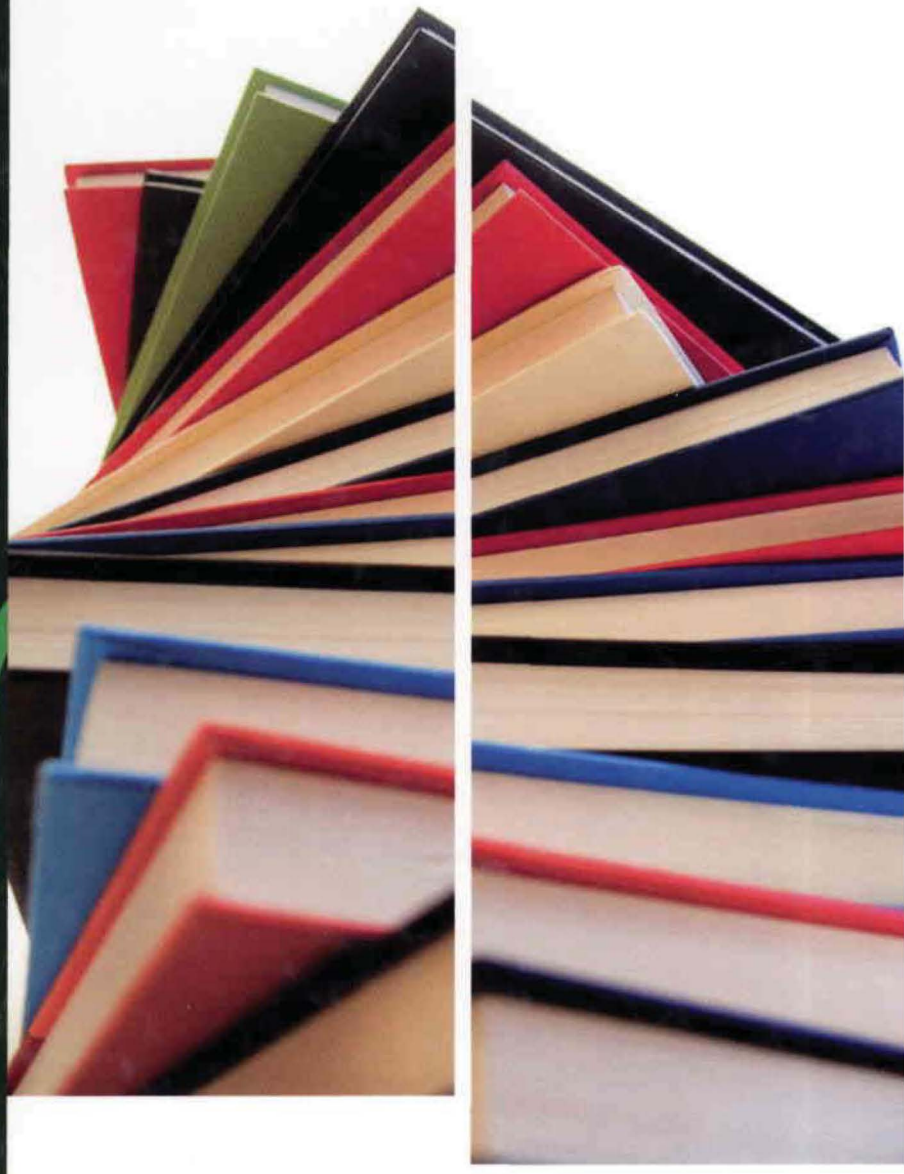
Proceeding of

IBOC 2012

INTERNATIONAL BIOLOGY CONFERENCE



Science for Energy, Food, and Environmental Sustainability

ITS Institut Teknologi Sepuluh Nopember

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PROCEEDING OF

INTERNATIONAL BIOLOGY CONFERENCE

Science for Energy, Food and Environmental Sustainability

December 6th, 2012
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Institut Teknologi Sepuluh Nopember
Sukolilo – Surabaya

IBOC 2012
INTERNATIONAL BIOLOGY CONFERENCE

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PREFACE

We would like to thank to God for blessing us until we are able to organize our first international annual event called The International Biology Conference (IBoC) 2012 at the end of this year. This conference is purposed to build a future and promising international networking between our department and other international parties which have a similar interest keeping environment balance for save the earth. Concerning to it, our first theme is "Science for Energy, Food and Environmental Sustainability".

The first outcome we would gain is having a direct and personal contact, sharing and discussion with scientists around the world. Further on we would like to build a real scientific networking doing a real work keeping the earth balance and save. We are really grateful realizing that they are from Egypt, Thailand, Bangladesh, Malaysia and Indonesia. Thank you for your participations.

We are also glad to inform that, this conference have been possible only because of the support from The Faculty of Mathematics and Natural Sciences and The Institut Teknologi Sepuluh Nopember (ITS), Surabaya-Indonesia. The Vice Rector, Prof. Darminto put several administrative and scientific advices. The big applause and thank is also going to our students from The Biology Department who has been managing the conference.

Thank you and best regards.

Surabaya, June 5th 2013
Head of Biology Department

Dr.rer.nat. Maya Shovitri

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CONTENTS

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The hypnotic effect of benzoylthioutea derivative (2.4 dichlorobenzoylthiourea) in mice

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Abstract

The structure modification of benzoylthiourea by adding Cl atoms at the position of C2 and C4 may result in 2,4-dichlorobenzoylthiourea. With the addition of two Cl atoms on the benzoiltiourea aromatic ring, log P value may increase to 2.24 and the synthesized compound is expected to have better biological activity as a central nervous system deppressant (hypnotic effect) than the lead compound. This compound has similar structure to barbiturates. In this study, the test of hypnotic effect was performed on white male mice by observing the mice sleep duration extension. The test animals were divided into 7 groups: the control group was given 0.5% Na-CMC suspension; the standard were given 125 mg/kg of Phenobarbital Na; while treatment group I, II, III, IV, and V were orally given 15 mg/kgBW, 30 mg/kgBW, 45 mg/kgBW, 60 mg/kgBW, and 75 mg/kgBW of suspensions of 2,4-dichlorobenzoylthiourea respectively, with the Time Peak Effect (TPE) : 30 minutes. The results showed that the average sleep duration of the control group, standards, treatment I, II, III, IV, and V were 0 minute, 33.37 minutes, 87.14 minutes, 178.67 minutes, 232.91 minutes, 272.60 minutes, and 304.66 minutes respectively. The ANOVA statistical results with α=0.05 showed significant differences between the groups and it can be concluded that 2,4- dichlorobenzoylthiourea has stronger hypnotic effect than Phenobarbital Na in white male mice.

Keywords: Hypnotic effect, 2,4-dichlorobenzoyilthiourea, *Barbiturate Sleeping Time.*

INTRODUCTION

Due to the advances in society, there are some inevitable consequences of the occurrence of various disorders. At first, stress will occur due to the inability of individuals to overcome challenges and adapt to the changes. If the stress continues and can not be overcome, it will lead to more severe disorders such as mental and psychiatric disorders. To overcome these disorders, the central nervous system suppressants such as sedative-hypnotic drugs are generally used (Maramis, 1994).

Sedative-hypnotics drugs are divided into two groups, benzodiazepines and non-benzodiazepines (e.g. barbiturates). The benzodiazepines are divided based on the duration of action, which are long-acting (Prazepam), intermediate-acting (Lorazepam) and short-acting (Alprazolam). Non-benzodiazepines (e.g. barbiturates) are also divided based on the duration of action, which are long-acting such as Na Phenobarbital (Luminal), intermediate-acting (amobarbital), short-acting (Pentobarbital), very short-acting (Thiopental Na) (Katzung & trevor's, 2002).

Siswandono (1998) has done benzoylurea synthesis through the acylation between a primary amine of urea and the benzoyl group of benzoyl chloride. Benzoylurea is an acyclic ureide with a similar structure to bromisovalum or barbiturate derivatives that have been known to have central nervous system suppressant effect, so it is expected that benzoylurea also have effects on central nervous system. Benzoilurea does not contain bromine in the structure so it does not cause bromism. The

results of benzoylurea activity test showed that it has central nervous system suppressant activities such as hypnotic, impaired coordination, and anticonvulsant effects.

Kesuma (2004) had synthesed the benzoythiourea by conducting acylation reaction between one of the amine groups of thiourea with benzoyl group of benzoyl chloride. In terms of the chemical structure, benzoythiourea is similar to benzoylurea. The substitution of O atom of the urea with S atom into thiourea, in which the O atoms are more electronegative than the S atoms. Based on the above, it is expected benzoythiourea has the central nervous system suppressant effect with better lipophilicity that its activity increases compared to benzoylurea. The results of activity assays show that benzoythiourea has central nervous system suppressant effect, so it can be used as the parent compound in further development to obtain new compounds with better central nervous system suppressant activity.

This study used 2,4-dichlorobenzoylthiourea which is a derivative of benzoythiourea which has central nervous system suppressant effect with a good lipophilicity and can be used as a lead compound for central nervous system suppressant drugs. In 2,4-dichlorobenzoyl thiourea, the addition of two Cl atoms on the C2 and C4 positions on the aromatic ring of benzoylthiourea will have a better activity as a central nervous system suppressant when compared to the parent compound, benzoylthiourea (Kesuma, 2004). Theoretically, benzoylthiourea has a lipophilicity value (log P) of 1.12. The optimal log P value of the central nervous system suppressants is 2. In 2,4-dichlorobenzoyl thiourea, log P value increases to 2.24 (Siswandono dan Soekardjo, 2000).

Based on the above data, a study was conducted to examine the hypnotic effect of 2,4-dichlorobenzoylthiourea compared to Phenobarbital-Na at various doses on white male mice. The pharmacological study method used was *barbiturate sleeping time*. The parameters measured were sleep duration of mice.

MATERIALS AND METHOD

Materials

The materials used for this study was 2.4-dichlorobenzoylthiourea synthesized by Dini Kesuma. The compound was synthesized through acylation reaction by reacting 2,4-dichlorobenzoylchlorida with thiourea in THF at a temperature of 90°C for 2 hours. The results of the analysis proved that the compound is 2,4-dichlorobenzoylthiourea.

The resulting compound was in the form of yellow fine crystal, with pungent characteristic odor, and tasted very bitter (Kesuma, 2004).

The other additional materials used in this study were Phenobarbital Na (Kimia Farma, Ltd. Co), water for injection (Otsuka Indonesia, Ltd. Co), aquadest (Laboratory of Qualitative Analysis, the Faculty of Pharmacy, University of Surabaya), Na-CMC (Bratako).

Instruments

The instruments used in this study were analytical balance (Sartorius ®), glass box, stopwatch (Alba ®), animal scales (Ohaus ®), modified oral syringe (gavage), laboratory glassware equipments (beaker glass, measuring cylinder, glass funnel, Pasteur pipette, glass stirrer, volumetric flask), spatula, mortar-pestle and water heater.

Animals

The animals used in this study were white male mice (*Mus muscullus*) BALB/c strain (70 animals), adult (2-3 months), 20.0 to 30.0 grams of weight, and obtained from the Laboratory of Experimental Animal, Faculty of Pharmacy, Airlangga University.

During maintenance in the cages, the mice were given standard food. The mice were adapted for 1-2 weeks prior to the study in the room where the study were conducted (the room was arranged to be noise-free and soundproofing). During the adaptation period, body weight of mice should not be reduced by 10%. The mice were visually healthy, characterized by red and clear eyes; the hair looked clean, smooth, and shiny; the nose and mouth were not runny and salivated continuously; the consistency of stools were dense and normal; not having diarrhea; no congenital defects; with normal behavior and never been used in any other experiment (naive).

Principles of the experiments

The principle of the experiments conducted in this research was the animals were divided randomly into seven groups (a control group, a standard group, and five treatment groups with different doses), with each group consisting of 10 mice. The control group was given 0.5% of Na-CMC, the standard group was given Phenobarbital Na solution, while the treatment groups were given the suspension of 2,4-dichlorobenzoylthiourea at different doses orally. After left for a certain time (based on the orientation of peak activity times result), the control group and the treatment group were given Phenobarbital Na solution orally, then they were observed from the time when the mice started to sleep (righting reflex negative) until they woke up (righting reflex positive).

The hypnotic test of 2,4-dichlorobenzoylthiourea against Phenobarbital Na

The mice were divided randomly into 7 groups, each group consisted of 10 mice. Each group were given 0.5% of Na-CMC suspension (control group), Phenobarbital Na 125 mg/kg (the standard group), 2,4-diklorobenzoiltiourea at the doses of 15 mg/kg peroral (treatment group I) , 30 mg/kg (treatment group II), 45 mg/kg (treatment group III), 60 mg/kg (treatment group IV), and 75 mg/kg (treatment group V). After a few minutes (according to TPE), the control group and the treatment group were given Phenobarbital Na 125 mg/kg via oral gavage. The sleep duration was calculated from the mice starting to sleep (righting reflex negative) to waking up (righting reflex positive).

Data analysis

The data obtained in hypnotic test were the mice sleep duration of the Phenobarbital and the treatment group, and the extension of sleep duration of the treatment groups were compared to Phenobarbital group. The data were presented in tables and charts between the treatment groups.

Data analysis method used in this study was One-way ANOVA using SPSS Version 19 with α = 5% or significance level of 0.05. One-way ANOVA was used to determine whether there were significant differences between the control group, the standard group and the treatment group in which the hypnotic effect of 2,4-dichlorobenzoythiourea at doses of 15 mg/kg, 30 mg/kg, 45 mg/kg, 60 mg/kg, and 75 mg/kg compared to Phenobarbital Na could be seen and to see the significant differences between the treatment groups.

RESULTS AND DISCUSSION

Table 1. The observation results of mice sleeping time on the treatment groups

Mouse number	Mice sleep duration (min)						
	Na-CMC 0,5% (Control)	Phenobarbital Na 125 mg/kg BW (Standard)	Compound 15 mg/kg BW (Treatment I)	Compound 30 mg/kg BW (Treatment II)	Compound 45 mg/kg BW (Treatment III)	Compound 60 mg/kg BW (Treatment IV)	Compound 75 mg/kg BW (Treatment V)
1	0	30.41	89.32	166.56	237.67	278.39	311.52
2	0	32.11	99.46	183.73	249.32	262.84	294.67
3	0	39.67	83.31	181.92	218.98	283.61	318.39
4	0	36.62	82.67	172.45	224.23	285.35	299.28
5	0	37.32	92.18	187.34	221.68	269.27	315.43
6	0	31.99	77.87	179.05	241.51	272.59	303.29
7	0	28.06	86.22	186.78	232.29	270.53	308.37
8	0	35.65	94.91	169.16	229.73	248.13	292.71
9	0	34.31	85.76	174.29	234.29	278.38	297.82
10	0	27.56	79.65	185.39	239.41	276.92	305.11
Mean ± SD	-	**33.37± 4.03**	**87.14± 6.85**	**178.67± 7.58**	**232.91± 9.52**	**272.60± 10.95**	**304.66± 8.73**

Table 2. The observation results of mice sleeping time on the treatment groups

Mouse number	Sleep duration extension (min)				
	Test Compound 15 mg/kg BW p.o	Test Compound 30 mg/kg BW p.o	Test Compound 45 mg/kg BW p.o	Test Compound 60 mg/kg BW p.o	Test Compound 75 mg/kg BW p.o
1	58.91	136.15	207.26	247.98	281.11
2	67.35	151.62	217.21	230.73	262.56
3	43.64	142.25	173.91	243.94	278.72
4	46.05	135.83	187.61	248.73	262.66
5	54.86	150.02	184.36	231.95	278.11
6	45.88	147.06	209.52	240.60	271.30
7	58.16	158.72	204.23	242.47	280.31
8	59.26	133.51	194.08	212.48	257.06
9	51.45	139.98	199.98	244.07	263.51
10	52.09	157.83	211.85	249.36	277.51
Mean ± SD	**53.77± 7.41**	**145.30± 9.14**	**199.00± 13.75**	**239.23± 11.39**	**271.29± 9.02**

The results from analysis using One-Way ANOVA (α=0.05) showed that sleep duration of the treatment groups on hypnotic test given 2,4-dichlorobenzoyilthiourea have significant difference with a significance value of 0.000 (< 0.05) compared to those with Phenobarbital. *Post hoc test* was done to see which other groups have significant difference, and it was found between the control group, the standard group, and the treatment groups (significance value of 0.000 < 0.05).

Then, the mice sleep duration extension was calculated by subtracting the sleep duration of the mice induced with the test compound with the sleep duration of the ones induced with Phenobarbital Na. To see if there is a significant difference between the treatment groups, One-Way ANOVA test (α=0.05) was used. The result showed that the mice sleep duration extension between the treatment groups on hypnotic test of 2,4-dichlorobenzoyilthiourea compared to Phenobarbital Na showed significant difference with significance value of 0.000 (< 0.05).

Based on the results from the statistical analysis, we can conclude that 2,4-dichlorobenzoyilthiourea at doses of 15 mg/kg BW, 30 mg/kg BW, 45 mg/kg BW, 60 mg/kg BW, and 75 mg/kg BW has stronger hypnotic effect than Phenobarbital Na by prolonging the mice sleep duration. At the dose of 75 mg/kg BW the compound gives the most hypnotic effect with the longest sleep duration extension, that is 271.29±9.02 minutes.

CONCLUSION

From the results of this study, it can be concluded that:

1. 2,4-dichlorobenzoyilthiourea has stronger hypnotic effect than Phenobarbital Na on white male mice.
2. At a dose of 75 mg/kg BW, 2,4- dichlorobenzoylthiourea gave the longest sleeping time of the mice.

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