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COMPARISON OF SUPPRESSIVE ACTIVITY OF THE CENTRAL NERVOUS SYSTEM FROM THE NEW DERIVATIVES *N*-BENZOYLPHENYLUREA

BAMBANG TRI PURWANTO

Department of Pharmaceutical Chemistry Faculty of Pharmacy, Airlangga University, Indonesia.
Email: bbg_tony@yahoo.com

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ABSTRACT

Object: To compare the suppressive activity test of the central nervous system and synthesis from the new compound *N*-benzoylphenylurea and its derivatives.

Methods: The Schotten Baumann method was used for the reaction between *N*-phenylurea and benzoyl chloride derivatives. The Central Nervous System (CNS) suppressive activity test was done for the *N*-benzoylphenylurea compound and its derivatives by Barbituric Sleeping Time (BST) method.

Result and discussion: The yield of the *N*-benzoylphenylurea was 86% , had a white crystal , the 4-tercierbutylbenzoyl-phenyl-urea was a white crystal and yield was 60%, the 2,4-dichlorobenzoylphenylurea yield was 81%, and the *p*-toluolbenzoylphenylurea was 65%. All of them gave one spot in Thin Layer Chromatography with two different eluent. The all compound melting point was different from the *N*-phenylurea and showed greater. The structure identification from the all new compounds were analysed by UV, IR, ¹H-NMR and MS, the result showed that the new compound were *N*-benzoylphenylurea and its derivatives. The CNS suppressive activity test from *N*-benzoylphenylurea and its derivatives had a greater activity if compare with the standard compound bromisoval.

Conclusion: The *N*-benzoylphenylurea and its derivatives, which were synthesized, had a higher suppressive activity on the central nervous system. The highest compound on suppressive activity in central nervous system is *p*-toluolbenzoylphenylurea.

Keywords: *N*-benzoylphenylurea and its derivatives; CNS suppressive activity.

INTRODUCTION

Development of compound substances having active moiety which is better of pharmacology activity than the lead compound need to get attention. This is to gain of compound substances can become active new candidate agents new drug to support public health. To gain new compounds will potentially as a candidate new drug a chemical synthesis takes a process in hopes of obtained new compounds having relatively pure and pharmacological activity higher compared to its lead compound [4;7].

Development of compound substances active may be conducted through a variety of methods of synthesis reaction (particularly through) which the synthesis reaction can be a reaction one step or some reaction phase. Methods of synthesis reaction one step has more excercises compared with some methods of synthesis more steps reaction, because the target compound directly obtained in one step reaction [8].

During this time, urea only known as the active material used as fertilizer plants, and now can already production by industrial plants in Indonesia, furthermore on development using the active ingredient urea as an active substance will produce compounds having an active diverse pharmacological activity among others as an central nervous system suppressants and as anti cancer [6].

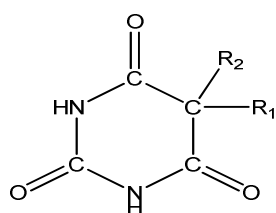
Some derivatives of urea which have been successfully made, have pharmacological activities, especially as the central nervous system

suppressants such as isovalerilurea who has successfully made by Reksোধadipodjo [11]; bromasilurea made by Tjipta Surasa [16]; benzoiurea made by Siswandono [14]; benzoiltiurea made by Suzanna [15]; and benzoiltiurea derivatives who has successfully made by Dini [5].

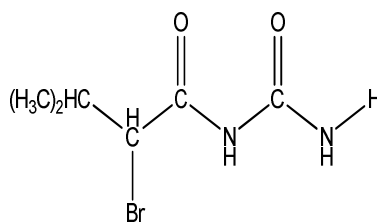
The urea derivatives which have a pharmacophoric moieties like acyclic ureid, have the central nervous system suppressants activities, the barbituric acids derivatives also have.

The urea derivatives which have the pharmacophoric moieties acyclic ureid can be seen on figure 1.

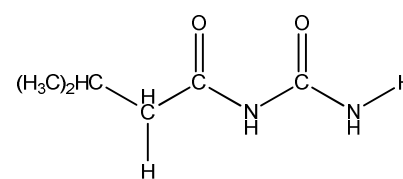
Further development of active compounds urea derivatives is *N*-benzoylphenylurea, which also has a ureid acyclic pharmacophoric moiety, and the new derivatives of *N*-benzoyl-phenylurea was obtained from the one step reaction through the Schotten - Baumann method. Some of the new *N*-benzoylphenylurea derived compounds that have been successfully synthe-sized are *N*-benzoylphenylurea, 4-tercierbutylbenzoylphenylurea, 2,4-dichlorobenzoylphenyl-urea and *p*-toluolbenzoylphenylurea. All of these compounds will have a different activities as the central nervous system suppressor so its needs to be tested the pharmacological activities of the compounds through Barbituric Sleeping Time (BST) method. The central nervous system suppressive activities from the *N*-benzoylphenylurea derivatives can be comparisson each other to known which ones of them has a higher pharmacological activity.



Derivative of Barbituric Acid



Bromisoval



Isovalerilurea



Fig. 1: The ureid acyclic pharmacophoric moiety in urea derivatives

MATERIALS AND METHODS

1. Synthesis of *N*-benzoylphenylurea active compounds and derivatives

Bambang Tri Purwanto [2], had successfully synthesis to make ampicillin derivative, namely *para*-bromobenzoylampicillin using Schotten - Baumann method , by reacting the ampicillin with *para*-bromobenzoylchloride compound .

Siswandono [14] , also using the method of Schotten Baumann to do reaction of urea compounds with benzoyl chloride derivatives in order to produce new compounds *N*-benzoylurea .

In the process of synthesis of *N*-benzoylphenylurea derivatives, the Schoten Baumann method is the elected method, by reacting a compound of *N*-phenylurea with benzoyl chloride derivatives (4-tercierbutylbenzoyl chloride, 2,4-dichlorobenzoyl chloride, *p*-toluolbenzoyl chloride) with the same concentration (0,01 mol) by using the solvent tetrahydrofuran . Mixing the reaction is carried out at a cool temperature for 60 minutes, after that the mix compounds was refluxed for 7 hours. Then separated and added to a saturated solution of sodium bicarbonate to form crystals [8] .

2. The purity test of the derivatives *N*-benzoylphenylurea

a. Thin Layer Chromatography To determine the purity of the compounds synthesized performed by thin layer chroma- tography using 2 different solvents [17]

b. Melting Point Determination The purity of the compounds synthesized can be determined from its melting point by Melting Point Apparatus [17].

3. The structure characterization of compounds synthesized

Identification of the molecular structure of compounds synthesized instrumentation is done by using a UV spectrophotometer, IR, ¹H-NMR and mass spectrometry [9;11].

4. The suppressants central nervous system activity test of the compounds

The test method suppressor activity of central nervous system is Barbituric Sleeping Time (BST), because this method is a standard method of testing activity in the central nervous system suppressant. BST method consists of two steps, first to measure the timing of peak activity of the test compound, which is the longest sleep of animal mice, then the second was the potentiation of the test compound at the time of peak activity after giving thiopental as inducer [16;18;1].

Experimental animals used were mice (*Mus musculus*) aged 2-3 months, weighing between 20-30g, Blab C strain, male, without physical disabilities, acquired from laboratory animals Airlangga University.

In the suppressing test activity of central nervous system, the compound derivatives of the *N*-benzoilfenilurea first steps was the determination of the peak activity time starts from minutes to 15, 30, 45, 60, 75, 90 and 120 with a single dose. The next steps was the determination of potentiation test using 5 different doses (10, 25, 50, 100 and 200 mg / kgBW) were administered intra-peritoneal (ip). As reference compound was used bromisoval compound (one of the urea derivative that have been used in practice by clinicians)

with the same dosage, whereas the inducer compound used thiopental compounds. Replication to test the activity of the central nervous system suppressant is performed 10 times.

RESULT AND DISCUSSION

1. Synthesis and structure identification of the active compounds and derivatives *N* - benzoilfenilurea

The results of the synthesis of compounds such as *N*-benzoilfenilurea white needle-shaped crystals with a yield of 86 %, the compound 4-tercierbutylbenzoilfenilurea be shaped white sugar crystals with a yield of 60%, the compound 2, 4-diklorobenzoilfenilurea cream-colored crystals with a yield of 81 %, whereas compound *p*-toluolbenzoilfenilurea a white powder with a yield of 65 %. Based on the yield of the synthesis, suggesting that the method of Schotten - Bauman has been chosen as the elected method of synthesis and deserves the right to perform the synthesis of the active compound *N*-benzoylphenylurea and derivatives.

The method for the purity test of the compounds was thin-layer chromatography (TLC) by using 2 different solvents (n-hexane : acetone = 4 : 2 and n-hexane : ethyl acetate = 4 : 2) . The TLC results from *N* - benzoylphenylurea compounds and derivatives provide a single spot with different Rf and it had different Rf too if compared by the base compound *N*-phenylurea. The TLC results above showed that the compounds synthesized in the form of *N*-benzoilfenilurea compounds and derivatives desired been formed and relatively pure and also different from the base compound.

At the melting point analysis of the compounds was synthesized *N*-benzoylphenylurea has a melting point (195°C), 4-tercierbutylbenzoylphenylurea (171°C), 2,4-dichlorobenzoylphenyl-urea (154°C) and *p*-toluolbenzoylphenylurea (151°C), which was different from the melting point the base compound *N*-Phenylurea (145°C).

At the melting point analysis has been proven that the compounds was synthesized *N*-benzoylphenylurea and its derivatives have been formed and has a relatively high purity because there were no other impurities in it.

On the characterization of the structure, the compounds synthesized *N*-benzoylphenylurea, UV (methanol), λmaks (nm) = 204, 232, 272 (sh), IR (KBr pellet), 3240 cm⁻¹ (secondary NH) , 1698 cm⁻¹ (- CO), 1600 cm⁻¹ (C = C arom); ¹H-NMR (DMSO - d₆ solvent), 7.00 to 8.10, m, (C₆H₅), 10.60, s, (NH), 11.20, s, (NH), MS (EI), 240(M)⁺, 93 (C₆H₅NH)⁺, 137 (C₆H₅NHCONH₂)⁺

The Compound, 4-tercierbutylbenzoylphenylurea, UV (methanol), λmaks (nm) = 236; IR (KBr pellet), 3467 cm⁻¹ (secondary NH), 2965 cm⁻¹ (CH alkane), 1685 cm⁻¹ (CO), 1610 cm⁻¹ (C = C aromatic); ¹H-NMR (DMSO - d₆ solvent, 7.00 to 8.20, m, (C₆H₅); 10.20, s, (NH) , 10.80, s, (NH, 1.0 to 1.6, m, (C (CH₃) , MS (EI), 246 (M)⁺; 163 (C (CH₃)C₆H₅COH)⁺ The compound, 2,4-dichlorobenzoylphenylurea, UV (methanol), λmaks nm) = 234; IR (KBr pellet) , 3468 cm⁻¹ (NH secondary) ; 1697 cm⁻¹ (CO), 1552 and 1581 cm⁻¹ (C = C aromatic) ; ¹H-NMR (DMSO - d₆ solvent), 7.00 to 8.40, m, (C₆H₆); 12.00, s, (NH), 13.00, s, (NH); MS (EI) , 310 (M)⁺; 240 (N(C₆H₅) N'COC₆H₅)⁺; 180 (C₆H₅NHCONHCOOH)⁺

The compound, *p*-toluolbenzoylphenylurea, UV (methanol), λmaks (nm) = 236, IR (KBr pellet) , 3427 cm⁻¹ (NH secondary); 1694 cm⁻¹

(CO), 1611 cm^{-1} (CO), 1556 and 1498 cm^{-1} (C = C aromatic); $^1\text{H-NMR}$ (DMSO- d_6 solvent), 6.80 to 8.80, m, (C_6H_5), 6.0, s, (NH); 9.40, s, (NH), 3.80 to 4.00 (CH_3), MS (EI), 254 (M^+)

The compound, *N*-phenylurea, UV, λ_{maks} (nm) = 204, 238; IR (pellet KBR), 3428 cm^{-1} (NH Primary), 1655 cm^{-1} (CO), 1553 cm^{-1} (C = C aromatic); $^1\text{H-NMR}$ (solvent DMSO- d_6), 6.80 to 8.00, m, (C_6H_5), 5.60, s, (NH), 6.20, s, (NH), 8.60, s, (NH_2).

On the characterization of the compounds structure with a variety of spectrophotometer instruments showed that structure compounds synthesized *N*-benzoylphenylurea and its derivatives have been different from the base compound *N*-phenylurea, especially on the number of hydrogen atoms contained in the compounds synthesized *N*-benzoylphenylurea and its derivatives with *N*-phenylurea ($^1\text{H-NMR}$) and the presence of 2 peaks of the

carbonyl group from the compounds synthesized *N*-benzoylphenylurea and derivatives (IR).

On the characterization of the structure with mass spectrometry showed that the compounds synthesized *N*-benzoyl-phenylurea and derivatives was already different from the base compounds *N*-phenyl urea especially looked from the molecular weight of each compound have been synthesized, and structural characterization results for all compounds synthesized *N*-benzoylphenylurea and its derivatives have been accordance with that shown by the reference [9;11], so based on the characterization structure from the compounds had been synthesized could be performed, namely *N*-benzoylphenylurea, 4-tercierbutylbenzoylphenilurea, 2,4-dichlorobenzoylphenylurea and *p*-toluolbenzoylphenylurea compounds. The structure of all compounds which were been synthesized can be seen on Figure 4. following

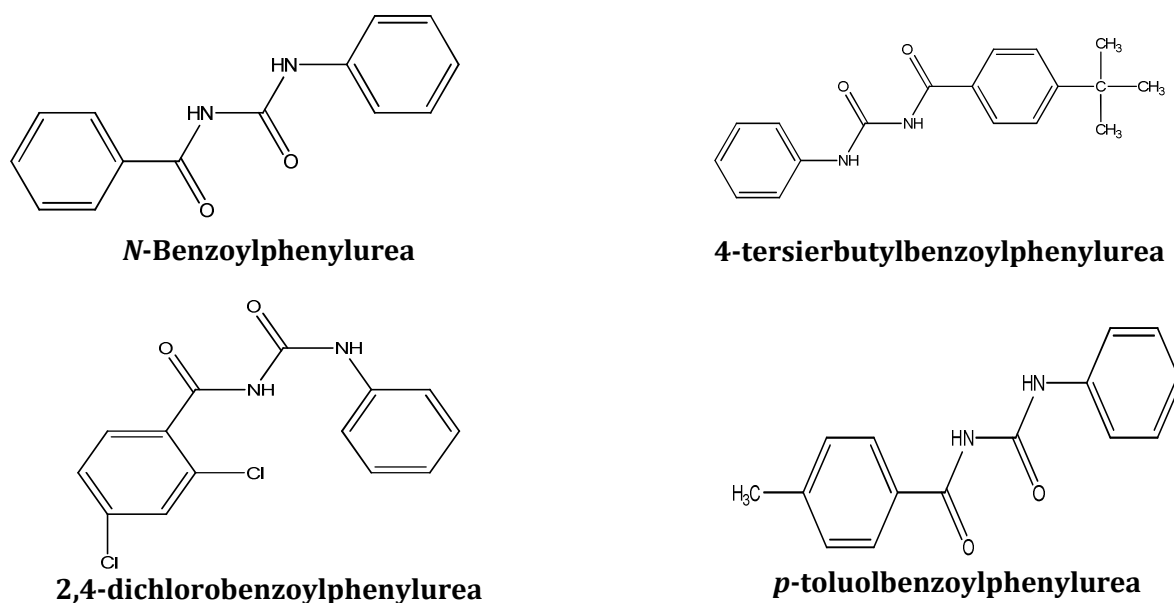


Fig. 4: Compounds synthesized *N*-benzoylphenylurea and derivatives

2. The suppressor activity test of the central nervous system

Test suppressor activity of central nervous system was done by using Barbituric Sleeping Time (BST) method. It has 2 steps, first the test suppressor activity of central nervous system looked for the highest time peak activity from the active substances, the

compounds synthesized have time peak activity at 30 min to mice with prolonged sleep the longest, being the peak activity times for reference compounds bromisoval 60 minute sleep mice showed the longest time. To test the potentiation of thiopental inducer compound, administered intra-peritoneal with 5 different doses can be seen in Figure 5 below

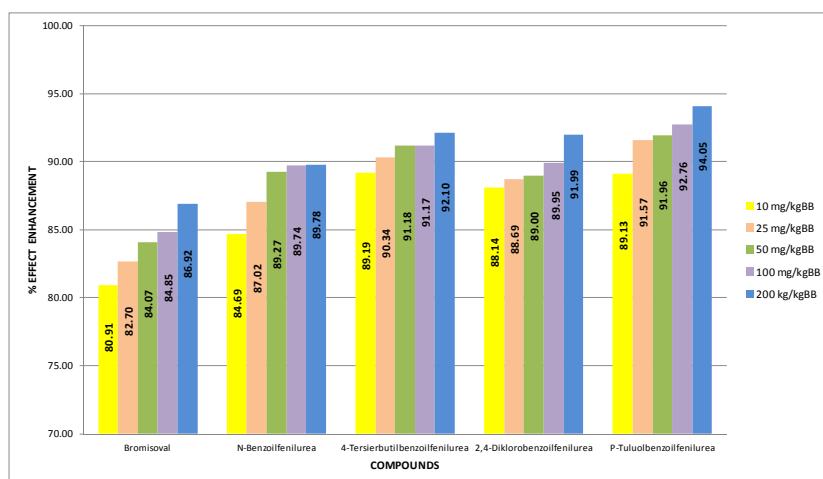


Fig. 5: % Effect enhancement of suppressive of the central nervous system from *N*-benzoylphenylurea and derivatives

Based on Figure 5 shows that the activity of all the compound *N*-benzoylphenylurea and its derivatives have suppressor activity of central nervous system higher than reference compounds bromisoval at the same dose, it is due to the addition of benzoyl group led to compound becomes more non polar nature so it is easy to penetration into the membrane biological.

Based on these results, *N*-benzoylphenylurea and its derivatives can be developed into a new drug candidate which have suppressing activity in the central nervous system.

CONCLUSION

All compounds which have been synthesized, *N*-benzoylphenylurea and its derivatives has a nervous system suppressive activity higher than bromisoval, and *p*-toluolbenzoylphenylurea is the highest suppressive compound in the central nervous system compared with other derivatives of *N*-benzoylphenylurea.

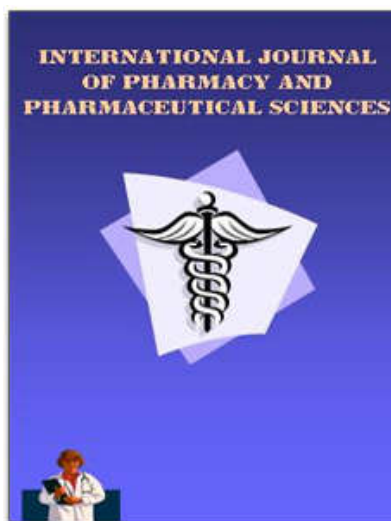
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