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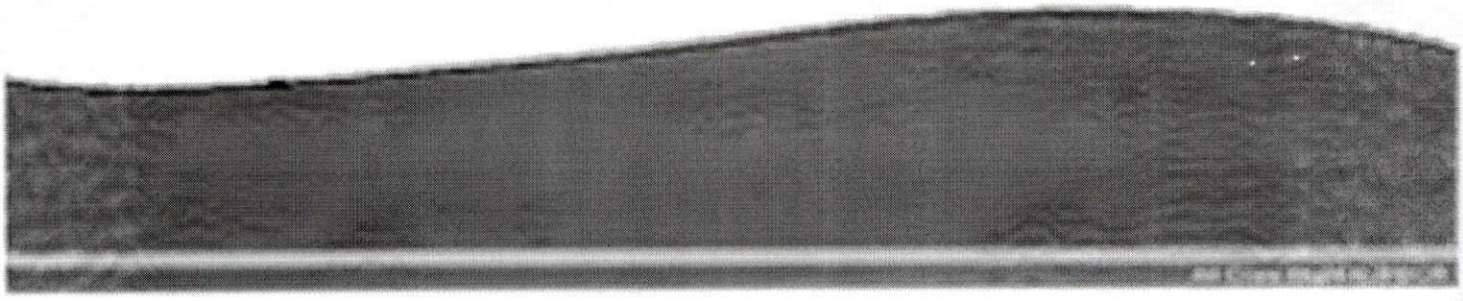

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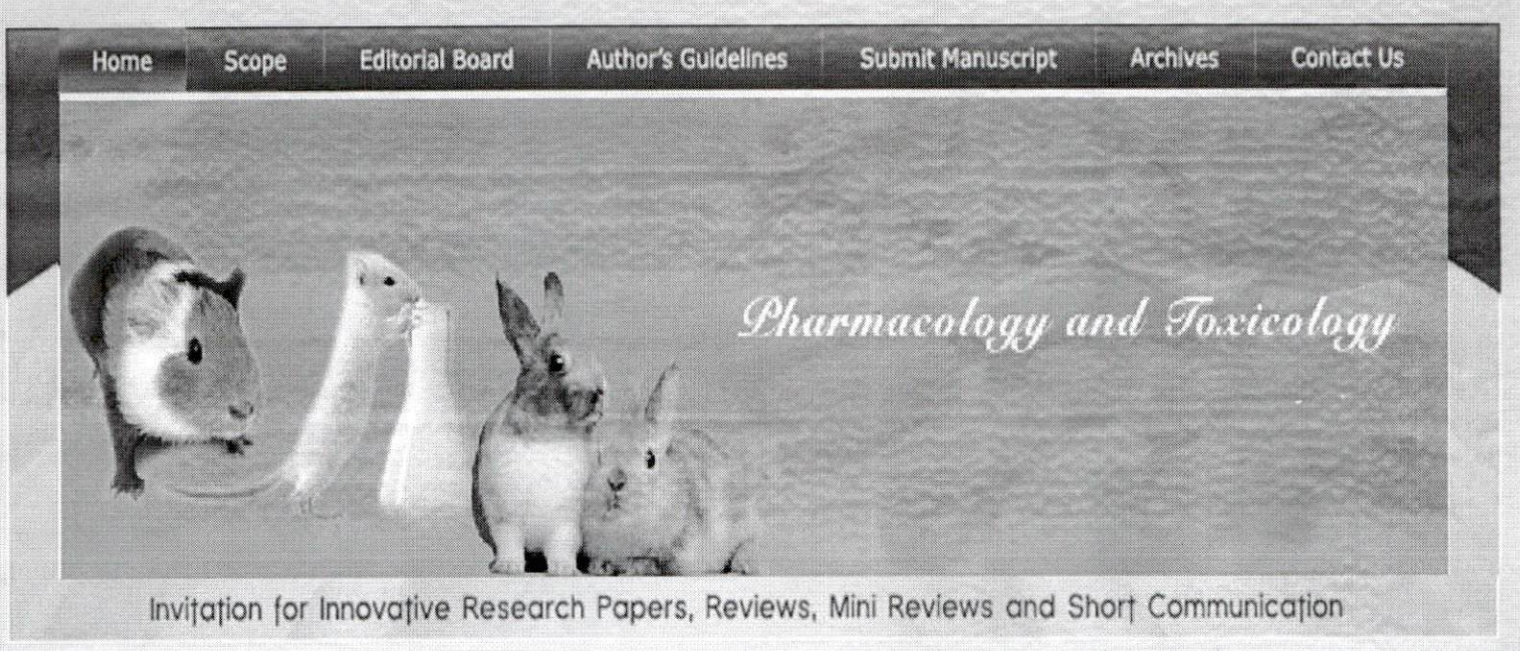

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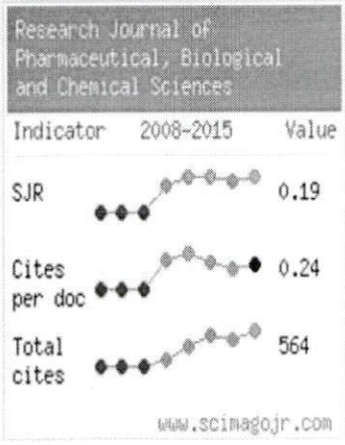

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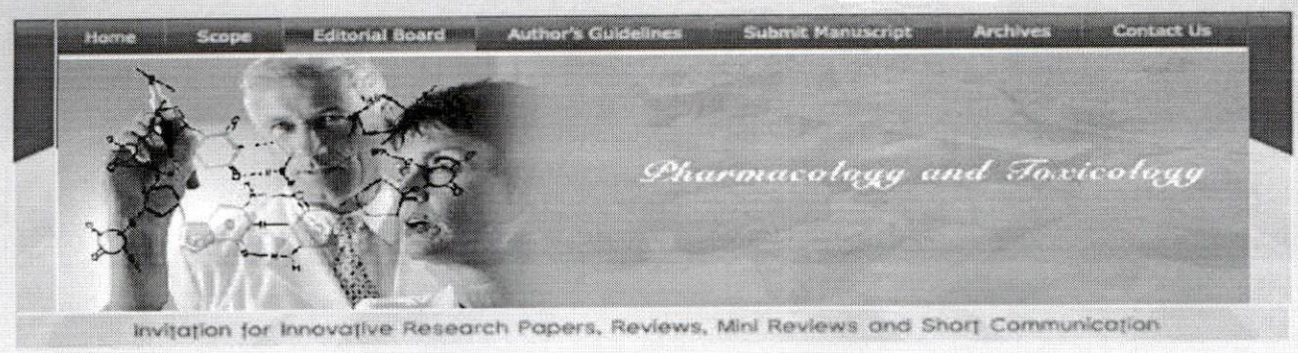

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Docking and Cytotoxicity Test on Human Breast Cancer Cell Line (T47d) of *N*-(Allylcarbamothioyl)-3-chlorobenzamide and*N*-(Allylcarbamothioyl)-3, 4-dichlorobenzamide.

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ABSTRACT

The specific objective of this research is to investigate the biological activity of thiourea derivativesby in silico study and the cytotoxicity test on human breast cancer cell lines. In this present study, the molecular docking of the new compound *N*-(allylcarbamothioyl)-3-chlorobenzamide (BATU-02) and *N*-(allylcarbamothioyl)-3,4-dichlorobenzamide (BATU-04) were evaluated on EGFR (1M17.pdb) using MVD v5.5 and showed that the re-rank scores of BATU-02 and BATU-04 are smaller than5-fluorouracil (5-FU). From the docking result, we can predict that the compounds have a higher biological activity. The cytotoxicity test were evaluated on human breast cancer cell lines (T47D) using MTT assay. Relevant result showed that these compounds(BATU-02 and BATU-04) demonstrated are more potent compared to 5-FU as the commercial anticancer drug, with respective IC_{50} were 128µg/mL (BATU-02); 86 µg/mL (BATU-04); and 213 µg/mL (5-FU). It can be concluded that the modification compounds of thiourea can be further developed as a potential anticancer drug.

Keywords: Docking, thiourea, cytotoxicity, T47D, 1M17

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INTRODUCTION

Cancer is one of the leading causes of death in the world and it is becoming a serve problem in the world of wellness. The success of cancer treatment is a challenge in the 21st century, so it underlines the most urgent to develop novel and safe chemotherapeutic agents with greater anticancer [1].

In the development of anticancer drugs, especially to obtain a better activity, it is performed modification of the structure of thiourea derivatives [2-4]. Thiourea derivatives constitute one class of anticancer promising for further development. This derivative work as inhibitors of EGFR to inhibit receptor tyrosine kinases (RTKs) in the intracellular region [5]. In addition, thiourea can also serve as a conjugate of the anti-EGFR monoclonal antibody[6,7].

In this study, the compoundshave been made by reacting allylthiourea with benzoyl chloride derivatives (3-chloro and 3,4-dichloro) to form the compound *N*-(allylcarbamothioyl)-3-chlorobenzamide (BATU-02) and *N*-(allylcarbamothioyl)-3,4-dichlorobenzamide[9].To predict the anticancer activity of the compounds, it is conducted in silico (docking) test using Molegro Virtual Docker version 5.5 that uses epidermal growth factor receptor (EGFR) kinase with pdb code 1M17, i.e. a receptor model of erlotinib constituting GFR a receptor inhibitor [5,10].

The BATU-02 and BATU-04 are investigated for cytotoxicity activity in human breast cancer cell line (T47D) [12, 15].The cytotoxicity test is determined through MTT method or 3-(4,5-dimethylthiazole-2-yl)-2-5-diphenyltetrazolium bromide. $IC_{50}$is determined based on 50% T47D cells alive. For comparability, it is used 5-fluorouracyl, an anticancer compound that has been used clinically for the treatment of cancer.

MATERIALS AND METHODS

In Silico

Materials: A computer (HP, core i7), Epidermal Growth Factor Receptor (1M17.pdb).

Method: The docking method is using Molegro Virtual Docker version 5.5. The docking began with a preparation on EGFR receptor with 1M17 code taken from the Protein Data Bank. The test ligand was prepared by making the 2-D and the 3-D structure of the compound using ChemBioOffice program Ultra 11.0 and its energy was minimized using MMF94. The resulted in Rerank Score describing the minimal energy required by the compound in interaction with the receptor [9].

In Vitro

Materials: (*N*-(allylcarbamothioyl)-3-chlorobenzamide (BATU-02), *N*-(allylcarbamothioyl)-3,4-dichlorobenzamide (BATU-04), 5-fluorouracyl(Sigma, USA); DMSO (Sigma, USA); *Rosewell Park Memorial Institute* (RPMI) 1640; 3-[4,5-dimetiltiazol-2-il(-2,5-difenil tetrazolium bromida)]= MTT (Sigma, USA); *Fetal Bovine Serum* (FBS) 10% (Gibco, USA).

Method: The Cytotoxicity assay was determined in vitro using cell lines T47D usingthe MTT method. This method required four test groups, namely the compound (A), a positive control (B), a control cell (C), and a control medium (D). It was prepared seven concentrations obtained from stratified dilution with RPMI medium (1000; 500; 250; 125; 62.5; 31.25; 15.625) µg/mL. T47D cell culture was prepared in the microplate of 96 wells, with a density of 5 x 10^3 cells/well.

MTT assay

The assay was carried out following Mossman method [16] with modification in stopper reagent. T47D cells were distributed into 96 well plates, then were incubated for 24 hours under CO_2. Test solutions in the series of concentrations were added, then the mixtures were incubated again for 24 hours. At the final stage of incubation, to each well was added MTT in PBS, then incubation was continued for 4 hours at 37 °C until formazan was formed.MTT reaction was stopped by addition of stopper reagent (SDS 10% in 0.01N HCl)

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followed by overnight incubation at room temperature. Absorbance was read with an ELISA reader at 595 nm. The absorbance was converted to percentage of living cells (cell viability) [17].

RESULTS AND DISCUSSION

Docking

The result of preparing the structure of 2-Dimensional and 3-Dimensional of the *N*-(allylcarbamothioyl)-3-chlorobenzamide (BATU-02), *N*-(allylcarbamothioyl)-3,4-dichlorobenzamide (BATU-04)using ChemBio3D Ultra 11.0 program is shown in Figure 1 [12].

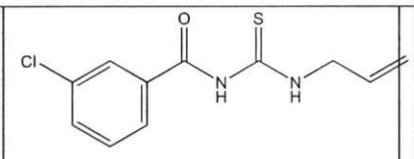
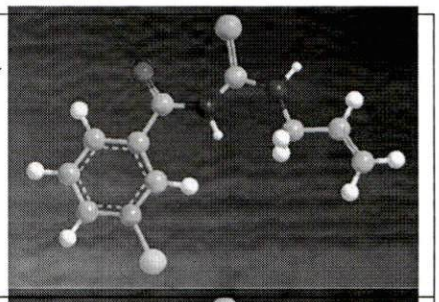
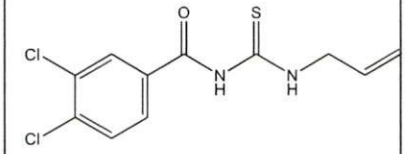
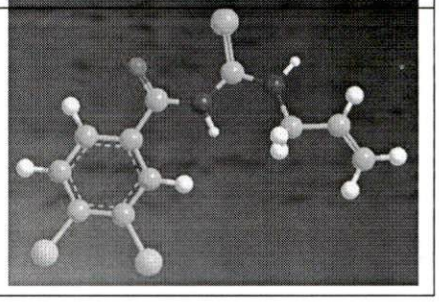
N-(Allylcarbamothioyl)-3-chlorobenzamide (BATU-02)		
N-(Allylcarbamothioyl)-3,4-dichlorobenzamide (BATU-04)		

Figure 1: The structure of 2-Dimensional and 3-Dimensional of the *N*-(allylcarbamothioyl)-3-chlorobenzamide (BATU-02), *N*-(allylcarbamothioyl)-3,4-dichlorobenzamide (BATU-04) of which the energy has been minimized using MMFF94.

Docking results of 5-FU and BATU-02 and BATU-04on EGFR kinase (1M17.pdb) can be seen in Table 1 [13].

Table 1: Docking Results of 5-FU and BATU Derivatives

Compound Code	Rerank Score
BATU-02	-94.4187
BATU-04	-93.1137
5-FU	-48.9354

From the docking results, the Rerank Score of all BATU derivatives is smaller than the Rerank Score of 5-FU. This suggests that the modified compound has smaller bond energy and so thatthe binding is more stable [14]. Thus, the modified compound can be expected to have greater biological activity by in silico test.

The overview of the amino acids involved in the interaction process of compounds between BATU-02 and BATU-04 versus EGFR is presented in Figure 2 [13].

Cytotoxicity Assay

Cell viability is converted from the absorbance of formazan formed after MTT treatment. Percentage of living cells (cell viability) after treatment of test compounds in different concentrations are presented in Table 2.

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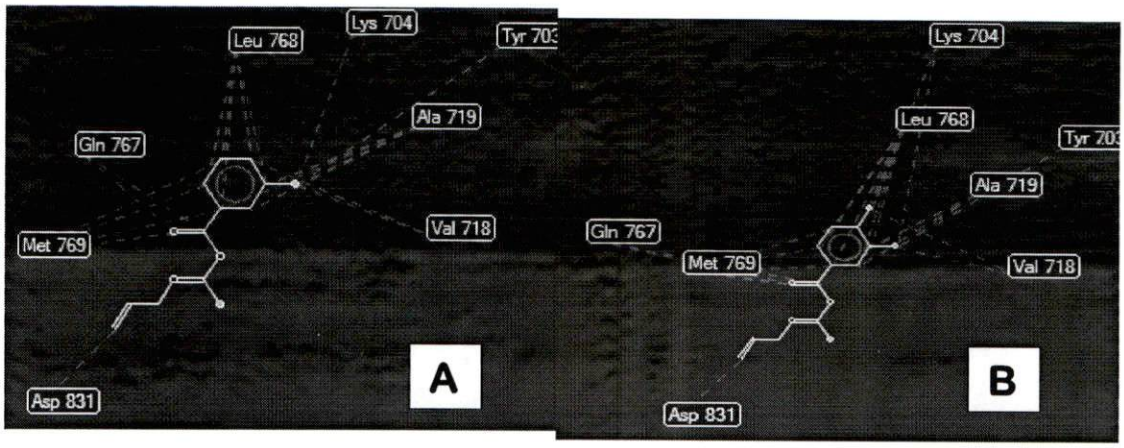

Figure 2: The amino acids involved in the interaction process of compounds between BATU-02 (A) and BATU-04 (B) and EGFR kinase (1M17)

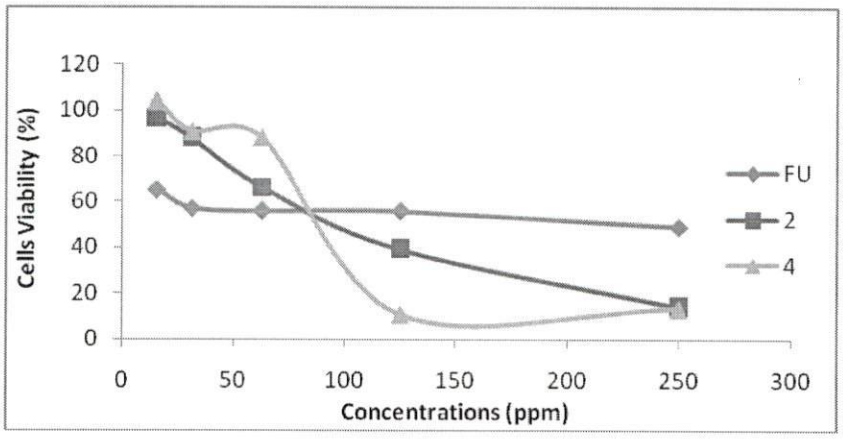

Figure 3: The cells viability overlay profile of 5-FU and BATU-02 and BATU-04

To provide clearer pictures on the influence of different concentrations of 5-FU and BATU-02 and 04 on cell viability, the data is plotted as profiles of cell viability as can be seen in Figure 3. It is obvious that cell viability is getting decreased as the concentration of 5-FU and BATU-02and BATU-04 increased.

Table 2: T47D cells viability (%) after treatment at various concentrations

Compound	The percentage of living cells T47D (%) at various concentrations						
	15.625 µg/mL	31.25 µg/mL	62.5 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL
BATU-02	96.86±1.26	88.23±2.67	82.86±13.89	50.29±31.49	30.94±13.02	12.10±1.98	14.19±2.61
BATU-04	103.97±9.35	90.73±1.96	88.22±4.97	10.84±0.48	13.42±1.43	13.17±0.11	16.82±1.92
FU	61.70±7.98	54.23±4.07	52.91±2.66	52.85±4.58	46.58±4.96	39.41±2.19	27.28±1.05

Table 3: IC_{50} of the test compounds on T47D

Compound	IC_{50} (µg/mL)
BATU-02	128±11.0
BATU-04	86±3.6
FU	213±2.2

The results of probitanalysis to obtain the score of IC_{50} BATU-02; 04 and 5-FU as a comparison can be seen in Table 3.

Based on the cytotoxicity results on IC_{50}, it has been known that *N*-(allylcarbamothioyl)-3-chlorobenzamide (BATU-02)and *N*-(allylcarbamothioyl)-3,4-dichlorobenzamide (BATU-04) havehigher cytotoxicity activity comparedwith 5-fluorouracyl. This result is linear with in silico approach. Therefore, these compounds are feasible to be developed as a potential anticancer drug.

CONCLUSION

Based on the results of this study can be concluded that the new compounds of thiourea derivatives (BATU-02 and BATU-04) can be further developed as a potential anticancer drug.

ACKNOWLEDGEMENTS

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