Original Article

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Docking, synthesis, and cytotoxic activity of *N*-4-methoxybenzoyl-*N'*-(4-fluorophenyl)thiourea on HeLa cell line

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ABSTRACT

Introduction: Thiourea derivatives such as phenylthiourea, benzoylthiourea, tenovin, and much more have been reported as anticancer agents. However, the discovery of new anti-cancer compound is still a challenge. **Objective:** To obtain another promising anti-cancer compound, we have done some initial study (docking, synthesis, and cytotoxic assay) of thiourea derivatives, *N*-4-methoxybenzoyl-*N'*-(4-fluorophenyl)thiourea. **Methods:** First, it has been docked in the SirT1 receptor with PDB ID: 4I5I using Molegro Virtual Docker v5.5. Then, synthesized by two-step reaction using ammonium thiocyanate, 4-methoxybenzoyl chloride, and 4-fluoroaniline, respectively, as precursors. The structure of the desired compound obtained by ultraviolet, infrared,1H-nuclear magnetic resonance (NMR), 13C-NMR, and mass spectrometer. Last, the new compound was screened for the *in vitro* cytotoxic activity against HeLa cell line by MTT Method. **Result:** The docking result showed that its re-rank score was lower than hydroxyurea (HU), which predicted its higher biological activity. It corresponded with MTT method result that *N*-4-methoxybenzoyl-*N'*-(4-fluorophenyl)thiourea has lower inhibitory concentration 50% more potent than HU as a reference. **Conclusion:** Thus, it can be developed for the next step of anticancer drug discovery.

INTRODUCTION

Cancer drug development has still become an important task for researchers because of the continuously increasing number of cancer patients. It was predicted that 14 million new cases of cancer will threat the world from 2012 to 2022 and lead the dead cause [1].

Thiourea derivatives have already known as an anticancer agent [2-4]. It was investigated that modifications of phenyl group of phenylthiourea by adding electron withdrawing groups such as *p*-nitro, *p*-trifluoromethyl, *p*-cyano, and *p*-fluoro increased cytotoxic activity [5]. The other study exposed that adding benzoyl moiety to thiourea increased its activity by increasing lipophilic property [6,7]. Lipophilic is one of the physicochemical properties in Quantitative Structure-Cytotoxic Activity Relationship (QSAR). It has a role in drug penetration through cell membrane, so increasing the amount of drug into receptor which increases its activity. The others are electronic which has the role in drug solubility at distribution and steric which has the role in strengthen drug-receptor interaction [8].

In this research, we have modified thiourea at the two aromatic rings, which are called phenyl and benzoyl groups. Phenyl and benzoyl groups might have a role in increasing lipophilic and steric properties. The phenyl group with *p*-fluoro and the benzoyl with *p*-methoxy are designed to obtain *N*-4-methoxybenzoyl-*N'*-(4-fluorophenyl)thiourea. Adding *p*-fluoro and *p*-methoxy groups might increase cytotoxic activity like the previous study by electronic property role [5].

The other rational design to develop the designed compound was *in silico* study [9]. Docking is one of *in silico* methods that obtains the best orientation pose and score prediction in drug-receptor interaction [10]. In the docking study, we used sirtuin1 (SirT1) enzyme as a receptor (PDB ID:4I5I) and Molegro Virtual Docker (MVD) v5.5. Sirtuin-1 was one of histone deacetylase enzymes that has a role in the deactivated p53 genome. However, p53 genomic produces a p53 protein to kill the tumor cell. While cancer occurs, p53 mutates, and high content of sirtuin-1 is produced [11]. Tenovin-1, one of thiourea derivatives, has been shown to

inhibit sirtuin-1 mechanism as anticancer [12]. Hydroxyurea (HU) was used as a reference because of similarity structures with thiourea, and it has been used as an anticancer agent since a long time ago.

Re-rank score (RS) was used as the score prediction platform. Lower RS compound has stable drug-receptor bond interaction, so it can be predicted that compound has higher biology activity [13]. Synthesis has been done by (Xu *et al*, 2003) (Figure 1) modification [14] two-step reaction between ammonium thiocyanate and 4-methoxybenzoyl chloride followed by 4-fluoroaniline. Then, we evaluated cytotoxic activity of *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea by 3-(4,5-dimethylthyazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) method on HeLa cell line and compared with HU.

MATERIALS AND METHODS

Materials

In silico study used a computer with specification processor Intel Core i5, 4GB memory, Windows 10.1, and NVIDIA GEFORCE 740 M VGA. All synthesis reagents were purchased from Sigma Aldrich® Chemical, Riedel de Haën®, and Merck®. Then, purification was determined by BARNSTEAD® melt-temp electrothermal and TLC CAMAG chamber. Confirmation of structures was done by HEWLETT PACKARD 8452A Diode Array, PERKIN ELMER spectrum one Fourier-transform infrared, JEOL RESONANCE 400 MHz, and high-resolution mass spectrum (HRMS) waters LCT premier ESI TOP. Cytotoxic activity was performed using CO_2 incubator, HeLa cell line (CCRC-UGM), and Biorad Microplate Reader (enzyme-linked immunosorbent assay [ELISA]).

Method

Docking study

Docking study was started by preparation of ligand (converted to three-dimensional form and minimized energy in ChemBio3D v12) and preparation receptor, which downloaded from protein data bank (PDB: 4I5I) and detects cavity in MVD v5.5. Then, the ligand was docked to the receptor for 3 times in 10 iterations until we obtained the RS. Pharmacophore visualization has done by Ligand Scout v4.0.

Synthesis

Thereafter, synthesis has been done by the reaction from 10 mmol of 4-methoxybenzoyl chloride with 15 mmol of ammonium thiocyanate and seven drops polyethylene glycol 400 in 20 ml dichloromethane with refluxed and heated at 40°C until yellow color appeared. After that, 10 mmol 4-fluoroaniline was added in the same mixture pot with refluxed and heated 40°C for 4-5 h. Then, air dry at room temperature. When the powder is formed, washed it with 10% sodium bicarbonate and recrystallized with absolute ethanol. As soon as crystal formed, heated at 50°C for 10-15 min in the oven. Compounds were evaluated its purity with thin-layer chromatography, and the melting point was measured. Afterward, characterization was performed using ultraviolet (UV), infrared (IR), ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and mass spectrometer to confirm structure of *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea.

Cytotoxic assay

The cytotoxic assay was done by MTT method against HeLa cell line and compared with HU as a reference compound. Cells were seeded with 104 cells in 100 μL per well into 96-well plates which contain RPMI, followed by treatment with the test compound at concentrations between 500 and 625 μg/ml for 48 h at 37°C and CO2 atmosphere. Cell viability was assessed with the MTT. The absorbance at 595 nm was recorded using ELISA MICROPLATE READER. The inhibitory concentration 50% (IC50) value was defined as the drug concentration required to inhibit 50% of cells after 48 h of treatment in comparison with untreated controls. Each experiment was repeated 3 times under identical conditions. The IC50 values were calculated with regression between % cell proliferation and concentration (mM).

RESULTS AND DISCUSSION

In Silico Study

The result from *in silico* study was RS and compound-receptor interaction compared with HU. Table 1 showed that the RS of *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea lower than HU, so it can be predicted that *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea has higher activity in receptor SirT1. Then, *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea is feasible to be synthesized. Visualization pharmacophore and

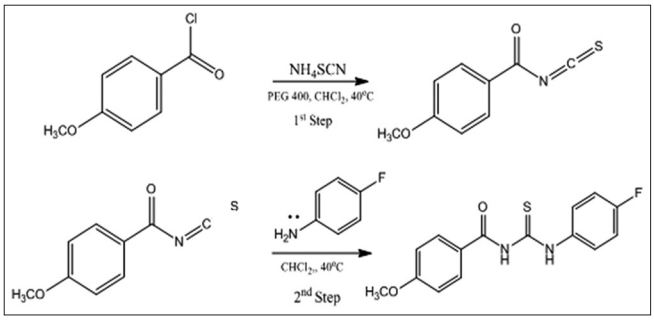
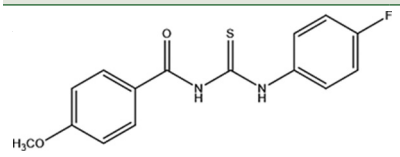
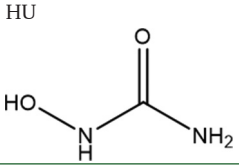


Figure 1: Synthesis of *N*-methoxybenzoyl-*N*'-(4-fluorophenyl) thiourea

Table 1: RS of *N*-methoxy benzoyl-*N*'-(4-fluorophenyl)thiourea and HU

Compound	RS (kcal/mol)
	−112.545±2.439
HU 	−39.008±0.377

HU: Hydroxyurea, RS: Re-rank score

some interactions of *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl) thiourea can be seen in Figure 2 and Table 2. Figure 2 shows the drug-receptor interaction of *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea that might be affected by some interactions as summarized in Table 2. Hydrogen interactions type in the compound pharmacophore was presumably to be an acceptor which accepting electron from three amino acids in receptor cavity (SER4 42A, SER 443A, and LEU 443A). The other was steric interaction which strengthening drug-receptor bound to inhibit SirT1. However, it might prolong the duration of action too with that interaction.

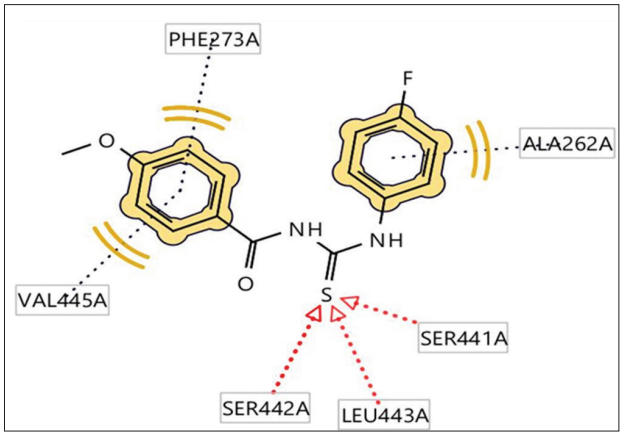


Figure 2: Pharmacophore interaction of *N*-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea with SirT1 (PDB ID: 4I5I)

Synthesis

This was the result of structure confirmation of *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea: Square and flat transparent crystal, yield 49%, melting point 159-161°C; UV spectrum, λ_{max} (nm) in ethanol 218 and 286; IR spectrum, ν (cm^{-1}) in KBr pellet: 3219 and 1606 (-NH amide), 1577 and 1510 (C=C aromatic), 1666 (C=O amide), 1097 and 814 (C=S), and 1212 (C-F) 1256 and 1023 (-O-C ether); ^{1}H-NMR, δ (ppm) in dimethyl sulfoxide (DMSO) D_6: 3.817 (s, 3H, OMeH), 7.012-7.049 (m, 2H, ArH), 7.182-7.243 (m, 2H, ArH), 7.614-7.649 (m, 2H,ArH), 7.971(d, 2H, ArH, J= 9.2 Hz), 11.366 (s, 1H, NHC=O), 12.548 (s, 1H, NHC=S); ^{13}C-NMR, δ (ppm) in DMSO $D_{6:}$ 55.426 (OCH_3)

Table 2: Interaction of *N*-methoxy benzoyl-*N*'-(4-fluorophenyl) thiourea with SirT1 (PDB ID: 4I5I)

Interaction	Type	Pharmacophore	Amino acids
Hydrogen	Acceptor	S in-CS	(N)SER441A
		S in -CS	(N)SER442A
		S in -CS	(O)LEU443A
Steric		Environment of benzoyl	PHE273A
			VAL445A
	---	Environment of phenyl	ALA262A

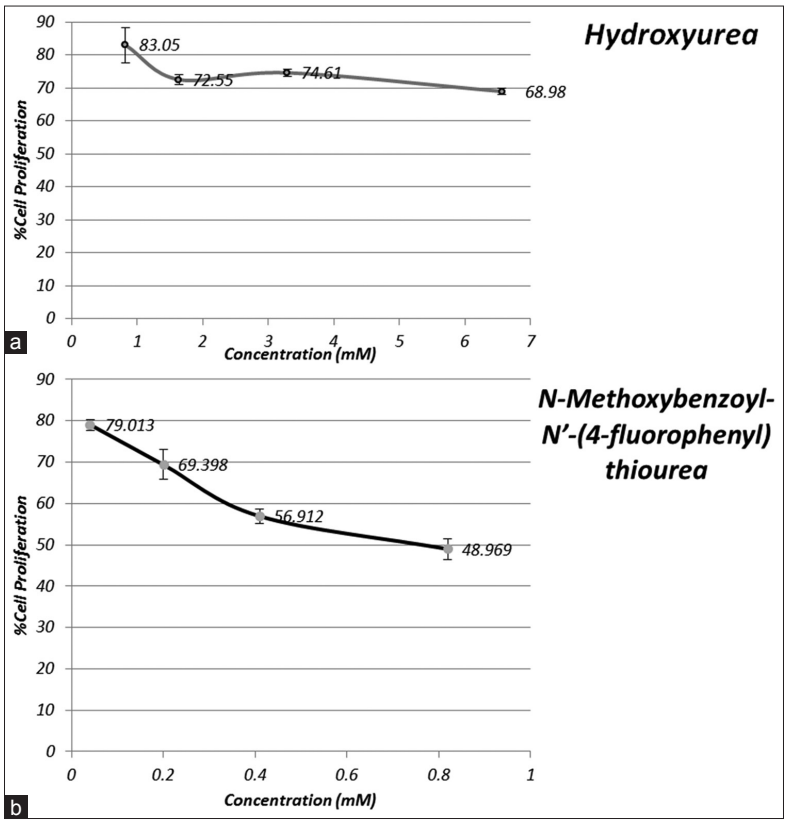


Figure 3: (a and b) Inhibitory concentration 50% determination from hydroxyurea and *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea

114.340, 115.761, 124.361, 127.336, 131.521, 134.944, 161.171,163.796 (C_6H_5), 168.020 (C=O), 180.348 (C=S); and HRMS: calculated for $C_{15}H_{13}FN_2O_2S$ $[M+H]^+$ = 305.0760, $[M+H]^+$ found = 305.0770.

Cytotoxic Assay

Figure 3 shows cell proliferation of HU which did not reach 50%. That was opposite to the synthesized compound which has less cell proliferation than HU at low concentration. To assure the activity level, calculation of their IC_{50} values was done for *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea (0.720 ± 0.07 mM) and HU (16.535 ± 2.092 mM). The increase lipophilic, electronic, and steric properties of *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea appeared to contribute to its activity. The lipophilic property could enhance the penetration through the membrane and increase drug receptor interaction, which resulted in the increased biological activity of the compound [8]. Other than that, an electronic property of methoxy moiety (σ_{para}^{-} = −0.27) would contribute in drug-receptor interaction and oxygen at methoxy might increase solubility in the blood (contained 70% of water), so it can distribute easily. Last, steric property strengthened drug-receptor interaction, so it would prolong its activity with a rigid structure. Thus, we concluded that *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea has better cytotoxic activity than HU.

CONCLUSION

The new compound, *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl) thiourea, has been docked and synthesized. It has better cytotoxic activity than hydroxyurea and it was in line with the docking result. So the docking result can be one way in the rational drug discovery of new active anti cancer compound. Furthermore, *N*-4-methoxybenzoyl-*N*'-(4-fluorophenyl)thiourea can be developed as an anticancer drug.

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REFERENCES

1. WHO. 2012. Available from: http://www.who.int/mediacentre/factsheets/fs297/en. [Last accessed on 2015 Jul 28].
2. Bielenica A, Stefanska J, Stepien K, Napiórkowska A, Augustynowicz-Kopec E, Sanna G, *et al.* Synthesis, cytotoxicity and antimicrobial activity of thiourea derivatives incorporating 3-(trifluoromethyl)phenyl moiety. Eur J Med Chem 2015;101:111-25.
3. Li HQ, Yan T, Yang Y, Shi L, Zhou CF, Zhu HL. Synthesis and structure-activity relationships of N-benzyl-N-(X-2-hydroxybenzyl)-N'-phenylureas and thioureas as antitumor agents. Bioorg Med Chem 2010;18:305-13.
4. Saeed S, Rashid N, Jones PG, Ali M, Hussain R. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. Eur J Med Chem 2010;45:1323-31.
5. Vega-Pérez JM, Periñán I, Argandoña M, Vega-Holm M, Palo-Nieto C, Burgos-Morón E, *et al.* Isoprenyl-thiourea and urea derivatives as new farnesyl diphosphate analogues: Synthesis and *in vitro* antimicrobial and cytotoxic activities. Eur J Med Chem 2012;58:591-612.
6. Ruswanto R, Miftah AM, Tjahjono DH, Siswodihardjo S. Synthesis and *in vitro* cytotoxicity of 1-benzoyl-3-methyl. Proc Chem 2015;17:157-61.
7. Widiandani T, Arifianti L, Siswodihardjo S. Docking, Synthesis and cytotoxicity test on human breast cancer cell line (T47D) of *N*-(allylcarbamothioyl) benzamide. Int J Pharm Clin Res 2016;8 Suppl 5:372-6.
8. Siswandono SB. Kimia Medisinal. Vol. 1. Surabaya: Airlangga University Press; 2016. p. 88-9.
9. Blundell TL, Sibanda BL, Montalva RV, Brewerton S, Chelliah V, Worth C L, *et al*. Structural biology and bioinformatics in drug design: opportunities and challenges for target identification and lead discovery. Philos Trans R Soc Biol 2006;361:413-23.
10. Sotriffer CA. Protein-ligand docking: From basic principles to advanced applications. In: Cavasotto CN, editor. *In Silico* Drug Discovery and Design: Theory, Methods, Challenges, and Applications. New York: CRC Press; 2016. p. 155-88.
11. Wang Z, Sun Y. Targeting p53 for novel anticancer therapy. Transl Oncol 2010;3:1-12.
12. McCarthy AR, Pirrie L, Hollick JJ, Ronseaux S, Campbell J, Higgins M, *et al.* Synthesis and biological characterisation of sirtuin inhibitors based on the tenovins. Bioorg Med Chem 2012;20:1779-93.
13. Hardjono S, Siswodihardjo S, Pramono P, Darmanto W. Quantitative structure-cytotoxic activity relationship 1-(benzoyloxy)urea and its derivative. Curr Drug Discov Technol 2016;13:101-8.
14. Xu X, Qian X, Li Z, Huang Q, Chen G. Synthesis and insecticidal activity of new substituted N-aryl-N 1-benzoyl thiourea compounds. J Fluor Chem 2003;121:51-4.