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Original Article

Synthesis and activity evaluation of a novel lead compound 1-benzyl-3-benzoylurea as antiproliferative agent

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ABSTRACT

This research deals with designing and synthesizing a novel lead compound 1-benzyl-3-benzoylurea using the modified Schotten Baumann method and the objective is to find a more potent drug.In order to achieve the goal , the in-silico activity againts 1-UWH was calculated by Molegro Virtual Docker. Then, in-vitro antiproliferative activity against MCF-7 cell line was tested by MTT assay and compared with Hydroxyurea as a reference compound. The result showed that both in-silico and in-vitro test of 1-benzyl-3-benzoylurea were more potent than Hydroxyurea. It is highly recommended that 1-benzyl-3-benzoylurea be developed further as an antiproliferative agent.

Keywords: 1-benzyl-3-benzoylurea, designing, synthesizing, antiproliferative activity

INTRODUCTION

According to WHO data (2013), there is an increasing on cancer incidences from 12.7 millions cases to 14.1 millions between 2008 – 2012. On the other side mortality rate increase from 7.6 millions in 2008 to 8.2 millions in 2012. Cancer is a second major leading cause of death (13 %) in the world. Indonesian Fundamental Health Research (Rikesdas, 2013) reported that prevalence on cancer diseases in Indonesia is 1.4 of every 1000 citizens. Indonesian Hospital Information System statedthat breast cancer (28,7 %) has ranked at the first place. Based on Globocan estimation, International Agency for Research on Cancer (IARC) year 2012, incidents on breast cancer were 40 per 100.000 women. It was predicted at the year 2030 , 26 millions people will suffer from cancer and 17 millions will die , especially from the developing country [14]. Many efforts still constitutes a major challenge to develop in medicinal chemistry field. In this field of study, designing a novel lead compound is the first priority in order to find another alternative for theurapeutic goals. Hydroxyurea has been established and used for many decades and is still valued to cure cancer[1]. Chhttisgark (2011) reported that IC_{50}Hydroxyurea on breast cancer is 307,15 mc M. Research on urea derivative compounds has been done based on mechanism of action, kind of cell lines, receptor and activity test methods. Antiproliferative activity test using MTT assay was reported by Li et al. (2009), Lokhwani et al. (2011) and El-Shawy et al. (2012). Related to many prior research on urea derivates, urea is a pharmacophore for anticancer activity. More over, Lokhwani et al. (2011) and Lu et al. (2013) underlined that benzylurea is the key pharmacophore to inhibit tumor cells. El-Shawy et al. (2012) stated that benzyl moeity keep a role play as antiproliferative on MCF-7 cell line. In the present study , the novel lead compound was designed and synthesized as urea derivative compound. Increasing liphophilicity aspect are expected to better penetration through biological membran in order to find more potent drug candidate. 1-benzyl-3-benzoylurea was designed and synthesized using the Schotten Baumann methods[2,8]. 1-benzyl-3-benzoylurea is more liphophyl compared to hydroxyurea. In-silico activity on 1-UWH was calculated by Molegro Virtual Docker[15]. In vitro antiproliferative activity againts MCF-7 cell line was tested by MTT assay and compared with Hydroxyurea as a reference compound. The novel lead compound is expected to have more potent antiproliferative activity on MCF-7 cell line compared with Hydroxyurea as a reference compound.

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MATERIALS AND METHODS

General: The novel lead compound was synthesized using Schotten Baumann method[2,8] with modification. Triethylamine substituted Sodium Hydroxide to avoid side reaction in synthesis. Benzylurea p.s (Merck) as a starting meterial is reacted with Benzoyl chloride p.s (Merck) based on nucleophylic substitution reaction. Purity test was performed using TLC(Thin Layer Chromatography) and Fisher John Melting Point Apparatus. NMR (Nuclear Magnetic Resonance) spectroscopy was performed using AGILENT 500 MHz spectrometer. HRMS (High Resolution Mass Spectra) was determined using ESI-TOF MS (Electron Spray Ionization - Time of Flight Mass Spectrometer) Waters LCT Premier XE with direct injection . In- silico activity againts 1-UWH was calculated by Molegro Virtual Docker. In-vitro antiproliferative activity against MCF-7 cell line was tested by MTT assay and compared with Hydroxyurea as a reference compound. The absobance at 595 nm was recorded using ELISA MICROPLATE READER[13]. The IC_{50} values were calculated with probit analysis using IBM SPSS 20.

Synthesis of 1-benzyl-3-benzoylurea[2,8,10]: First, dissolve benzylurea 0,025 mol in 20 ml THF (Tetrahidrofuran) and 4 ml TEA (Threethylamine) into a 200 ml conical flask, pour the solution under temperature 0 – 5 ° C and run in drop wise benzoyl chloride 0,0275 mol in 20 ml THF from separating funnel . Then continue to stir the mixture for 30 minutes. Next reflux the mixture at temperature 70 ° C for 6- 8 hours then evaporatethe mixture under reduced pressure using rotavapor. After that add saturated sodium bicarbonate to the residu , filter the solid product on a buchner funnel and wash it with a little cold water. Finally, recrystallize it with hot ethanol, filter off in hot condition, allow to stand by night, filter off the crystals and dry them upon filter paper at temperature 50 ° C.

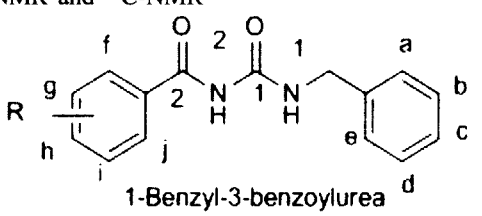
Identification of 1-benzyl-3-benzoylurea[9]: Purity test was performed using TLC(Thin Layer Chromatography with solvent ethyl acetat : chloroform 7 : 3) on pre-coated silica gel 60 F-254 layer thickness 0,2mm, Merck and Fisher John Melting Point Apparatus. ^{1}H-NMR and ^{13}C-NMR (Nuclear Magnetic Resonance) spectroscopy were performed using AGILENT 500 MHz spectrometer in $CDCl_3$(δ 77,0) with TMS (Tetramethylsilane) as an internal standard (δ 0,00) . HRMS (High Resolution Mass Spectra) was determined using ESI-TOF MS (Electron Spray Ionization - Time of Flight Mass Spectrometer) Waters LCT Premier XE with direct injection .

In-silico activity againts 1-UWH was calculated by Molegro Virtual Docker [15]

In-vitro antiproliferative activity against MCF-7 cell line was tested by MTT assay[5,7, 12, 13] and compared with Hydroxyurea as a reference compound. Cells were seeded into 96- well plates with 1X 10^4 cells in 100 µL per well, followed by treatment with test compound at concentration between 250 – 7,8125 µg/ml for 72 hours at 37 °C. Cell viability was assessed with the 3-(4,5-dimethylthyazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT).The absobance at 595 nm was recorded using ELISA MICROPLATE READER. The IC_{50}value was defined as the dug concentration required to inhibit 50 % of cells proliferation after 72 hours of treatment in comparison with untreated controls. Each experiment was repeated three times under identical conditions. The IC_{50} values was calculated with probit analysis using IBM SPSS 20 and was conversed into µM.

RESULT AND DISCUSSION

1-benzyl-3-benzoylurea: Using the previous procedure , yield : 36.09 %, white needle, Rf 0.88; mp 78 – 78.5 °C . **^{1}H NMR** : δ 7.96 ppm (d, 2 H, J=8Hz) , δ 7.43 ppm (t, 2 H, J=7.5 Hz) , δ 7.56 ppm (t, 1 H, J= 7.5 Hz), δ 7.35 - 7.27 ppm (m, 5H), δ 9.71 ppm (s,1H,br), δ 9.17 ppm (s,1H,br), δ 4.57 ppm, (d, 2H, J= 5.5 Hz). **^{13}C NMR :**δ 138.1 ppm, 133.1 ppm, 132.2 ppm, 128.7 ppm, 128.7 ppm, 127.8 ppm, 127.5 ppm, 127.4 ppm (C_6H_5) ; δ 154.6 ppm (C=O) , δ 168.3 ppm (C=O), δ 43.8 ppm (CH_2). **IR (KBr)** :υ 1600,1537,1428 cm^{-1}(C=C) , υ 3086,3063,3032,2948 cm^{-1}(=C-H), υ 3349 cm^{-1}(NH), υ 3304 cm^{-1}(NH), υ 1692 cm^{-1} (C=O), 1666 cm^{-1} (C=O), υ 1469 cm^{-1}(CH_2). **HRMS** : calcd for $C_{15}H_{14}N_2O_2$[M + H]$^+$ = 255.1128, [M + H]$^+$found = 255.1124



1-Benzyl-3-benzoylurea

Figure 1 : The structure of 1-benzyl-3-benzoylurea (R = H)

RS(Rerank Score): Hydroxyurea –23.553 , 1-benzyl-3-benzoylurea – 90.5615
IC_{50}(μM) : Hydroxyurea 1228.96, 1-benzyl-3-benzoylurea 384.87

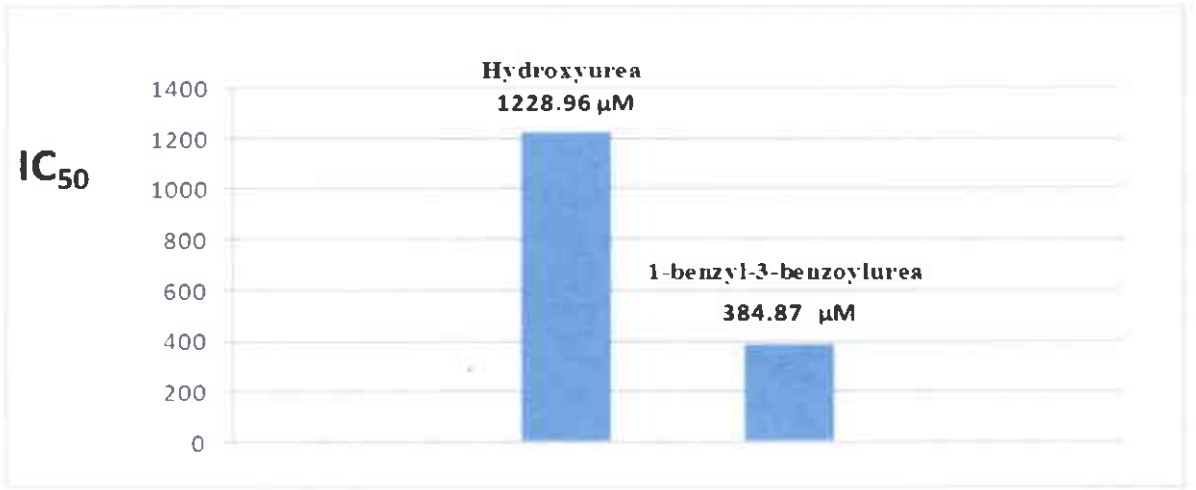


Figure 2 : Anti proliferative activity of Hydroxyurea and1-benzyl-3-benzoylurea

The yield of synthesis showed single spot which Rf is different from a starting material on TLC and also a narrow range of melting point. These indicatethat the Scotten Baumann method is still valued and valid in the synthesis of a novel compound.Modification in synthesis by presenting Triethylamine as a base is supposed to avoid side reaction product that could become a problem in purifying the target compound. Structure identification spectra supported its statement. To prove phenyl group , ^{1}H NMR showed chemical shift signal between δ 7.96 ppm -7.27 ppm, supported with^{13}C-NMR ($CDCL_3$)δ 138.1 ppm - 127.4 ppm and IR (KBr) spectra υ1600,1537,1428 cm^{-1}(C=C aromatic) ; υ 3086,3063,3032,2948 cm^{-1}(=C-H aromatic)

To prove amine group, ^{1}H-NMR ($CDCl_3$) showed chemical shift signal at δ 9.71 ppm NH_2 and δ 9.17 ppm NH 1,supported with IR (KBr) spectra υ 3349 cm^{-1}NH 1,υ 3304cm^{-1}NH To prove carbonyl group^{13}C-NMR (($CDCL_3$)showed chemical shift signal at δ 154.6 ppm C=O 1; δ 168.3ppm C=O 2, supported withIR (KBr) spectra υ 1692 cm^{-1}C=O 1, 1666 cm^{-1}C=O 2.To prove etylen group^1 H-NMR showed chemical shift signal at δ 4.57 ppm supported with^{13}C-NMR ($CDCL_3$) δ 43.8 ppm and IR (KBr) spectra υ 1469 cm^{-1}. From the spectra, it appears signal at downfield area cause of electronegativity environment. These all spectras are strenghtened with HRMS (ESI-TOF) m/z analysis data :[M + H]$^+$Calcd = 255.1128, [M + H]$^+$found = 255.1124 concluded that a novel compound is 1-benzyl-3-benzoylurea.RS(Rerank Score):Hydroxyurea –23.553 , 1-benzyl-3-benzoylurea – 90.5615.IC_{50} (μM) :Hydroxyurea 1228.96 , 1-benzyl-3-benzoylurea 384.87. More negative RS value indicates more stable drug – receptor interaction that means activity will increasae. In-silico data seems in line with in-vitro data which shows that 1-benzyl-3-benzoylurea more potent than Hydroxyurea. It is also in line with the theory that lipophilicity will increase biological membran penetration and the final result is that pharmacological activity will increase[11]. Based on the result 1-benzyl-3-benzoylurea can be developed in the future as a novel anti proliferativelead compound .

CONCLUSION

The 1-benzyl-3-benzoylurea which has been designed and synthesized has a higher activity against MCF-7 cell line as anti proliferative compared with the established drug Hydroxyurea.

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