PROCEEDINGS OF THE NEUROSCIENCE SOCIETY OF NIGERIA (NSN) CONFERENCE, JOS, 2006

Anticonvulsant Activity of Some N-phenothiazyl and N-Carbazyl Para-Substituted Benzamides

By

Afolabi, E. O.*[1] Wannang, N. N.[2] ; Gomos, L. P.[1] and Iyoryisa, S.[1]

[1]*Department of Pharmaceutical Chemistry, University of Jos, Jos.*

[2]*Department of Pharmacology, University of Jos, Jos.*

Abstract

Some Nphenothiazyl and Ncarbazyl-parasubstituted benzamides were synthesized and evaluated for anticonvulsant activity in mice against pentelentetrazoleinduced seizures. The study was designed to determine the relationship between the structure of the benzamides and their anticonvulsant activity. In both series, the chloro- analogues were the most potent. The study showed that substituted benzamides are potential anticonvulsants. Thus they may be exploited for therapeutic usefulness in the treatment of epilepsy and seizures.

Key Words: Anticonvulsant, Benzamide, Epilepsy and Seizures.

Introduction

The evolution of drug development and discovery has led to screening of compounds for possible pharmacological activity. The substituted-benzamides have generated a lot of interest, as neuroleptics, in recent years (Blaney *et al.,* 1983). The benzamides have a unique behavioural profile and they appear to exert their pharmacological activity selectively at the D_2 dopamine receptors. This D_2 receptor subtype is responsible for their therapeutic use as anti-schizophrenic. Clark *et al.,* (1984) demonstrated that substituted-aminobenzamides show a high level of protection against maximal electrostatic induced convulsions in animal models.

Epilepsy affects about 0.5-1% of the world's population (Daniels and Jorgensen, 1982). There are many drugs used in the management of epilepsy. Among these anticonvulsants armamentarium, the benzamides have shown some promising evidence in anticonvulsant properties. Structurally, compounds possessing anticonvulsant activities, e.g. Valproate, are carboxylic acids and their amides (Murray and Kier, 1977). However the basic event responsible for epilepsy should be the rational approach to the development of any anticonvulsant drug.

In this study, some N-phenothiazyl, and N-carbazyl para substituted benzamides were prepared, screened and evaluated for their anticonvulsant activity.

Materials and Method

Animals

Mice (25-40 g) were obtained from National Veterinary Research Institute, Vom, Nigeria. The animals were kept in the Animal house of the Department of Pharmacology, University of Jos, Nigeria for 2 days to acclimatize to laboratory condition before the commencement of experiment. They were fed with standard feed and water *ad libitum*.

Synthesis of 10-(4-chlorobenzoyl)-10H-phenothiazine

A mixture of 2.623g (0.01M) triphenylphosphine, 9.6ml (0.1M) carbon

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Univeristy of Jos, PMB 2048, Jos.Nigeria.
Tel. +234-73-6105514 Ext. 211 GSM: 08035889579, E-mail: afolabie@unijos.edu.ng.

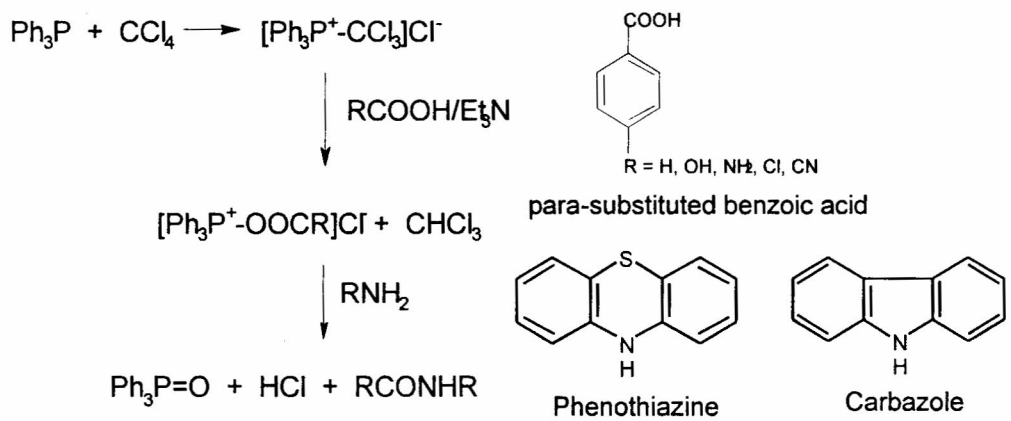
tetrachloride and 30ml of tetrahydrofuran (THF) was refluxed for thirty (30) minutes. The solution was cooled in an ice bath to 5°C and a mixture of 1.565g (0.01M) 4-chlorobenzoic acid and 1.4ml (0.01M) triethylamine was added and allowed to stand at 5°C for ten (10) minutes. 1.993g (0.01M) of phenothiazine was added and the mixture was heated under refluxed for forty-five (45) minutes. The precipitated triphenylphosphite was removed by filtration while the solvents were removed under vacuum. The crude product was purified with preparative TLC on silica, and its melting point was found to be 85 °C. The CHN analysis agreed within ± 0.04% range from the expected formula.

Anticonvulsant test

Animals were pretreated with the compounds for 30 minutes and later pentylenetetrazol (PTZ) (85mg/Kg) was administered intraperi-toneally. The animals were observed for 30 minutes. Failure to observe a single episode of clonic spasms for at least 5 seconds duration was considered as protection, and the results expressed as number of animals protected/number of animals tested.

Result

The synthesis of 10-(4-chlorobenzoyl)-10H-phenothiazine was followed as a typical procedure in a parallel synthesis of the two series of para-substituted benzamides (*see* Tables 1 and 2). Five carboxylic acids (*i.e.*: benzoic acid, 4-hydroxyl benzoic acid, 4-amino benzoic acid, 4-chloro benzoic acid, and 4-cyano benzoic acid) were reacted in parallel with the Wittig reaction of triphenylphosphine and carbon tetrachloride. Each of the esters formed were exchanged with the appropriate amine (*i.e.*: phenothiazine or cabarzole). In all five N-phenothiazyl and four N-carbazyl para-substituted benzamides were synthesized. The results of the anticonvulsant screening test for both series are presented in Tables 1 and 2.



Wittig Reaction with Ester-Exchange

Discussion

The chloro-substituted analogues in both N-phenothiazyl and N-carbazyl benzamides series were found to give better protection for PTZ-induced seizures in mice (see Tables 1-2, and Figures 1-6) than all the other para-substituted analogues. At higher doses, it was found that compounds in both series, produced convulsion.

The N-cabazyl series generally appear to give better protection against convulsion than the N-phenothiazyl series when each of the para-substituted analogues is considered on a one to one basis (*see* Figures 1-6).

Swinyard *et al.* (1989) demonstrated that some compounds are termed "preventing

seizure spread" rather than "raising seizure threshold". In this experiment, the compounds are seen to increase seizure threshold, probably through GABA pathway. Therefore, the observed activity of the compounds (see Tables 1 and 2) may not be phenytoin- or carbamazepine-like. Pentylenetetrazole blocks Gamma amino butyric acid (GABA) receptors, thus, interfering with the function of this inhibitory neurotransmitter (Kupferberg, 1989). The anticonvulsant activity of the compound suggests that it is mediated, at least in part, through a GABAagonist effect.

References

Blaney, F. E.; Michael S. *G* Clark, D. V. Gardner, M. S. Hadley, David Middleton, and White T. J. (1983): "Anilides Related to Substituted Benzamides. Potential Antipsychotic Activity of N-(4-Amino-5-chloro-2-mehtoxy phenyl)-1-(phenylmethy1)-4 piperidinecarboxamide" ***J.*** Med. Chem. 1983,26, 1747-1752

Clark, C. R., M. J. M. Wells, R. T. Sansom, G. N. Norris, R. C. Dockens, And W. R. Ravis. (1984). "Anticonvulsant activity of 4-aminobenzamide." ***J.*** Med. Chem.. 27: pp. 779-782.

Daniels, T. C.; Jorgensen, E. C. (1982): In "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry", 8th ed.; Doerge, **R.** F. Ed.; J. B. Lippincott: Philadelphia, p 375.

Kupferberg, H.J. (1989): "Antiepileptic drug development program: A cooperative effort of government and industry". *Epilepsia* 30 (suppl.1), S51-S56.

Murray, W. J.; and Kier, L. B. (1977): "Structure-Activity Studies on Hallucinogenic Amphetamines Using Molecular Connectivity" J. Med. Chem. 1977, *15,* 591.

Swinyard, E.A., Woodhead, J.H., White, H.S. & Franklin, M.R. (1989): "General principles: experimental selection, quantification, and evaluation of anticonvulsants". In: *Antiepileptic Drugs,* Third Edition R.H. Levy, R.H. Mattson, B. Melrum, J.K. Penry and F.E. Dreifuss eds, pp. 85-102. New York: Raven Press.

Table 1: Anticonvulsant Activity of 4-Substituted-N-phenothiazylbenzamide

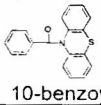
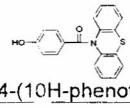
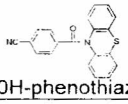
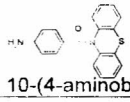
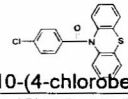
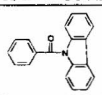
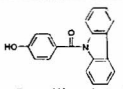
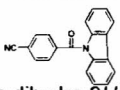
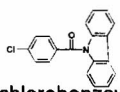
4-Substituted-N-phenothiazylbenzamide	Dose	Onset of Seizure (s)	Episode of Seizure	Time of Death (s)	Number Survived
 **4-H-PHB** mp 92 °C 10-benzoyl-10H-phenothiazine	30mg/kg	61.25 ± 22.2	4	193.50 ± 68.1	0/4
	100mg/kg	54.00 ± 13.6	5	159.25 ± 24.2	1/4
	300mg/kg	61.25 ± 33.0	5	167.50 ± 36.2	0/4
 **4-OH-PHB** mp 101 °C 4-(10H-phenothiazin-10-ylcarbonyl)phenol	30mg/kg	49.25 ± 24.8	3	138 ± 18.2	0/4
	100mg/kg	50.25 ± 23.5	4	185 ± 15.2	1/4
	300mg/kg	54.75 ± 1.35	3	104.75 ± 1.2	0/4
 **4-CN-PHB** mp 90 °C 4-(10H-phenothiazin-10-ylcarbonyl) benzonitrile	30mg/kg	76.5 ± 2.8	4	192.5 ± 63.1	0/4
	100mg/kg	84.0 ± 3.3	3	64.5 ± 1.8	0/4
	300mg/kg	89.5 ± 2.3	4	193.5 ± 58.1	0/4
 **4-NH_2-PHB** mp 120 °C 10-(4-aminobenzoyl)-10H-phenothiazine	30mg/kg	40.75 ± 5.9	2	115.0 ± 48.1	0/4
	100mg/kg	78.5 ± 2.2	2	62.25 ± 1.6	0/4
	300mg/kg	19.25 ± 20.2	2	90.25 ± 8.1	0/4
 **4-Cl-PHB** mp 85 °C 10-(4-chlorobenzoyl)-10H-phenothiazine	30mg/kg	110 ± 33.9	3	186.7 ± 47.7	¼
	100mg/kg	140 ± 52.1	4	375.0 ± 85.7	0/4
	300mg/kg	120 ± 10.1	3	177.5 ± 85.7	0/4
(-)C {NEGATIVE CONTROL (ipPTZ only)}	90mg/kg	64.0 ± 7.0	7	222.5 ± 56.6	0/4
(+)C {POSITIVE CONTROL (Carbamazepine + ipPTZ)}	10mg/kg	33.75 ± [illegible]	4	646.25 ± 50.3	0/4

Table 2: Anticonvulsant Activity of 4-Substituted-N-carbazylbenzamide

4-Substituted-N-carbazylbenzamide	Dose	Onset of Seizure (s)	Episode of Seizure	Time of Death (s)	Number Survived
 **4-H-CBZ** mp 110°C 9-benzoyl-9,9a-dihydro-4a*H*-carbazole	30mg/kg 100mg/kg 300mg/kg	66.67± 4.7 94.00 ± 2.3 86.00 ± 3.3	4 3 5	333.67 ± 73.5 244.75 ± 76.0 246.75 ± 76.2	0/4 ¼ 0/4
 **4-OH-CBZ** mp >300 °C 4-(4a,9a-dihydro-9*H*-carbazol-9-ylcarbonyl)phenol	30mg/kg 100mg/kg 300mg/kg	66.00 ± 6.1 58.00 + 1.0 98.00 ± 5.9	4 4 3	213.0 ± 10.2 213.0 ± 13.0 310.50 ± 13.3	0/4 ¼ 0/4
 **4-CN-CBZ** mp 226 °C 4-(4a,9a-dihydro-9*H*-carbazol-9-ylcarbonyl)benzonitrile	30mg/kg 100mg/kg 300mg/kg	60.75 ± 1.3 53.00 ± 1.0 45.67 ± 2.1	4 4 4	83.5 ± 1.4 149.25 ± 47.4 269.00 ± 56.6	0/4 0/4 0/4
 **4-Cl-CBZ** mp 150 °C 9-(4-chlorobenzoyl)-9,9a-dihydro-4a*H*-carbazole	30mg/kg 100mg/kg 300mg/kg	103.5 ± 5.1 74.5 ± 2.8 77.75 ± 1.4	4 2 6	472.75 ± 74.2 123.5 ± 35.3 222.5 ± 127.0	¼ 0/4 0/4
(-)C {NEGATIVE CONTROL (ipPTZ only)}	90mg/kg	64.0 ± 7.0	7	222.5 ± 56.6	0/4
(+)C {POSITIVE CONTROL (Carbamazepine + ipPTZ)}	10mg/kg	33.75 ± 5.9	4	646.25 ± 50.3	0/4

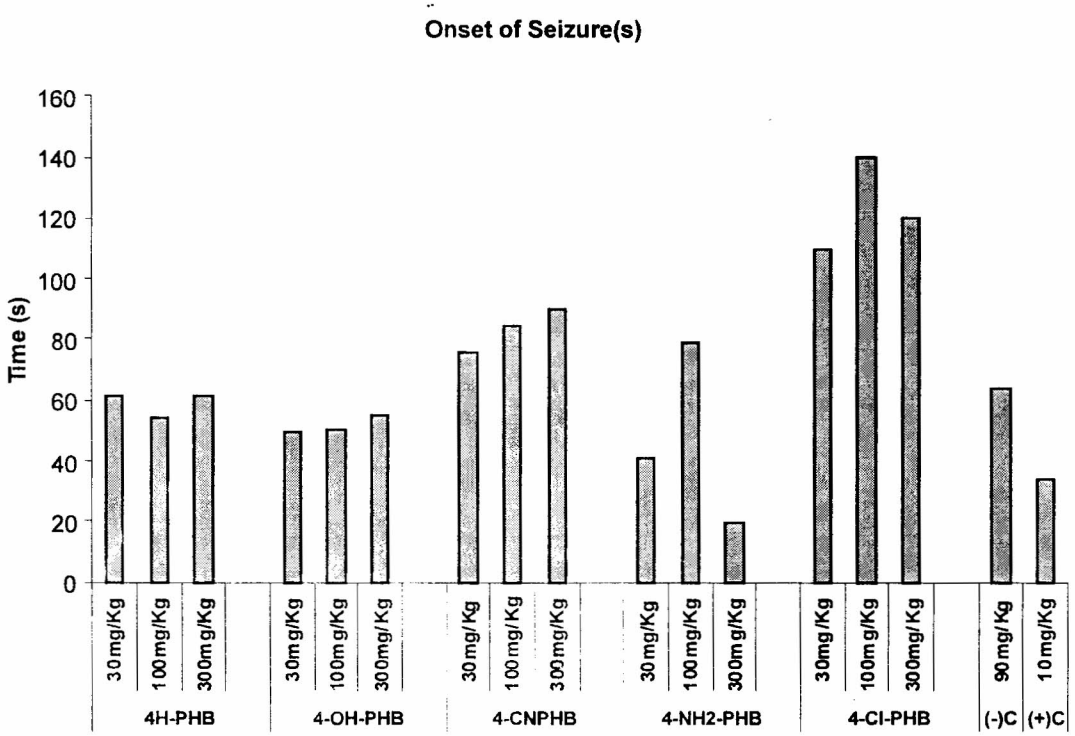


Figure 1: Onset of Seizure(s) of 4substituted-N-phenothiazylbenzamides

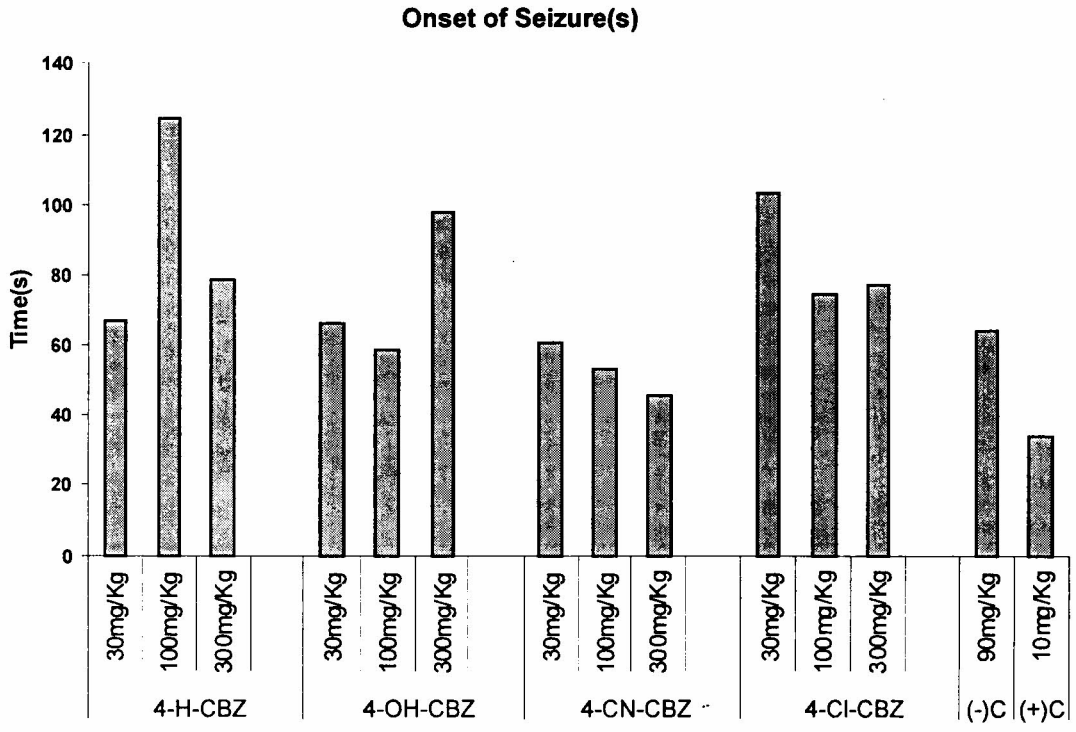


Figure 2: Onset of Seizure(s) of 4-substituted-N-carbazylbenzamides

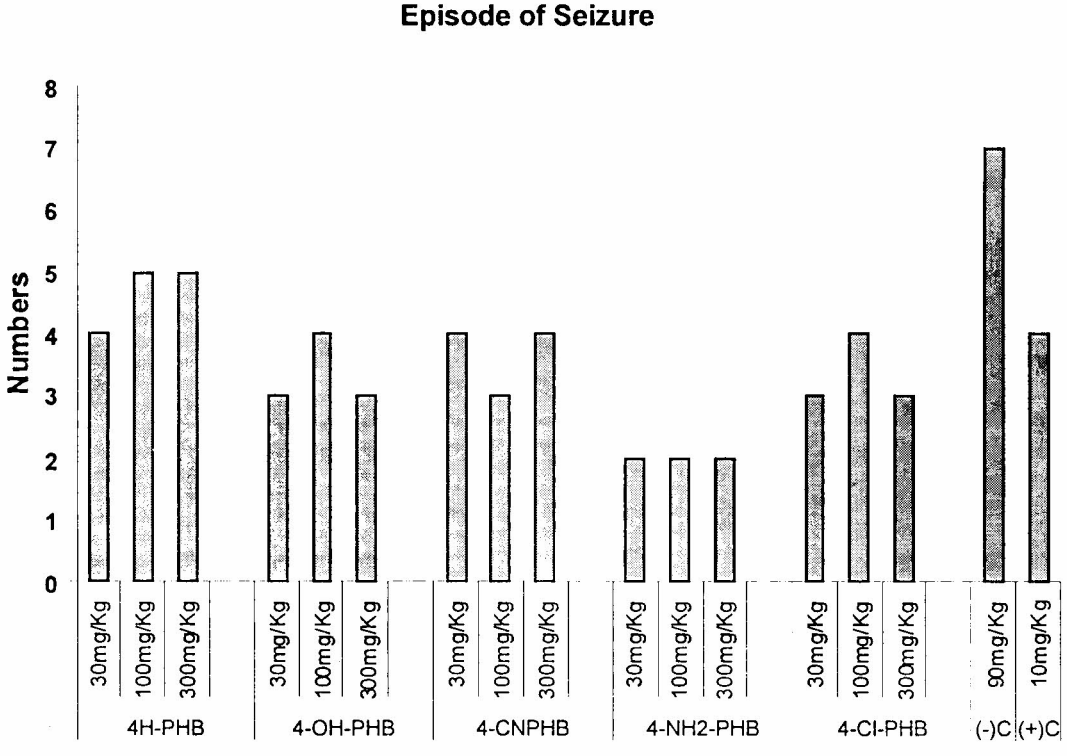


Figure 3: Episode of Seizureof 4-substituted-N-phenothiazylbenzamides

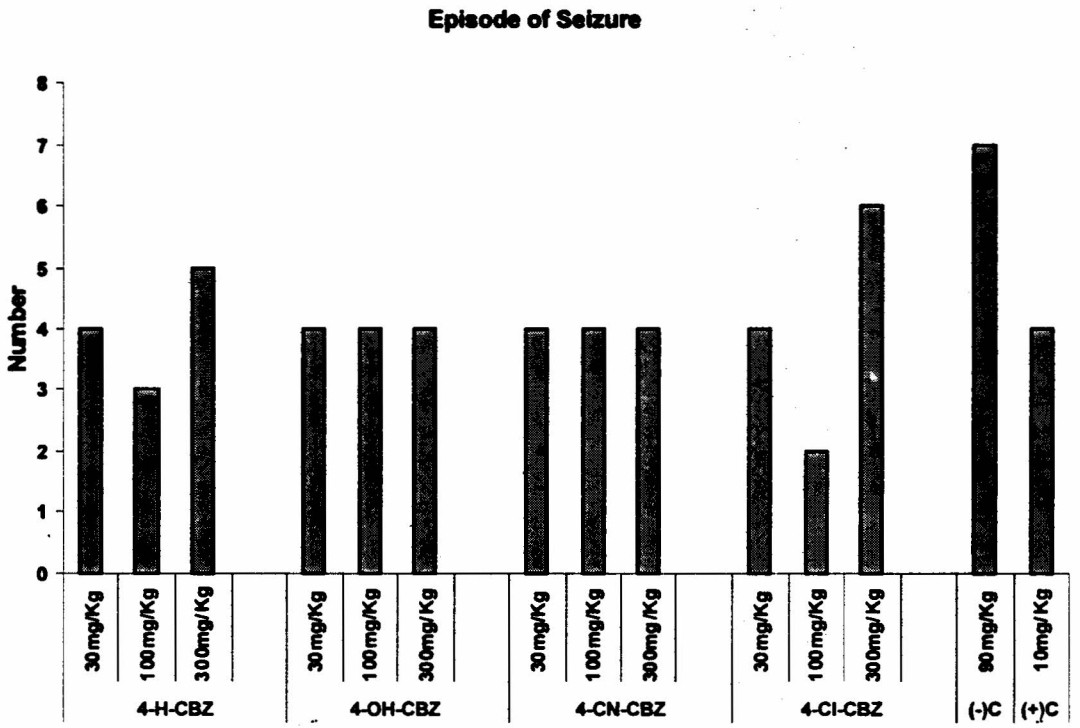


Figure 4: Episode of Seizure of 4substituted-N-carbazylbenzamides

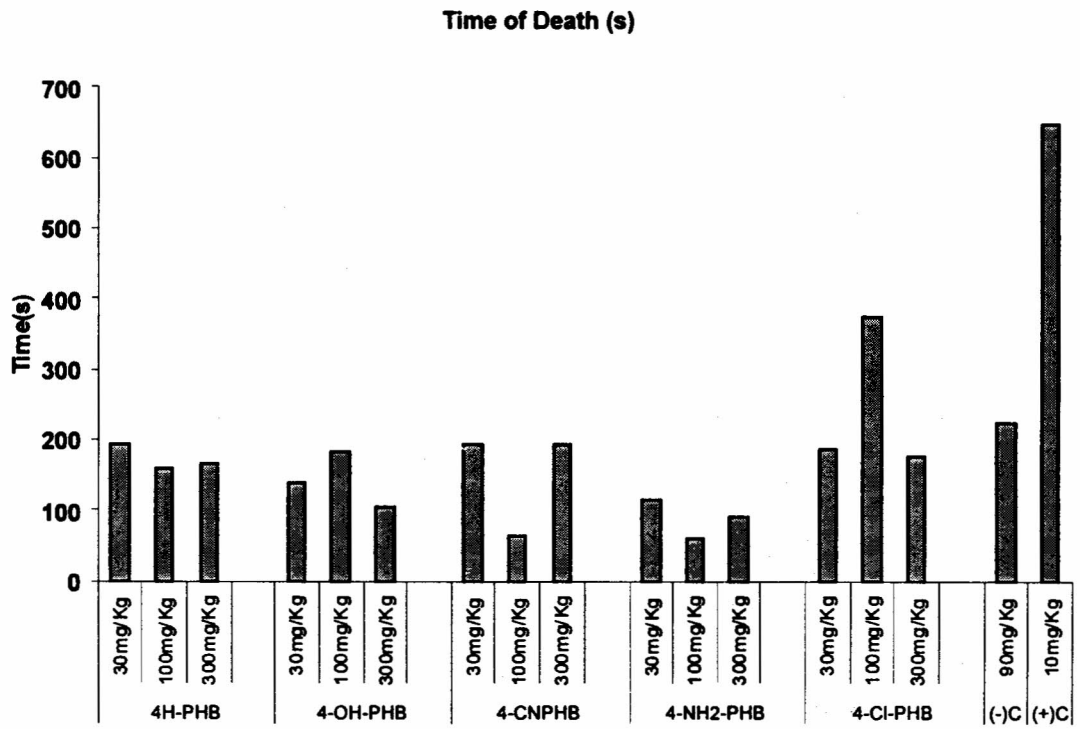


Figure 5: Time of Death(s) by 4-substituted-N-phenothiazylbenzamides

Figure 6: Time of Death(s) by 4-substituted-N-carbazylbenzamides