ascertained by TLC on precoated silica F_{254} plates (Merck, Mumbai, India) using iodine vapors and UV light as detecting agents. The melting points of the synthesized compounds were determined by open capillary method and are uncorrected. The IR spectra of synthesized compounds were recorded on a SHIMADZU FT-IR spectrophotometer in KBr. The ^{1}H-NMR were recorded in $CDCl_3$ and DMSO-d_6 using a NMR Varian-Mercury 300 MHz spectrometer and chemical shifts (δ) are given in parts per millions (ppm) downfield from tetramethylsilane (TMS) as an internal standard from University of Pune, Pune, India.

Synthesis of methyl ester hydrochloride[6] of various amino acids, i.e. L-alanine (**1a**), L-phenylalanine (**1b**), and L-tyrosine (**1c**), was carried out by the procedure described below. Freshly distilled thionyl chloride (0.06 mol) was slowly added to methanol (100 mL) with cooling, followed by addition of the amino acid (0.05 mol). The mixture was refluxed for 8 h at 60-70 °C with continuous stirring on a magnetic stirrer. Excess thionyl chloride and solvent were removed under reduced pressure giving crude amino acid methyl ester hydrochloride. It was recrystallized from hot methanol and solvent ether mixture (5:2). The crystals were collected the next day and washed twice with an ether:methanol mixture (5:1) followed by pure ether and dried under vacuum to give pure product.

Synthesis of benzhydrol (**3a**) and chlorobenzhydrol (**3b**) was carried out by the method described earlier.[7,8]

For synthesis of phenytoin-3-carboxylic acid (**2**), the 5,5-diphenyl-N-carboethoxyhydantoin (0.01 mol) was dissolved in 10% sodium hydroxide (20 mL) in a 100 mL round bottom flask and refluxed for 3 h. The solution



Scheme

was cooled and neutralized by adding dilute hydrochloric acid. The product separated was filtered, washed and dried in air, and recrystallized by ethanol. The physicochemical characteristics of all the intermediates are summarized in Table 1.

The synthesis of phenytoin-3-carboxylic acid (**2**) derivatives with methyl esters of alanine (**4a**), phenylalanine (**4b**), and tyrosine (**4c**) and with benzhydrol (**5a**) and chlorbenzhydrol (**5b**) was carried out as described below. The amino acid methyl ester hydrochloride (0.004 mol) or benzhydrols (0.004 mol) and phenytoin-3-carboxylic acid (**2**) (0.003 mol) were added to the pyridine, which was placed in a 100 mL conical flask. N,N Dicyclohexyl carbodiimide (DCC) (0.004 mol) was added to the above solution with continuous stirring at 0 °C. After the addition was complete, the reaction mixture was stirred at room temperature for 24 h and the formed dicyclohexyl urea was removed by filtration. The solvent was then distilled out under reduced pressure. The residue thus obtained was then recrystallized from 2-propanol.

The log P of the synthesized compounds was determined to see the effect of the attached moiety on

Table 1. Physicochemical characteristics of intermediates.

Compounds	Mol. Formula	Mol. Weight	m.p (°C)	Yield (%)	R_f
2	$C_{16}H_{12}N_2O_4$	296	285-288	85	0.58
1a	$C_4H_9NO_2.HCl$	139.5	-	80	-
1b	$C_{10}H_{13}NO_2.HCl$	215.5	-	75	-
1c	$C_{10}H_{13}NO_3.HCl$	230.5	-	79	-
3a	$C_{13}H_{12}O$	184	65-67	90	0.68
3b	$C_{13}H_{11}ClO$	218.5	68-70	94	0.70

the lipophilicity of phenytoin. Except for **4a** all derivatives showed an increase in lipophilicity, a positive modification in the physicochemical properties of phenytoin.

Methyl 2-(2,5-dioxo-4,4-diphenyl imidazolidine-1-carboxamido) propanoate (4a) Yield 70%, mp 280-282 °C, R_f = 0.56 (benzene:MeOH, 9:1), log P: 1.80, λ_{max} (EtOH): 208.5 nm. IR (KBr) cm^{-1}: 669, 761, 1608, 1645, 1172, 3088, 3288. ^{1}H-NMR (DMSO-d_6): δ 11.06 (s, 1H, imidazolidine NH), 9.21 (s, 1H, NH), 7.05-7.77 (m, 10H, Ar-H), 5.05-5.10 (q, 1H, CH), 4.26 (s, 3H, OCH_3), 1.24-1.28 (d, 3H, CH_3)."

Methyl 2-(2,5-dioxo-4,4-diphenylimidazolidine-1-carboxamido)-3-phenylpropanoate. (4b) Yield 72%, mp 265-266 °C, R_f = 0.6 (benzene:MeOH, 9:1), log P: 2.74, λ_{max} (EtOH): 209 nm. IR (KBr) cm^{-1}: 656, 770, 1699, 1734, 1772, 3038, 3239. ^{1}H-NMR (DMSO-d_6): δ 11.06 (s, 1H, imidazolidine NH), 9.21 (s, 1H, NH), 7.23-7.8 (m, 10H, Ar-H), 5.0-5.09 (q, 1H, CH), 4.24 (s, 3H, OCH_3), 1.2-1.27 (d, 3H, CH_3)."

Methyl 2-(2,5-dioxo-4,4-diphenylimidazolidine-1-carboxamido)-3-(4-hydroxyphenyl) propanoate. (4c) Yield 69%, mp 270-273 °C, R_f = 0.62 (benzene:MeOH, 9:1), log P: 2.99, λ_{max} (EtOH): 209 nm. IR (KBr) cm^{-1}: 656, 746, 1717, 1748, 1773, 3038, 3072, 3271. ^{1}H-NMR (DMSO-d_6): δ 11.06 (s, 1H, imidazolidine NH), 9.21 (s, 1H, NH), 7.2-7.85 (m, 10H, Ar-H), 5.0-5.1 (q, 1H, CH), 4.24 (s, 3H, OCH_3), 1.21-1.28 (d, 3H, CH_3)."

Benzhydryl 2,5-dioxo-4,4-diphenyl-imidazolidine-1-carboxylate. (5a) Yield 76%, mp 290-293 °C, R_f = 0.66 (chloroform: ethyl acetate, 9:1), log P: 2.61, λ_{max} (EtOH): 213.5 nm. IR (KBr) cm^{-1}: 642, 787, 1717, 1721, 1742, 1773, 3038, 3273. ^{1}H-NMR (DMSO-d_6): δ 9.29 (s, 1H, imidazolidine NH), 7.14-7.40 (m, 20H, Ar-H), 5.86 (s, 1H, CH)."

4 Chlorobenzhydryl 2,5-dioxo-4,4-diphenyl-imidazolidine-1-carboxylate. (5b) Yield 65%, mp 285-287 °C, R_f = 0.66 (chloroform: ethyl acetate, 9:1), log P: 2.66, λ_{max} (EtOH): 214 nm. IR (KBr) cm^{-1}: 656, 770, 1721, 1742, 1773, 3032, 3273. ^{1}H-NMR (DMSO-d_6): δ 9.29 (s, 1H, imidazolidine NH), 7.14-7.40 (m, 19H, Ar-H), 5.86 (s, 1H, CH)."

The log P values of the target compounds were calculated by the following method,[9] 10 mL of octanol and 10 mL of phosphate buffer (pH 7.4) were taken in a separating funnel. Then 10 mg of compound **4a-c**,

5a-b was added to the mixture, which was shaken for 3 h and then kept undisturbed overnight. The aqueous layer was separated out and the concentration of the synthesized compounds (**4a-c**) (**5a-b**) was determined using a UV spectrophotometer. The log P values of compounds were calculated using the formula

log p = log (conc in octanol phase/conc in phosphate buffer)

Anticonvulsant activity screening

The suspensions of test compounds were prepared in sterile 0.9% NaCl solution. In all cases the control received the same quantity of sterile 0.9% NaCl solution as the vehicle.

Electro-shock induced seizure test[10]

Groups of 3 male Wister albino mice (18-30 g) were used. An apparatus with ear electrodes was used to deliver the stimuli 30 min and 4 h after i.p. injection with the test compound or the vehicle. The intensity of stimulus was 12 mA, 50 Hz for 0.2 s. Under these conditions all vehicle treated mice showed the characteristic extensor tonus. The animals were observed closely for 2 min. Disappearance of the hind leg extensor tonic convulsion was used as positive criterion. Percent of inhibition of seizures relative to controls was calculated.

Pentylenetetrazole (Metrazole) induced seizure test[10]

Wistar albino mice of either sex with a body weight between 18 and 30 g were used. The test compound or the reference drug was injected i.p. to groups of 4 mice. Another group of 4 mice served as controls. Some 30 min after i.p. injection, 60 mg/kg MTZ (Metrazole) was injected subcutaneously. Each animal was placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions were recorded. The number of protected animals in the treated groups was noted.

Rotorod method[10]

The animal motor impairment was measured in mice by the rotorod test. Groups of 3 Wistar albino mice of either sex with a body weight between 18 and 30 g were used. The mice were trained to stay on an accelerating rotorod that rotates at 6 revolutions per minute. The rod diameter was 3 cm. Trained animals were injected i.p. with the test compounds at doses of 30, 100, and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min.

The results are given in Table 2.

Results and discussion

In the present study the starting material, phenytoin-3-carboxylic acid (**2**), was obtained by hydrolysis of 5,5-diphenyl-N-carboethoxyhydantoin, which was synthesized as per the reported procedure.[5] IR spectra of compound **2** reveal a characteristic aromatic (C-H) stretch observed at 3072 cm^{-1}, amino (N-H) stretch at 3209 cm^{-1}, and sharp carbonyl peaks observed at around 1618-1775 cm^{-1}.

Table 2. Anticonvulsant screening project (ASP): phase 1 test result by MES, PTZ, Tox.

Sr. No.	Compounds	Dose (mg/kg)	MES[a]		PTZ[b]	Tox[c]	
			0.5 h[d]	4 h	0.5 h	0.5 h	4 h
1	4a	30	1/3	0/3	2/4	0/3	0/3
		100	2/3	2/3	3/4	0/3	0/3
		300	2/3	1/3	2/4	0/3	0/3
2	4b	30	3/3	2/3	1/4	0/3	0/3
		100	3/3	2/3	1/4	0/3	0/3
		300	3/3	1/3	1/4	0/3	0/3
3	4c	30	1/3	1/3	0/4	0/3	0/3
		100	3/3	2/3	1/4	0/3	0/3
		300	3/3	2/3	1/4	0/3	0/3
4	5a	30	3/3	1/3	2/4	0/3	0/3
		100	3/3	2/3	2/4	0/3	0/3
		300	3/3	2/3	1/4	0/3	0/3
5	5b	30	1/3	0/3	1/4	0/3	0/3
		100	3/3	1/3	1/4	0/3	0/3
		300	3/3	1/3	2/4	0/3	0/3

[a]MES test (number of animals protected/number of animals tested). [b]PTZ test (number of animals protected/number of animals tested).[c]Toxicity (number of animals exhibiting toxicity/number of animals tested).
[d]Time after drug administration.

The title compounds (**4a-c** and **5a-b**) were prepared by using dehydrating agent like DCC with the help of pyridine. The IR spectrum of **4a-c** and **5a-b** in KBr showed carbonyl stretching vibrations at around 1608-1775 cm^{-1}, aromatic stretch at 3037-3088 cm^{-1}, amino (N-H) stretch at 3072-3288 cm^{-1} and the out of phenyl vibrations of phenyl ring were observed at 645-770 cm^{-1}.

In the ^{1}H-NMR (δ ppm, DMSO) spectrum of **4a-c**, aromatic protons showed peaks at 7.057-7.776 ppm. The protons of the methyl groups appeared at 1.243-1.280 as a doublet and the methoxy protons as a singlet at 4.261 ppm. The methylene proton revealed a quartet at 5.052-5.105 ppm. The amide protons of the compound show peaks at 9.214 (side chain) and 11.64 ppm (ring nitrogen) as a singlet. In the ^{1}H-NMR (δ ppm, DMSO) spectrum of **5a-b**, aromatic protons revealed characteristic peaks as a multiplet at 7.142-7.403 ppm. The methylene proton revealed a singlet at 5.86 ppm. The amide proton of the hydantoin ring appeared at 9.285 ppm as a singlet.

The results of the pharmacological screening of the synthesized compounds indicate that the amino acids (especially phenylalanine and alanine) and benzhydrol linked with phenytoin are actually increasing the anticonvulsant activity of phenytoin and decreasing the neurotoxicity. The linkage with chlorobenzhydrol and tyrosine though was found ineffective in modifying the anticonvulsant activity of phenytoin positively.

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