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Synthesis and pharmacological activity of some new α N-Phthalimido amino acids analogues

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Abstract
The aim of the present study was to synthesize and investigate the pharmacological activity of some α N-Phthalimido derivatives of amino acids 2-8. The chemical structures of the titled compound were confirmed by physical and spectra analysis. All the synthesized compounds were evaluated *in vivo* for analgesic and antiepileptic activities by using standard experimental models. Compound 4 showed significant (P<0.05-0.0001) analgesic effect with hot plate test in mice. The anticonvulsant activity of all compounds were evaluated by picrotoxin -induced seizure . Compounds 3-5 and 7 significantly (P<0.01-0.0001) delayed the onset and antagonized picrotoxin -induced seizures.

1. Introduction

Nitrogen heterocycle is an important part of the chemical structures of many natural and synthetic products with a variety of properties and applications in medicine[1].

Compounds containing phthalimide subunit have been described as an scaffold (biophoro) to design new prototypes drug-candidates with different biological activities and they are used in different diseases such as leprosy, AIDS, tumor, diabetes, multiple myeloma, convulsion, inflammation, pain and bacterial infection [2, 3].

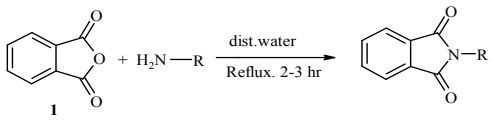
Non-steroidal anti-inflammatory drugs find the most clinical importance in the management of inflammation, pain and fever[2]. These drugs exert anti-inflammatory activity and relieve inflammation associated pain by the interacting and inhibiting the enzymatic activity leading to the inhibition of prostaglandins [4]. Design and development of NSAIDs with enhanced safety profile is still a necessity and challenge for the pharmaceutical industry. Moreover NSAIDs are getting lot of attraction because of their utility in early phases of many serious disorders like Alzheimer's dementia, cancer and heart vascular disease.

Epilepsy is a relatively common neurological condition affecting 0.5-1% of the

world is population. The classical antiepileptic drugs comprise phenytoin, phenobarbital, carbamazepine, ethosuximide, valproic acid and various benzodiazepines still widely utilized in the treatment of the various forms of epilepsy. In recent years several new drugs have been employed, e.g. felbamate, fosphenytoin, gabapentin, lamotrigine, tiagabine, topiramate, vigabatrin and zonisamide. However, about 25% of patients are resistant to the available medical therapies. All the currently approved antiepileptic drugs have dose-related toxicity and idiosyncratic side effects [6,7]. Antiepileptic drugs belong to many different classes of chemical compounds and act *via* various mechanisms [8]. 4-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain [9]. It has been estimated that approximately 40% of synapses in the central nervous system (CNS) are GABAergic[10].

2. Experimental Section

A series of N-substituted-Phthalimido amino acids were synthesized for the purpose of determining the analgesic and antiepileptic activity. The compounds were synthesized using phthalic anhydride with various appropriate amino acids in reflux synthesizer.



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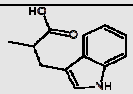
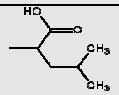
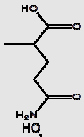
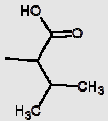
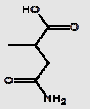
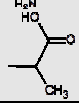
2		5		7	
3		6		8	
4					

3. Materials and Methods

All chemicals and solvents, reagents used in the present study were of analytical grade purchased from Sigma and Fischer. All the solvents were used after distillation. The melting points were determined by open capillary method and were uncorrected. The purity of compounds was confirmed by thin layer chromatography using Silica coated aluminum sheets(silica gel 60. F_{254}). The IR spectra of samples were recorded by Varian FT-IR spectrophotometer using KBr pellet technique and C, H, N analysis (not described).

4. General Procedure for the Synthesis of Compounds (2–8)

Equimolar amounts of the phthalic anhydride with appropriate amino acid in distilled water, the reaction mixture were refluxed for 2-3h. After cooling the precipitate was filtered off, washed with water and then recrystallized from dist. water (Table 1).

Table 1. *Physicochemical properties of the new synthesized compound 2-8.*

Comp. No	M. Formula (M. Wt)	Rec. solvent	Yield %	M.P °C	R_f cm	IR (KBr) cm^{-1}
(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)acetic acid 2	$C_{10}H_7NO_4$ (205.16)	Dist. Water	77	187-189	0.20	1550 cm^{-1} (-C=O) "carboxyl" 1700 cm^{-1} (-C=O) "imide" 2600 cm^{-1} (-C-H) 3000 cm^{-1} (-O-H) "carboxyl"
5-amino-2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-5-oxopentanoic acid. 3	$C_{13}H_{12}N_2O_5$ (276.24)	Dist. Water	37	198-199	0.51	3074 cm^{-1} (-N-H) 3009 cm^{-1} (-O-H) "carboxyl" 1700 cm^{-1} (-C=O) "imide" 1580 cm^{-1} (-C=O) "carboxyl"
2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propanoic acid. 4	$C_{11}H_9NO_4$ (219.20)	Dist. Water	37	199-200	0.56	3100 cm^{-1} (-O-H) 2600 cm^{-1} (-C-H) 1600 cm^{-1} (-C=O) "carboxyl" 1750 cm^{-1} (-C=O) "imide"
2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-3-(1H-indol-3-yl)propanoic acid. 5	$C_{19}H_{14}N_2O_4$ (334.33)	Dist. Water	30	155-156	0.25	3400 cm^{-1} (-N-H) 3000 cm^{-1} (-O-H) 2524&2652 cm^{-1} (-C-H) 1585 cm^{-1} (-C=O) "carboxyl" 1700 cm^{-1} (-C=O) "imide"

Comp. No	M. Formula (M. Wt)	Rec. solvent	Yield %	M.P °C	R_f cm	IR (KBr) cm^{-1}
2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-3-methylbutanoic acid. 6	$C_{13}H_{13}NO_4$ (247.25)	Dist. Water	41	201-202	0.46	796 cm^{-1} (substituted aromatic ring) 3000 cm^{-1} (-O-H) 2524&2652 cm^{-1} (-C-H) 1700 cm^{-1} (-C=O) "imide" 1585 cm^{-1} (-C=O) "carboxyl"
2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-4-methylpentanoic acid. 7	$C_{14}H_{15}NO_4$ (261.28)	Dist. Water	61	155-156	0.16	3000 cm^{-1} (-O-H) 2158,2362&2524 cm^{-1} (-C-H) 1750 cm^{-1} (-C=O) "imide" 1600 cm^{-1} (-C=O) "carboxyl"
4-amino-2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-4-oxobutanoic acid. 8	$C_{12}H_{10}N_2O_5$ (262.22)	Dist. Water	30	215-217	0.53	3400 cm^{-1} (-N-H) 3100 cm^{-1} (-O-H) 1400 cm^{-1} (-C=O) "carboxyl" 1700 cm^{-1} (-C=O) "imide" 2789 cm^{-1} (-C-H)

5. Pharmacology

New α N-Phthilimido Amino Acids Analogues 2-8, were tested for analgesic and antiepileptic properties and found to possess such activities. The results are shown in Table 2-5.

- CNS Activity Screening Tests:
- Screening analgesic activity by hot plate method:

The experiments were performed on male albino mice (15-30 g). The animals were kept at constant temperature facilities exposed to 12:12 h light: dark cycle. A standard pellet diet and tap water was given *adlibitum*. Each experimental group consisted 4 animals. The tested compounds were administered intraperitoneal (*ip*) 30min before the test, in a solution of 1% carboxy methyl cellulose (CMC), in dose 50mg/kg and volume of 1ml/100g. The animals were placed on hot plate after 30 minutes of injection test drug or CMC. The temperature is controlled from (55-56 □C). Record the time when the animal licks its fore limbs or jumps out of the plate as therapeutic end point [11]. Table 2. Fig.1

Table 2. *Analgesic Activity of new N- phthalimido amino acids analogues 2-8 by hot plate method.*

No of mice	Control	2	3	4	5	6	7	8
1	7.04	8.27	9.03	19	7.57	10.24	8.93	7.32
2	7.3	10	8.91	21	7.91	10.82	9.25	7.6
3	7.1	9.3	10	19.8	7.32	9.53	9.04	7.13
4	8	8.07	8.32	22.17	7.46	10.03	9.19	8.07
Mean	7.36	8.91	9.065	20.4925	7.565	10.155	9.1025	7.53
STDEV	0.440908	0.904691	0.6963	1.387885	0.251727	0.534072	0.145	0.408493
p value		0.021	0.006	<0.00000	0.45	<0.000	0.0002	0.59

Values are mean ± S.E.M. *p < 0.05, **p< 0.01,*** p < 0.001, significantly different from control, paired t-test (n = 4).

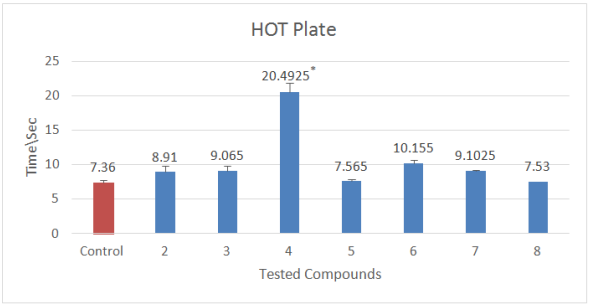


Figure 1. *Analgesic Activity of new N- phthalimido amino acids analogues 2-8 by hot plate method.*

- Screening antiepileptic activity by Picrotoxin induced seizures:

Picrotoxin in dose of 5mg/kg was injected to the mice either control or test groups by *i. p* route (30 minutes after drug injection). The animals were observed for 15 minutes after picrotoxin administration for the following observations [12] .

5.1. Time of seizures (onset). Table. 3, Fig.2.

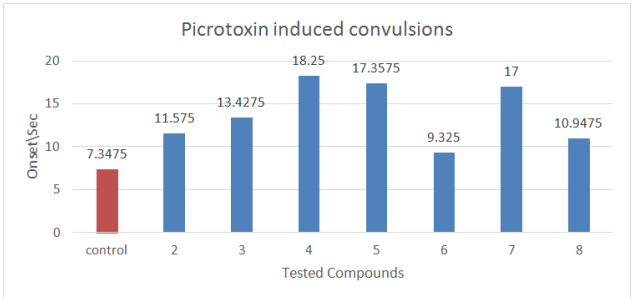


Figure 2. *Antiepileptic Activity of new N-phthalimido amino acids analogues 2-8 by picrotoxin induced convulsion (Onset time of seizure).*

Table 3. *Antiepileptic Activity of new N- phthalimido amino acids analogues 2-8 by pirotoxin induced convulsion (Onset time of seizure).*

No. of. mice	Control	2	3	4	5	6	7	8
1	7.2	10	13	10	17	9	17	11
2	8	12	14	19	15.43	8.3	19	9.34
3	7.06	11.3	12.3	23	17	9	15	11.45
4	7.13	13	14.41	21	20	11	17	12
Mean	7.3475	11.575	13.4275	18.25	17.3575	9.325	17	10.9475
STDEV	0.438739	1.2606215	0.9569178	5.737305	1.910818	1.164403	1.632993	1.147036
p value		<0.000	<0.0000	0.009	<0.0000	0.019	<0.000	0.001

*P < 0.01 when compared with control group

5.2. Number of seizures: Table. 4, Fig.3.

Table 4. *Antiepileptic Activity of new N- phthalimido amino acids analogues 2-8 by picrotoxin induced convulsion (Number of seizures).*

No. of. mice	Control	2	3	4	5	6	7	8
1	52	41	28	8	3	40	5	27
2	54	37	26	6	6	42	3	24
3	53	36	23	3	4	44	9	30
4	56	43	27	6	2	39	7	26
Mean	53.75	39.25	26	5.75	3.75	41.25	6	26.75
STDEV	1.707825	3.3040379	2.1602469	2.061553	1.707825	2.217356	2.581989	2.5
P value		<0.000	<0.0000	<0.0000	<0.0000	<0.000	<0.00000	<0.00000

*P < 0.01 when compared with control group

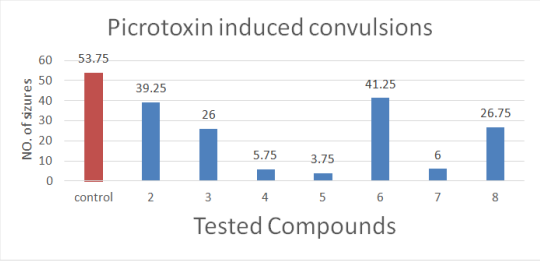


Figure 3*. Antiepileptic Activity of new N- phthalimido amino acids analogues 2-8 by picrotoxin induced convulsion (No. of seizures).*

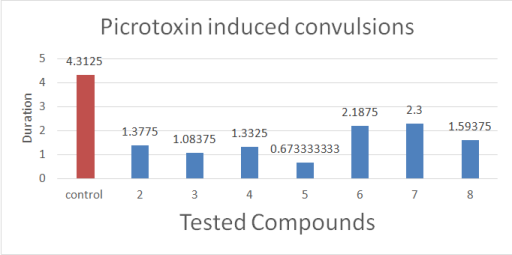


Figure 4. *Antiepileptic Activity of new N-phthalimido amino acids. analogues 2-8 by picrotoxin induced convulsion(duration of seizures).*

5.3. Duration of seizures: Table. 5, Fig.4.

Table 5. *Antiepileptic Activity of new N- phthalimido amino acids analogues 2-8 by picrotoxin induced convulsion(duration of seizures).*

No. of. mice	Control	2	3	4	5	6	7	8
1	4.41	1.26	0.94	1.32	1.2	2.5	2.1	1.3
2	3.77	1.56	0.875	1.4	0.37	1.8	3.1	1.59
3	4.27	1.13	1.58	1.26	0.45	2.07	1.8	1.97
4	4.8	1.56	0.94	1.35	0. 35	2.38	2.2	1.515
Mean	4.3125	1.3775	1.08375	1.3325	0.673333	2.1875	2.3	1.59375
STDEV	0.425549	0.2173131	0.3322493	0.058523	0.457857	0.315529	0.559762	0.279326
p value		<0.00005	<0.00002	<0.0000	0.0001	0.0002	0.001	<0.00005

*P<0.01 as compared to control. Values are expressed as Mean±SEM.

6. Result & Discussion

6.1. Chemistry

N-substituted-phthalimide derivatives were synthesized according to the preferred synthetic route. These derivatives were prepared from phthalic anhydride and amino acids via direct fusion, with a yield varying from 30-77%. The physicochemical properties of the synthesized compounds are reported in Table 1. The purity of these compounds was determined by TLC and their structures were confirmed by IR.

6.2. Pharmacology

All described, new phthilimido amino acids analogues 2-8, were evaluated *in vivo* for analgesic and antiepileptic activity by using standard experimental models.

The evaluation of analgesic activity was done by hot plate method, the reaction time is noted as therapeutic end

point. From the fig.1, among all the synthesized compounds 2-8 at dose of 50 mg/kg showed increase in reaction time (analgesic activity). It has found that the best analgesic activity was observed for the compound 4 that contains alanine when compared with control. Table.2, fig.1. This suggested that compounds 2-8 showed inhibitory actions on cyclooxygenase mediated pathway. We are trying to understand the mechanism of action of these compounds probably act as NSAIDs, and, so we intended to extend our studies using COX I and COX-2 enzymes, and to discover if these substances inhibit one or both isoforms.

The anticonvulsant activity of the synthesized compounds 2-8 were evaluated in mice using Picrotoxin induced convulsions at dose 5mg/kg. The test compounds were administered intraperitoneal (*i. p*) at a dose of 0.2ml/kg, half an hour after the administration of test compounds. The animals were observed after 15 minutes of picrotoxin administration. Table. 3-5, fig. 2-5. Among the newly synthesized compounds significantly indicative of their ability to prevent, decreasing and protection seizure spread. The best anticonvulsant activity was observed for the compounds 3-5 and 7 that contains glutamate, alanine, tryptophan and valine respectively.

The tested compounds at both doses (0.2ml/kg *i.p*) inhibited picrotoxin-induced convulsions. These observations indicate that the anticonvulsant effects of α N-phthalimido amino acid analogues are possibly mediated by chloride channels of GABA/benzodiazepine receptor complex and by chloride channel of glycine receptor [13]. GABA plays a critical role in the etiopathology of epilepsy [14]. The data obtained from the *in vivo* studies can be further evaluated for the side effects and mechanism of action.

7. Conclusion

Thus, we concluded that novel α N-Phthilimido Amino Acids Analogues exhibited good analgesic and antiepileptic activities. But it should be suggested that further exact mechanism of action is necessary.

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References

[1] S. Barman, E.I. Newhouse and W.C. Neely, Polym. Eng. Sci., 34,279, 2004.

[2] Miyachi ,H., Azuma, A ., Kitamoto, T ., Hayashi, K., Kato, S., Koga, M., Sato, B ., and Hashimoto, Y., Bioorg .Med .Chem. Lett., 7, 1483-1488, 1997.

[3] Chapman , J. M., Jr., Cocolas, G. H., and Hall , I. H., Journal of Pharmaceutical Sciences., 72, 1344-1347, 1983

[4] Singh G, Triadafilopoulos G., J. Rheumatol., 56, 18–24, 1999.

[5] Meade EA, Smith WL and DeWitt DL. , J. Biol. Chem., 268, 6610-6614, 1993.

[6] Greenwood R.S.: Epilepsia., 41, 42, 2000.

[7] Saxsena A.K., Saxsena M.: In Progress in Drug Research vol. 44, p. 185 Jucker E. Ed.; Birkhauser Verlag, Basel 1995.

[8] D.E. Robertis, in: G. Racagni, A. Donom (Eds.), GABA and Endocrine Function, Raven Press, New York., pp.1e12, 1987.

[9] L. Sivilotti, A., Nistri, Prog. Neurobiol., 36 , 35e 92, 1991

[10] F. Fonnum, in: H.Y. Melzer (Ed.), Psychopharmacology: the Third Generation of Progress, Raven Press, New York., pp. 173e182, 1987.

[11] Poupaert J., Hamoir G., Barbeaux P., Lambert D., Henichart P.J.: J. Parm. Pharmacol., 47,89, 1995.

[12] Eddy. N. B., and Leimbach, D., J. Pharmac. Exp. Ther., 107, 385-393, 1953.

[13] B. S. Meldrum. Epilepsia., 37: S4-S11, 1996.

[14] B. S. Meldrum., Int. Rev. Neurobion. 17: 1-36, 1975.