DESIGN, SYNTHESIS AND CENTRAL NERVOUS SYSTEM DEPRESSANT ACTIVITY TEST OF THE NEW COMPOUND N'-PHENYL-N-BENZOYLUREA

Bambang Tri Purwanto
Pharmaceutical Chemistry Departement, Faculty of Pharmacy Airlangga University, Surabaya, Indonesia

ABSTRACT
Design and synthesis the new compound N’phenyl-N-benzoylurea has been done. N’phenyl-N-benzoylurea was designed by In Silico by Molegro Virtual Docker Program to predict its activity as the central nervous system depressant. The Schotten Baumann method was used for the reaction between N-phenylurea and benzoyl chloride to produced N’-phenyl-N-benzoylurea. The CNS depressant activity test was done for the N’phenyl-N-benzoylurea compound and compared with bromisoval as the standard reference by Barbituric Sleeping Time (BST) method. The compound N’-phenyl-N-benzoylurea bound as well as the standard reference bromisoval with the protein Pro 85, In Silico method. The compound yield was 86% , had a white crystal and gave one spot in Thin Layer Chromatography with two different eluent. The compound melting point was different from the N-phenylurea and showed greater. The structure identification from the new compound was analysed by UV, IR, HNMR and MS, the result showed that the new compound was N’phenyl-N-benzoylurea. The CNS depressant activity test from N’phenyl-N-benzoylurea had a greater activity if compare with the reference compound bromisoval.

Keywords: Design synthesize by In Silico; N’phenyl-N-benzoylurea; CNS depressant activity test.

INTRODUCTION
The active moiety compound that has pharmacological activity better than the parent compound needs to be development, because it will be used as a new drug candidate.
materials to support public health. The chemical synthesis is the one of the process to obtain a new compound that would potentially as a new drug candidate, and the new expectations that the new compound has relatively pure and gives higher pharmacological activity than the parent compound 4,8. The development of compounds active moeity can be done through a variety of methods (primarily through the synthesis reaction), the first stages of the development of the active moeity compound are doing molecular structure design through a method of 'in silico' followed by a synthesis method that is appropriate for obtaining the active moeity compound 4,8. Urea only known as the active compound which is used as fertilizer for crops, and can already be in production by industry in Indonesia, and on the next development by using urea as raw material will produce the active ingredient compounds that have diverse pharmacological activities, as the central nervous system depressant and as an anti cancer 6. Some of the urea derivatives that has been successfully created and have pharmacological activity as a depressant of the central nervous system are isovalerilurea which has successfully made by Reksohadipodojo 11, bromasilurea was made by Tjipta Sura 16, benzoyleurea was made by Siswando 14, benzoylthiourea created by Suzanna 15, and benzoylthiourea derivatives who have successfully made by Dini 5.

The central nervous system depressant agents have the activity because the compounds have the pharmacophoric moeities named as ureida acyclic moeity, and also owned by the group of compounds nervous system depressant drugs known as barbiturate acid 7,13. The structure of the active compound that has pharmacophoric ureida acyclic moeities can be seen in Figure

![Chemical structures](image)

Fig 1: The CNS depressant with the ureide acyclic pharmacophore
Further development of active compounds are one of the urea derivatives which will be synthesized is N’phenyl-N-benzoylurea, which also has pharmacophoric ureida acyclic moiety and it is expected has the pharmacological activity as the central nervous system depressant too by theoretically modeling or designing with the virtual Docker Molegro program.

Through a search of the Protein Data Bank (PDB) can be known that one of the receptors of the central nervous system depressant agents is 2R2Q (Gabarap1)\(^{20}\). To illustrate the bonding compatibility between multiple active compounds of urea derivatives with receptor 2R2Q then used Virtual Docker Molegro program version 5.5 which will then rerank scores and grades obtained his mol.doc.score. By knowing these values, it can be done determining the feasibility of the synthesis of the compound N-phenylurea as urea derivatives with benzoylchloride compound that will produce the compound N’phenyl-N-benzooylurea.

MATERIALS AND METHODS

The design of compounds N’phenyl-N-benzooylurea

To perform design compound N’phenyl-N-benzooylurea early stages done first depiction of the molecular structure Chem.Draw program version 11.1, the next stage of making the design of compounds with a 3-dimensional model designs and find the form of the most stable compounds with Chem.Bio.Draw program 3D. version 11.1 Further search for the appropriate receptor structure using the Program Data Bank (PDB), then do the merging process (alignment) between the active compound N’phenyl-N-benzooylurea with appropriate receptors. 3-dimen-sional depiction of harmony between the active compound with the receptor N’phenyl-N-benzooylurea required to determine the presence of active compounds N’phenyl-N-benzooylurea in the room (cavity) as appropriate. The next stage is to verify bonding the active compound N’-phenyl-N-benzooylurea with several proteins associated with the observed hydrogen bonding. Then the final stage of the docking process is carried out using the program Molegro version 5.5, to obtain the value rerank score and mol.doc.score.

Synthesis of the active compound N’phenyl-N-benzooylurea

Bambang Tri Purwanto\(^2\), using Schotten - Baumann method, has synthesized to make ampicillin derivatives, namely p-bromobenzoylampicillin, by reacting the compound of ampicillin with p-bromobenzoylchloride.
Siswandono, also using the method of Schotten Baumann reaction of urea compounds with benzoylchloride derivatives in order to obtain new compounds N-benzyolurea. In the process of synthesis of N’phenyl-N-benzyolurea had been done with the elected Schoten Baumann method by reacting a compound of N-phenylurea with benzoylchloride in the same amount (0.01 mol) using tetrahydrofuran as the reaction solvent. Mixing the reaction is carried out at a cool temperature for 60 minutes, then the mixture of the compounds was refluxed for 7 hours. After that process the mixtures of the compounds was separated and added to a saturated solution of sodium bicarbonate to form crystals.

3. Test the purity of the compound N’phenyl-N-benzyolurea
   a. Thin Layer Chromatography
      To determine the purity of the compounds synthesized performed by thin layer chromatography using 2 different solvents.

   b. Melting Point Determination
      The purity of the compounds synthesized can be determined from its melting point. Compounds synthesized are determined by means of melting point apparatus.

4. Structure Identification of compounds synthesized
   Identification of the molecular structure of the compounds have been synthesized using UV spectrophotometer, IR, ¹HNMR and mass spectrometry.

5. The depressant activity test of the central nervous system
   The method of the depressant activity test of central nervous system was Barbituric Sleeping Time (BST), because this method is a standard method of testing activity in the central nervous system depressant. BST method consists of two stages, first is the highest timing of peak activity of the compound, which is the longest sleep of the mice, then the second is the potentiation of the compound at the highest time of peak activity by giving compound thiopental induction. Experimental animals used were white mice (Mus musculus) aged 2-3 months, weighing between 20-30 grams, without physical disabilities, acquired from the animals laboratory Airlangga University.

   In the depressant test activity of central nervous system the compound N’phenyl-N-benzyolurea first stage is the determination of the peak activity time starts from 15, 30, 45, 60, 75, 90 and 120 minutes with a single dose. The next stage is the determination of potentiation test using 5 different doses (10, 25, 50, 100 and 200 mg / kgBW) and the
N’phenyl-N-benzoylurea were administered by intra-peritoneal. As reference compounds used bromisoval (the urea derivative compounds that have been used in practice by clinicians) with the same dosage, whereas the inducer compound used thiopental compounds. Replication of the test is performed in 10 times.

RESULTS AND DISCUSSION

1. Design of active compounds N’phenyl-N-benzoylurea by In Silico method

The result of the design of the active compound N’phenyl-N-benzoylurea and was compared with bromisoval which were bound to amino acids in the receptor 2R2Q can be seen in Figure 2 and 3 below. The table below showing the types of amino acids that are bound by N’phenyl-N-benzoylurea as the active compound and reference compound bromisoval, also rerank score and mol.doc.score value.

![Figure 2](image1.png)

**Fig 2**: The N’phenyl-N-benzoylurea was bound with Pro 86 amino acid.

![Figure 3](image2.png)

**Fig 3**: The reference compound bromisoval was bound with Pro85 amino acid.

Table 1: The Amino acids and the value of mol.doc.score rerank score as well as the value of the active compound N’phenyl-N-benzoylurea and bromisoval as the reference
Bromisoval is the compound and one of urea derivatives which has a central nervous system depressant activity and used by the clinicians, thus fit for use as reference compound against depressant activity of the central nervous system active compounds N’phenyl-N-benzoyleurea. In figure 3 and table 1 shows that the bromisoval was bound to the Pro 85 amino acid as well as the active compound N’phenyl-N-benzoyleurea bound by the same amino acid. That were indicating that the active compound N’phenyl-N-benzoyleurea theoretically would have a central nervous system depressant activity as well as indicated by the reference compound bromisoval. When it was viewed from the rerank score and mol.doc.score values are presented in Table 1 it can be said that the active compound N’phenyl-N-benzoyleurea will have depressant activity of the central nervous system is higher than the reference compound bromisoval theoretically, because the value indicate that the active compound N’phenyl-N-benzoyleurea smaller than the comparator compounds. N’-phenyl-N-benzoyleurea and bromisoval was bound with the same protein (Pro 85), it means that the compounds have the same pattern for bound with the protein and the molecular changed not influenced to the aligment with the protein.

In addition, other researcher Lipinski have analyzed 2,245 drug and concluded that the compound would be difficult to be absorbed and low permeability when:
1. Has a molecular weight larger than 500.
2. Has a value of log partition coefficient octanol / water (log P) greater than 5.
3. Has H-bond donors, which is expressed by the number of OH and NH groups, greater than 5.
4. Have-H bond acceptors, which is expressed by the number of atoms O and N, greater than 10.

The above analysis is known as the law of five of Lipinski, since all values are multiples of five. So, based on the analysis of In Silico method, and based on the Lipinsky rule of the active compounds N’phenyl-N-benzoyleurea has Molecular Weight 240, log P of 2.46, H-
bond donor 2, H-bond acceptor 2. the active compound N’phenyl-N-benzoylelurea feasible for synthesis.

**Synthesis and structure identification of the active compounds N’phenyl-N-benzoylelurea**

Synthesis results in the form of white needle-shaped crystals with a yield of 86% , it shows that the method of Schotten - Bauman was the elected method of the synthesis process to produced the N’phenyl-N-benzoylelurea compound. Identification of the structure of the active compound N’phenyl-N-benzoylelurea can be seen in the following analysis . In the next stage test thin-layer chromatography on compounds synthesized by using 2 different solvents ( n-hexane : acetone = 4 : 2 and n-hexane : ethyl acetate = 4 : 2 ) gave a single spot with different Rf compound with the N-phenylelurea as the parent compound . The above shows that the desired compounds synthesized have been formed and relatively pure compounds also have different from the parent compound . At the melting point analysis of test compound that had been synthesized had a melting point ( 195°C ) and had a different with the parent compound which had the melting point ( 145°C ) . In this test has proven that the compound which was synthesized have been formed and has a relative purity because there were no other impurities in it.

Compound was synthesized , \( \lambda_{\text{maks}} \) (nm) = 204 , 232 , 272 (sh), IR (KBr pellet) , 3240 cm\(^{-1}\) ( secondary NH ) , 1698 cm\(^{-1}\) (- CO) , 1600 cm\(^{-1}\) (C = C arom) ; \(^1\)HNMR (solvent DMSO-d6) , 7.00 to 8.10 , m , (C\(_6\)H\(_5\)) ; 10.60, s , (NH) , 11.20, s , (NH) , MS (EI) , 240 (M\(^+\)) , 93 (C\(_6\)H\(_5\)NH\(^+\)) , 137 (C\(_6\)H\(_5\)NHCONH\(_2\)) \(^+\) The parent compound N-phenylelurea , \( \lambda_{\text{maks}} \) (nm) = 204 , 238 ; IR (KBr pellet) , 3428 cm\(^{-1}\) ( NH Primary) , 1655 cm\(^{-1}\) (CO) , 1553 cm\(^{-1}\) (C = C aromatic) ; \(^1\)HNMR (DMSO-solvent d6) , 6.80 to 8.00 , m , (C\(_6\)H\(_5\)) , 5.60, s , (NH) , 6.20 , s , (NH) , 8.60 , s , (NH\(_2\)) . On the characterization of the structure with a variety the spectrophotometer instrument, indicated that compound was synthesized have different structure with the parent compound, especially on the number of hydrogen atoms contained in the parent compound ( \(^1\)HNMR ) and the presence of 2 peaks from the compound was synthesized carbonyl group ( IR ) . On the characterization of the structure with mass spectrometry showed that the compounds synthesized are intact compound with a molecular weight of 240 and the results of the characterization of these structures are in accordance with that shown by the reference\(^ {10;12} \) , so it can be ascertained based on the characterization of the structure of the compound that had been synthesized have formed .
3. The depressant activity test of the central nervous system from the active N’phenyl-N-benzoylurea

The depressant activity of central nervous system were done by using Barbituric Sleeping Time (BST). The depressant activity test of central nervous system in the early stages, the compound was synthesized have a highest time peak activity at 30 min on mice with prolonged sleep the longest, being the peak activity times for reference compound bromisoval 60 minute sleep mice showed the longest time. To test the potentiation of thiopental inducer compound, administered by intraperitoneal with 5 different doses can be seen in Figure 4 below.

Fig 4 : % effect enhancement of depressant activity from N’phenyl-N-benzoylurea and Bromisoval compounds.

Based on Figure 4 showed that the activity of the compound N’phenyl-N-benzoylurea have the depressant activity of the central nervous system is higher than the reference compound 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 very easy to penetration into the membrane biological. Based on these results demonstrate that the synthesis of compounds obtained can be developed into a new drug candidate compounds depressing the central nervous system.

CONCLUSION

The compound which was designed by In Silico method and was synthesized was N’phenyl-N-benzoylurea has been formed and has a nervous system depressant activity higher than bromisoval.

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