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Synthesis and *in vitro* Cytotoxicity of 1-Benzoyl-3-methyl thiourea Derivatives

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Abstract

Thioureas are important sulfur and nitrogen containing compounds and they are useful substances in drug research. In an effort to improve potential anticancer activities, two novel 1-benzoyl-3-methyl thiourea derivatives were designed and synthesized with substitution at aromatic positions of C-3, and C-4 of 1-benzoyl-3-methyl thiourea. Their structures were confirmed using IR, MS and ^{1}H-NMR. The *in vitro* cytotoxicity against HeLa cell lines were evaluated by the standard MTT assay. All of the compounds exhibited more potent cytotoxicity with IC_{50} values of 160–383 Pg/mL stronger than hydroxyurea with IC_{50} value of 428 Pg/mL.

Keywords : Thiourea, MTT assay, cytotoxicity, 1-benzoyl-3-methyl thiourea, HeLa cell lines.

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1. Introduction

Cancer is one of the major causes of death in developing countries and also in the worldwide. In Indonesia, the cancer became the third largest contributor to death after heart disease. Since 2007 breast cancer reached first ranks for in-patients in hospitals, followed by cervical cancer[1]. It is still ongoing efforts to find new anticancer drugs because long used drugs have gradually become less effective and there is a tendency of resistance[2]. Thiourea is an organic compound that has carbon, nitrogen, sulfur and hydrogen group. This compound is similar

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to urea except that the oxygen atom is replaced by sulfur. Thiourea and its derivatives appear as white, lustrous crystal or flaky solids and they are useful in pharmaceuticals[3]. From some previous studies have observed that thiourea derivatives have potent pharmacological activities such as anti-HIV/antivirus[4], analgesic[5] and anticancer properties[6,7,8].

In previous studies, we have studied the interaction between 1-benzoyl-3-methylthiourea compound and COX-2[9]. We have also synthesized and characterized 1-benzoyl-3-methylthiourea[10]. In other study, we have synthesized and *in vitro* test of 1-benzoil-3-phenylthiourea compound in MCF-7 cells and 1-benzoyl-3-phenylthiourea compound have IC_{50} value in MCF-7 cells at 245 Pg/mL[11].

In order to find more potent thiourea anticancer agents, we modified the thiourea structure by attached one methyl group and substituted aromatic ring on the one *N*-atom generated some 1-benzoyl-3-methylthiourea derivatives. The anticancer activity of the new compounds were evaluated against HeLa cancer cell line.

2. Materials and Methods

2.1. Materials

The 1-benzoyl-3-methylthiourea (3a-c) were synthesized from the reaction of starting material *N*-Methylthiourea (2) with several ring-substituted benzoyl chlorides (1) (Figure 1). The structure of synthesized compound were identified by IR, ^{1}H-NMR, ^{13}C-NMR, and MS spectroscopy; whereas their purity were determined by melting point and TLC analysis.

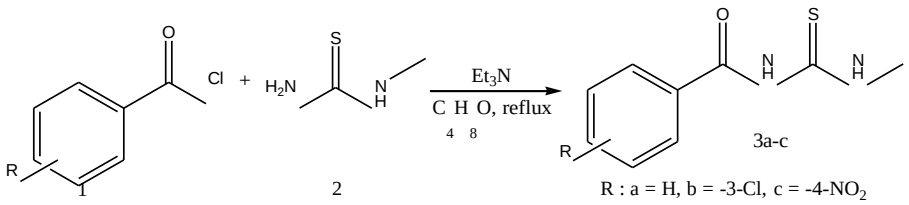

Fig. 1. Scheme of synthesis reaction of the 1-benzoyl-3-methylthioureas

2.2. General Experimental

All reagents and solvents were purchased from standard commercial suppliers. Melting points were measured with an electrothermal melting point apparatus without correction. IR spectra were recorded in KBr on Jasco FT-IR 5300 and major absorption was listed in cm^{-1}. ^{1}H-NMR spectra were taken at Agilent 500 MHz spectrometer, and chemical shift were reported in ppm on the G-scale from internal standard TMS. MS spectra were measured with Agilent 6890N spectrometer using EI methods. TLC was carried out on aluminium plates coated with silica gel F_{254} (Merck) using UV lamp 254 nm to spot detection.

2.3. Synthesis of 1-Benzoyl-3-Methylthiourea derivatives

To 0.05 mol *N*-Methylthiourea in tetrahydrofuran (THF) and triethylamine and then a few drops of 0.025 mol benzoyl chloride were added slowly at room temperature. Then the temperature was raised to 100 ^{0}C and the mixture were refluxed for 5 hours. The mixture was concentrated by evaporating THF and the product was washed with water and saturated sodium bicarbonate respectively. The resulting solid was recrystalised from hot ethanol to give 1-benzoyl-3-methylthiourea compounds **3a-c** (Figure 1).

2.4. Anticancer Activity

In vitro anticancer activity against HeLa cancer cell lines was assayed by MTT method and expressed in IC_{50} value, concentration of the compounds inducing a 50% inhibition of cell growth of treated cells compared to the growth of control cells. Hydroxyurea (HU) was used as the reference drug. Cancer cell lines were seeded at a density of $1x10^4$ cells/well in 96-well microplates. After 24 hours, exponentially growing cells were exposed to the test compounds in DMSO at final concentration ranging from 15.625 to 250 Pg/mL. After 48 hours incubated in a 5% CO_2 incubator at 37 ^{0}C, cell survival was determined by the addition of MTT solution (100 μL of 0.5 mg/ml MTT in PBS). Once formazan was formed, 100 μL 10% SDS in 0.1 N HCl was added and plates were incubated in the dark at 37 ^{0}C overnight. The absorbance was observed at 595 nm on ELISA-reader and survival ratio of living cells were exppressed in percentages with respect to untreated cells. Each experiment was performed at least three times. The IC_{50} value of the test compounds were shown in Table 1.

3. Results and Discussions

3.1. Synthesis of 1-Benzoyl-3-Methylthiourea derivatives

Three 1-Benzoyl-3-Methylthiourea derivatives **(3a-c)** were synthesized from *N*-Methylthiourea and benzoyl chloride in one steps. Two compounds are white solids (**3a** and **3b**), one compound is light yellow (**3c**) and all of them are water insoluble substances. The structure of the synthesized compounds were identified by IR, ^{1}H-NMR and MS spectroscopy as follows :

3.2. 1-Benzoyl-3-methylthiourea (3a).

Yield 54% as white crystal, m.p. 96-97 ^{0}C. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.23-3.24 (3H, d, *J*=5 Hz, CH_3), 7.47-7.50 (2H, t, *J*=15, H-Ar), 7.59-7.62 (1H, t, *J*=15, H-Ar), 7.82-7.83 (2H, dd, *J*=5, 6.5 Hz H-Ar), 9.18 (1H, s, NH), 10.7 (1H, s, NH). IR (KBr), Q_{max} (cm^{-1}) : 3243 (N–H), 1666 (C=O amide), 1565 (C=C Ar), 1195 (-C=S). MS *m/z* EI : 195 (M^+), Calculated Mass $C_9H_{10}N_2OS$: 194.05.

3.3. 1-(3-Chlorobenzoyl-3-methylthiourea (3b)

Yield 27% as white crystal, m.p. 65-67 ^{0}C. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.23-3.24 (3H, d, *J*=5 Hz, CH_3), 7.47-7.50 (2H, t, *J*=15, H-Ar), 7.59-7.62 (1H, t, *J*=15, H-Ar), 7.82-7.83 (2H, dd, *J*=5, 6.5 H-Ar), 9.18 (1H, s, NH), 10.7 (1H, s, NH). IR (KBr), Q_{max} (cm^{-1}) : 3386 (N–H), 1666 (C=O amide), 1581 (C=C Ar), 1172 (-C=S). MS *m/z* EI : 229 (M^+), Calculated Mass $C_9H_9ClN_2OS$: 228.01.

3.3. 1-(4-Nitrobenzoyl-3-methylthiourea (3c).

Yield 27% as white crystal, m.p. 165-166 ^{0}C. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.24-3.25 (3H, d, *J*=5 Hz, CH_3), 7.57-7.59 (3H, m, *J*=9, H-Ar), 7.84 (1H, s, H-Ar); 9.25 (1H, s, NH), 10.20 (1H, s, NH). IR (KBr), Q_{max} (cm^{-1}) : 3255 (N–H), 1646(C=O amide), 1562 (C=C Ar), 1052 (-C=S). MS *m/z* EI: 240 (M^+), Calculated Mass $C_9H_3N_3O_3S$: 239.04.

The 1-benzoyl-3-methylthiourea was produced by nucleophilic acylation between benzoyl chlorides and *N*-Methylthiourea, where the nucleophile was *N*-Methylthiourea. The structural change of **(2)** to 1-benzoyl-3-methylthioureas was characterized by the conversion of $–NH_2$ moeity of *N*-Methylthiourea to –N–C=O(Ar). It

could be observed in ^{1}H-NMR spectra which showed proton peak at –NH region, and supported by IR spectra with N-H bands. The characteristic Q (N-H) stretching vibrations of 1-benzoyl-3-methylthiourea derivatives are appeared in the 3243-3386 cm^{-1}. This difference between the Q (N-H) stretching frequencies is due to intramolecular hydrogen bonding[12]. The strong absorption of Q (C=O) amide band in the IR spectra of the compounds are observed in the region 1646-1666 cm^{-1}, apparently decreasing in frequencies comparing with the ordinary carbonyl absorption (1700 cm^{-1}). This is interpreted as being a result of its conjugated resonance with phenyl ring and the possible formation of intramolecular hydrogen bonding with N-H. The frequencies of 1052-1195 cm^{-1} are assigned to the Q (C=S) vibration for all compounds respectively. In addition to the infra red spectrum, the synthesized compounds have also been confirmed by ^{1}H-NMR and MS.

3.4. Anticancer Activity

Intending to find anticancer agent for cervical cancer, we examined anticancer activity of the compounds *in vitro* on HeLa cell line. The results were listed in Table 1.

Table 1. IC_{50} *in vitro* cytotoxicity of the tested compounds against HeLa Cell line.

Compounds	IC_{50} cytotoxicity (Pg/mL)
3a	383
3b	363
3c	160
Hydroxyurea (HU)	428

Table 1 show that all of the tested compounds have IC_{50} lower than anticancer drug hydroxyurea. Those indicated that all of the tested compounds had anticancer activity against HeLa cells higher than HU. It seems that benzoyl and methyl group on the structure of thiourea may increase anticancer activity. HU that does not contain benzoyl substituents. Compound without substituent on benzene ring **(3a)** showed the lowest activity among the synthesized compounds.

Compound with nitro substituents on the paraposition **(3c)** exhibit the highest activity with IC_{50} of 160 Pg/mL and this compound more active than HU with IC_{50} of 428 Pg/mL. Electron withdrawing atom substitution such as chloro at the metaposition and electron-repelling such as nitro at the paraposition of the aromatic ring in the compound structure increases the lipophilicity of molecules and is responsible for enhanced cytotoxicity in MTT model[13]. These results indicated that the 1-Benzoyl-3-methylthiourea derivatives were potential as anticancer agents against HeLa cell line. However, the mechanism of thiourea toxicity to HeLa cells is not fully understood.

Conclusions

The research concluded that three derivatives of 1-Benzoyl-3-methylthiourea showed *in vitro* anticancer activity against HeLa cell line higher than hydroxyurea. One of them (1-(4-Nitrobenzoyl-3-methylthiourea compound-**3c**) displayed the highest activity among the tested compounds with IC_{50} 160 Pg/mL higher than hydroxyurea with IC_{50} value of 428 Pg/mL.

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